Microbes and Infection 16 (2014) 362–366
www.elsevier.com/locate/micinf

Short communication

# Molecular characterization of hepatitis A outbreak in the province of Rome, Lazio region, Italy, January–July 2013

Maria R. Capobianchi [a,*], Anna Rosa Garbuglia [a], Chiara Agrati [a], Alessia Rianda [a], Pasquale Noto [a], Angela Corpolongo [a], Maria Adriana Cataldo [a], Silvia Rosati [a], Paola Zaccaro [a], Mariarosaria Loffredo [b], Maria Grazia Pompa [c], Enrico Girardi [d], Paola Scognamiglio [d], Giuseppe Ippolito [e], the GEAS Group[1]

[a] *Laboratory of Virology, National Institute for Infectious Diseases "L. Spallanzani", Via Portuense 292, 00149 Rome, Italy*
[b] *Local Health Unit ASL Roma D, Via dell'Imbrecciato, 71b/73, 00149, Rome, Italy*
[c] *Ministry of Health, DG for Prevention, V Office, Via G. Ribotta 5, 00144, Rome, Italy*
[d] *Department of Epidemiology, National Institute for Infectious Diseases "L. Spallanzani", Via Portuense 292, 00149 Rome, Italy*
[e] *Scientific Direction, National Institute for Infectious Diseases "L. Spallanzani", Via Portuense 292, 00149 Rome, Italy*

Received 23 September 2013; accepted 21 January 2014
Available online 28 January 2014

## Abstract

Reduced circulation of hepatitis A virus lead to an increase of susceptible individuals, and outbreaks occurred recently. In Northern Italy an outbreak is ongoing, attributed to a monophyletic genotype IA strain, with mixed frozen berries as probable source. From 01/01/2013 to 07/15/2013, 30 cases were diagnosed at National Institute for Infectious Diseases, Rome, Italy, representing about twice the number of cases in whole 2012. Phylogenetic analysis indicated that most, although not all, infections were attributable to the same monophyletic genotype IA strain identified in the contemporary Northern Italy outbreak. This strain is also very similar to previous isolates from Venezuela.

*Keywords:* Hepatitis A; Molecular characterization; Food-borne infections; Outbreak; Vaccine-preventable diseases

## 1. Introduction

In Europe the incidence of hepatitis A (HA) has decreased from 15.1 in 1996 to 3.9 per 100,000 inhabitants in 2006, due to improved sanitary and living conditions [1,2]. However, an increase of susceptible individuals has paralleled the reduced circulation of hepatitis A virus (HAV), and several outbreaks occurred in recent years [3–6]. We have previously reported in Lazio region, Italy, a long lasting (2008–2010) outbreak of HA among homosexual males, many of whom HIV-infected, involving a unique monophyletic strain of genotype IA HAV, circulating around the same period also in other European countries [7].

In October 2012–July 2013, Denmark, Finland, Norway and Sweden reported an HA outbreak due to genotype IB strains. Epidemiological investigations in Denmark and Sweden pointed towards frozen strawberries as the vehicle of infection [8,9]. Another genotype IB outbreak related to frozen soft fruits supplemented with pomegranate seeds has been reported in the same period in US [10]. The US and European IB strains presumably originated from the same geographic region, but there is no evidence so far that they are related [10].

The alarm raised by HA outbreaks in Northern European countries recalled attention in other European countries. In particular, in Italy an increase of notified HA cases have been observed in the first months of 2013 at national level, and, in

---

* Corresponding author. Tel.: +39 0655170434; fax: +39 065594555.
*E-mail address:* maria.capobianchi@inmi.it (M.R. Capobianchi).
[1] The members of the GEAS Group are listed at the end of this article.

1286-4579/$ - see front matter © 2014 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.micinf.2014.01.003

particular, in Trento and Bolzano provinces. Cases resulted from exposure during travel to these provinces were reported in other European countries (Germany, the Netherlands and Poland). In May 2013 the Italian Ministry of Health issued an alert, requiring intensified surveillance and recommending molecular typing of HAV strains nationwide. Molecular investigation indicated a unique genotype IA strain involved in the Trento and Bolzano provinces outbreak [11]. Autochthonous cases associated with the same strain, whose geographical origin has not been identified so far, have been reported also in Ireland. Epidemiological, microbiological and environmental investigations indicate mixed frozen berries as the most likely vehicle of infection for Northern Italy and Ireland outbreaks [12,13]. Prompt recall of the implicated products from the market has been undertaken by Italian health authorities, but, due to the incubation period of HA, cases are expected to occur for some time, despite the adoption of this preventive measure.

