An outbreak of hepatitis A virus infection with a high case-fatality rate among injecting drug users

Enea Spada1,*, Domenico Genovese2, Maria Elena Tosti1, Andrea Mariano1, Marco Cuccuini3, Laura Proietti4, Cinzia Di Giuli5, Alessandro Lavagna5, Giuseppe Edoardo Crapa5, Graziella Morace2, Stefania Taffon2, Alfonso Mele1, Giovanni Rezza2, Maria Rapicetta2

1Istituto Superiore di Sanità, National Center of Epidemiology, Surveillance and Health Promotion, Clinical Epidemiology Unit, Viale Regina Elena 299, 00161 Rome, Italy
2Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy
3Drug Dependency Department, ASL 4 Terni, Terni, Italy
4Prevention Department, ASL 4 Terni, Terni, Italy
5Infectious Diseases Clinic, Santa Maria Hospital, Terni, Italy

Background/Aims: In 2002, the first reported outbreak of hepatitis A virus (HAV) infection involving mostly intravenous drug users (IDU) occurred in Italy. We attempted a thorough evaluation of the outbreak, including epidemiological, clinical and virological analyses.

Methods: We conducted an epidemiological investigation, including a case-control study, to identify the source and the modes of HAV transmission. Hepatitis B and C (HCV) viruses and human immunodeficiency virus (HIV) coinfections were clinically analysed. Sequence analysis of the VP1/2A junction of the HAV isolates was also performed.

Results: Of the 47 symptomatic cases, 35 were IDUs. The only associated risk factor was contact (not related to injecting practices) with a jaundiced person (odds ratio: 5.8; 95% confidence interval: 1.3–29.9). Of the cases, 58% were anti-HCV positive and 4.7% anti-HIV positive. Three individuals died of acute liver failure: 2 were HCV-coinfected alcohol abusers, with underlying liver cirrhosis; 1 was HCV/HIV-coinfected. HAV-RNA was found in 15 of the 24 tested patients: genotype IB (8 cases) and IIIA (7 cases) were detected.

Conclusions: HAV was probably transmitted through the fecal-oral route, although parenteral transmission cannot be excluded. The high fatality rate was probably due to severe underlying liver damage. The occurrence of this outbreak highlights the need for routine HAV vaccination for IDUs.

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Keywords: Hepatitis A; Intravenous drug use; Outbreak; Risk factors; Genotype

1. Introduction

 Injecting drug users (IDU) are at high risk of infection with bloodborne viruses, such as hepatitis B and C viruses (HBV and HCV), the human immunodeficiency virus (HIV), and, though not classically bloodborne, hepatitis A virus (HAV) [1–3]. In non-endemic countries, both large and small outbreaks of HAV infection among IDUs have occurred [4–13]. In some outbreaks, the prevalent variant was genotype IA [4,5] or IIIA [7,11]; co-circulation of different strains was also reported [7]. Nonetheless, the mode of transmission was not determined. HAV is mainly transmitted through the fecal-oral route, which, among IDUs, can be favored by poor hygiene and living conditions [4–10]. The implication of blood products in HAV transmission [14–15] suggests that the virus can also be
transmitted through needle sharing during the viremic period, which can exceed two months [16,17].

We describe an outbreak of HAV infection with an unusually high case-fatality rate among drug users in the city of Terni, Central Italy. We attempted to identify risk factors through a case-control study, to identify the genotypes, and to evaluate the role of coinfections in disease outcome.

2. Patients and Methods

2.1. Epidemiological investigation

Terni (105,000 inhabitants) is located in an area with low HAV endemicity. Although an outbreak (probably water-borne) occurred in the late 1980s, an yearly average of only five new cases have since been reported.

Beginning in September 2002, an unexpectedly high number of cases of acute HAV infection were reported to the Local Health Unit (LHU) by the Infectious Disease Unit of Terni Hospital, by the local Drug Dependency Unit (DDU), and by general practitioners. Most cases occurred among IDUs. In January 2003, an investigation was performed to identify the source of infection and the modes of transmission and to adopt appropriate control measures. A case was defined in the presence of an acute illness source of infection and the modes of transmission and to adopt appropriate control measures. A case was defined in the presence of an acute illness.