In this study, we report molecular characterization of all available HAV strains detected at the Laboratory of Virology of the National Institute for Infectious Diseases (INMI) in Rome from January to mid July 2013.

## 2. Materials and methods

### *2.1. Case definition*

A case of acute HA was defined as an individual with acute hepatitis symptoms and the presence of immunoglobulin M antibodies to HAV and/or HAV genome in blood or feces.

### *2.2. Molecular methods*

HAV RNA was detected by RT-PCR as previously described [7]. The VP1/2A amplicons from 22 samples were sequenced and underwent phylogenetic analysis. All sequences were deposited in the GenBank nucleotide database (accession numbers KF475774–KF475780), and were contributed to the national acute hepatitis surveillance system (SEIEVA), Istituto Superiore di Sanità, Rome, Italy.

## 3. Results

In the present year, from first of January to July 15, 30 HAV infections were diagnosed at our Institute (Fig. 1). The number of HA diagnosed in the study period represent 45.4% of total acute viral hepatitis cases (HAV; HBV; HCV; HEV) reported by INMI to regional health authorities in the same period. This figure represents more than 2 times the total number of HAV infections notified by INMI in the whole year 2012, paralleling the increase of cases observed in other Italian regions.

All the patients lived in the province of Rome, Lazio region, that is located in Central Italy, about 600 km apart from the provinces of Trento and Bolzano, where the outbreak was initially identified. All the patients (median age: 38 years; IQR: 29–43 years) were hospitalized and presented with elevated transaminase levels and other signs of hepatic damage. All patients recovered and were discharged in good clinical conditions. The ratio male/female was 1.1, suggesting that the epidemiological characteristics of the present outbreaks are different from those observed in the 2008–2010 outbreak [7]. The nationality of the patients was non Italian in 4 (13.3%) cases (1 India, 1 Poland, 2 Romania).

Twenty one out of 22 HAV sequences were genotype IA, differing by 0–5.4% from each other in the 225 nt stretch analyzed. Of note, the sequences from 17 patients (Pt3, Pt8, Pt12, Pt15, Pt17, Pt19, Pt21, Pt22, Pt23, Pt24, Pt25, Pt26, Pt28, Pt29, Pt30, Pt31, including the 4 non Italian patients) were highly related to each other (mean similarity: 99.56%), and formed a unique monophyletic cluster including the prototype strain of the Italian outbreak (RIVM-HAV13-060, isolated from a Dutch case imported from Italy), and two Venezuelan strains described in 2006 (accession numbers GU001141 and GU001127) [14]. The Pt11 isolate had 98.2% sequence identity and clustered with an HAV strain isolated in 2010 in Rome (accession number JN002370) [7]. Other genotype IA sequences form the present study were inter-dispersed in the phylogenetic tree, and none of them clustered with the monophyletic strain associated with the 2008–2010 Italian outbreak (accession numbers JN002365–JN002368) [7]. Genotype IB was detected only in one patient (Pt20), showing a sequence identity of 98.2% and

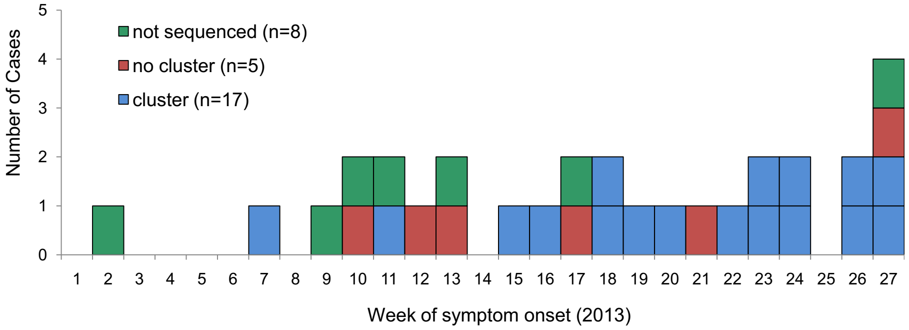

Fig. 1. Hepatitis A cases, identified at National Institute for Infectious Diseases "L. Spallanzani", Rome, Italy, by week of symptom onset. A total of 30 cases were diagnosed in the period 01/01/2013–07/15/2013; in 22 cases sequence analysis could be performed. Sequences included or not included in the monophyletic cluster are indicated in blue and red, respectively; cases not sequenced are indicated in green.