In January 2003, a program was created to prevent and control infection among drug users attending the DDU, in addition to the prevention measures already in place for the contacts of cases [18]. HAV vaccination was conducted among drug users with acute infection and, as potential controls, apparently uninfected drug users consecutively attending the DDU in the same period, selected randomly. We investigated travel history, shellfish consumption, contact with a jaundiced person, sexual behavior, history of drug use, types of drugs, and drug-using practices. Information was also collected from HAV-infected non drug users, identified by the LHU.

The study was approved by the Regional Ethics Committee; written informed consent was obtained from all participants.

2.2. Prevention and control measures

In January 2003, a program was created to prevent and control infection among drug users attending the DDU, in addition to the prevention measures already in place for the contacts of cases [18]. HAV vaccination was conducted among drug users with acute infection and, as potential controls, apparently uninfected drug users consecutively attending the DDU in the same period, selected randomly. We investigated travel history, shellfish consumption, contact with a jaundiced person, sexual behavior, history of drug use, types of drugs, and drug-using practices. Information was also collected from HAV-infected non drug users, identified by the LHU.

The study was approved by the Regional Ethics Committee; written informed consent was obtained from all participants.

2.3. Virological assays

All suspected cases of acute hepatitis A were tested for IgM (ETI-HAV IgM Plus, DiaSorin, Saluggia, Italy) and total anti-HAV (ETI-AB-HAVK Plus, DiaSorin). These tests were also used to identify susceptible controls in the case-control study. Hepatitis A cases were also tested for HBsAg (ETI-MAK-2 PLUS, DiaSorin), IgM anti-HBc (ETI-CORE-IgM, DiaSorin), anti-HCV (Ortho HCV3.0, Ortho Clinical Diagnostic, Raritan, NJ), and anti-HIV (AxSYM HIV 1/2G0, Abbott Diagnostic Division, Delsheim Germany). Immunoblot assay was used as a confirmatory test for anti-HCV (RIBA HCV 3.0, Chiron Corporation, Emeriville, CA) and anti-HIV (RIBA HIV1/HIV2, SIA, Chiron Corporation). Anti-HCV-positive samples were also tested for HCV RNA (COBAS Amplicor HCV-Monitor, Roche Diagnostic, Hoffmann-La Roche Ltd, Basel, Switzerland). Serum of IgM anti-HAV-positive patients was further tested for HAV RNA.

HAV RNA was extracted from 200 µl of serum using the QIAamp MiniElute Virus Spin Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer’s instructions. The RNA was recovered in 40 µl of elution buffer and a 266 bp region of the VP1/2A junction of HAV genome was amplified by RT-PCR reaction [19].

All HAV RNA-positive samples were sequenced using the BigDye 1.1 terminator kit (Applied Biosystems, Foster City, CA), following the manufacturer’s instructions, and an ABI 310 automatic sequencer. Genotyping was performed by sequence analysis after alignment of the outbreak sequences with reference strains [20]. The Neighbor-Joining method implemented in the software MEGA2 [21] with 1000 bootstrap replications was applied for the phylogenetic analysis.

Samples of heroin confiscated just before the outbreak were obtained from the Terni police department and tested for HAV RNA. Two aliquots of 100 mg each were suspended in 500 µl PBS, vortexed three times for 60 s, and briefly centrifuged to sediment undissolved material; an equal volume of the suspended drug and negative human serum was mixed, and RNA was extracted, as described above. To establish the sensitivity of the method, different amounts of positive sera from the outbreak were added to the drug.

2.4. Statistical analysis

The association between HAV infection and risk factors was assessed using odds ratios (OR); 95% confidence intervals (95% CI) were also calculated. Differences in proportions were tested by Chi-square or Fisher’s exact test, when necessary. A P-value <0.05 was considered as significant. All statistical analyses were conducted using STATA software (version 8.0) (Statacorp, College Station, TX, USA).

3. Results

3.1. Epidemiological characteristics of the outbreak

From September 2002 to June 2003, 47 cases of acute HAV infection were identified, with a peak between December and February (Fig. 1). The epidemic began among IDUs. Thirty-five persons (74.5%) were known to be IDUs. Contact with a jaundiced person, raw shellfish consumption and travel to highly endemic countries (India) were the other risk factors for non-IDUs (Table 1).

3.2. Case-control study

Twenty-one (60%) of the 35 IDUs with acute HAV infection and 37 (75.5%) of the 49 potential controls participated in the case-control study. All controls had been
vaccinated during the outbreak. Of the 37 potential controls, 19 (51.4%) were tested for IgM and IgG anti-HAV before vaccination: 17 were susceptible to infection (negative for both markers); 2 were IgG-positive and considered as ineligible. The remaining 18 potential controls had already been vaccinated when tested for IgM and IgG anti-HAV and were all anti-HAV IgG-positive and IgM-negative one month or more after vaccination. Since these persons may have already been IgG-positive before vaccination, their inclusion in the case-control study could have led us to underestimate the risk of HAV infection associated with the selected characteristics. We thus performed separate case-control analyses for all 35 controls and for the 17 ascertained susceptible controls.

Only contact with a jaundiced person (i.e. any type of person-to-person contact, excluding that related to injecting practices) was significantly associated with HAV infection (Table 2). Cases were more frequently unemployed than controls, with a nearly statistically significant difference (OR = 3.64; 95%CI 0.95–14.25). The results were essentially unchanged when limiting the analysis to the 17 susceptible controls, although none of the associations were significant (data not shown).

### 3.3. Clinical characteristics

Forty-three of the 47 cases were tested for anti-HCV: 25 (58%) were positive (all were IDUs), including one patient who was also positive for both HBsAg and anti-HIV and another patient who was anti-HIV-positive yet HbsAg-negative. Twenty of these patients were tested for HCV RNA: 12 (60%) resulted positive.

Three of the 47 cases (6.4%) died of acute liver failure. Two of them were anti-HCV-positive; they also reported excessive alcohol consumption and showed clinical evidence of liver cirrhosis before the onset of HAV infection. The only other patient with clinical evidence of underlying cirrhosis was anti-HCV-positive; this patient also developed hepatic encephalopathy yet survived after a prolonged course of disease. The other fatal case was HCV/HIV-coinfected; he had been an IDU for 25 years, had never had an opportunistic infection, and had been receiving highly active antiretroviral therapy (HAART) for 5 years, with a good immuno-virological response (HIV RNA < 200 copies/ml and CD4 count = 690/mmc, a few months before the onset of HAV infection). The only other HIV-positive patient had uncomplicated HAV infection; he had been an IDU for 14 years, had never had an opportunistic infection, consistently had a CD4 count > 350/mmc (yet had not received HAART), and was also HCV/HBV-coinfected.

### 3.4. HAV viremia and genotyping

Serum samples for HAV RNA testing were obtained from 24 patients (51%), a median of 15.5 days after disease onset (range: 0–87); 15 (62.5%) were HAV RNA-positive. One patient was positive 87 days after disease onset; this was the above-described HIV/HBV/HCV-coinfected patient. A serum sample for HAV RNA testing was not available for the other HIV-positive patient.

All HAV RNA-positive samples were sequenced and analyzed together with reference strains from GenBank. The phylogenetic tree is shown in Fig. 2. Seven of the 15 isolates were classified as belonging to genotype IIIA and seemed closely related: they were isolated from 5 IDUs, 1 contact of a case, and 1 individual who had traveled to India. The remaining eight isolates (six from IDUs and two from persons who had consumed raw shellfish) belonged to genotype IB. Of the genotype IB isolates, one (T16) appeared to belong to a different strain and was closely correlated with isolates from an outbreak in the City of Bari (southern Italy), which was traced to a food handler [22]. The genotype IB cases occurred between December 2002 and February 2003, whereas all, but 1 of the genotype IIIA cases (which occurred in January 2003) occurred between late February and late April 2003. It was possible to perform HAV genotyping for only 1 of the deceased patients, who was infected with genotype IIIA. Both samples of heroin were HAV RNA-negative.

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**Table 1**

<table>
<thead>
<tr>
<th>Characteristics of acute hepatitis A cases during an outbreak in Terni (Central Italy); September 2002–June 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>Number of cases</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age (in years)</td>
</tr>
<tr>
<td>≤ 14</td>
</tr>
<tr>
<td>15–29</td>
</tr>
<tr>
<td>30–44</td>
</tr>
<tr>
<td>≥ 45</td>
</tr>
<tr>
<td>Median (range)</td>
</tr>
<tr>
<td>Years of schooling</td>
</tr>
<tr>
<td>≤ 8</td>
</tr>
<tr>
<td>9–13</td>
</tr>
<tr>
<td>&gt; 13</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Risk factors</td>
</tr>
<tr>
<td>Intravenous drug user</td>
</tr>
<tr>
<td>Non drug users</td>
</tr>
<tr>
<td>Contact with a jaundiced case</td>
</tr>
<tr>
<td>Shellfish consumption</td>
</tr>
<tr>
<td>Travel to an endemic area</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Median (range) duration of drug use (in years)</td>
</tr>
</tbody>
</table>

*With or without other possible exposure.*
3.5. Control measures

From January to June 2003, HAV vaccine was administered to 267 IDUs, 30 sexual/household contacts of cases, and 18 healthcare workers. Seven of the vaccinated IDUs developed acute HAV infection: 4 had been vaccinated 15 days earlier; the remaining 3 IDUs had been vaccinated 26, 33, and 35 days earlier. HAV vaccination resulted in a reduced number of new cases in the following months (Fig. 1). No additional cases of HAV infection have been reported since July 2003, and HAV vaccine is now routinely offered to IDUs attending the DDU.

4. Discussion

This is the first report of an outbreak of HAV infection among IDUs in Italy. Outbreaks among IDUs have been reported more often in low endemic areas, compared to highly endemic areas [4–13], yet it is not known whether the differences are a result of the efficiency of surveillance systems. It is possible that in highly endemic countries infection occurs early in life and that a low proportion of the population thus remains susceptible in young adulthood, when injecting drug use usually begins. In recent decades, Italy, which was previously highly endemic, has shown a low-medium endemicity [23], which could explain why outbreaks of acute HAV may be observed among IDUs.

### Table 2

<table>
<thead>
<tr>
<th>Selected characteristics of intravenous drug user (IDU) hepatitis A cases and controls. Terni (Central Italy), 2002–2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis A cases (No. 21)</td>
</tr>
<tr>
<td>Age &gt; 30 years</td>
</tr>
<tr>
<td>Duration of abuse &gt; 10 years</td>
</tr>
<tr>
<td>Male sex</td>
</tr>
<tr>
<td>Household size &gt; 3</td>
</tr>
<tr>
<td>Less than 9 years of schooling</td>
</tr>
<tr>
<td>Being unemployed</td>
</tr>
<tr>
<td>More than one sexual partner</td>
</tr>
</tbody>
</table>

| Contact with a jaundiced case | 9 | 42.8 | 4 | 12.9 | 5.80 (1.30–29.90) |
| Shellfish consumption | 4 | 19.0 | 14 | 40 | 0.35 (0.07–1.43) |
| Use of white heroin (+ other drugs) | 18 | 85.7 | 30 | 85.7 | 1.00 (0.20–7.20) |
| Intravenous drug intake (+ smoke/inhalation) | 16 | 76.2 | 28 | 80 | 0.80 (0.20–3.80) |
| Drug intake at least once a day | 13 | 61.9 | 24 | 68.6 | 0.70 (0.20–2.70) |
| Injecting equipment sharing | 16 | 100 | 25 | 96.4 | NSc |
| No hand-washing before drug use | 18 | 85.7 | 27 | 77.1 | 1.80 (0.40–11.70) |
| One person prepared drugs for everyone | 12 | 57.1 | 27 | 77.1 | 0.40 (0.10–1.50) |
| Use of drugs with > 2 persons | 9 | 42.8 | 10 | 28.6 | 1.90 (0.50–6.70) |
| Having the same supplier of other known cases | 11 | 52.4 | 13 | 37.1 | 1.90 (0.50–6.40) |

a OR, odds ratio.
b CI, confidence interval.
c Excluding contact related to injecting practices.
d In those who injected drugs.
e Not significant.
The case-fatality rate was extremely high (6.4%, compared to the national surveillance system’s estimate of 0.01% in 1995–2000) [24]. This discrepancy could be explained by underlying chronic liver disease. HAV infection superimposed on chronic liver disease has been found to be associated with both a higher case-fatality rate and more severe disease, including acute liver failure [25], although the results for patients with chronic HCV infection are conflicting [26,27].

All three patients with clinical evidence of cirrhosis were anti-HCV-positive and had a complicated course of HAV infection: it was fatal in two patients who were also alcohol abusers; the third fatal case was HCV/HIV-coinfected.

Although HIV infection accelerates HCV-related liver disease, few data are available on its effects on HAV infection. It may be responsible for a higher HAV load and a longer duration of viremia [28,29], which is consistent with our finding that an HCV/HBV/HIV-coinfected patient was still HAV RNA-positive 87 days after the onset of symptoms. However, it is not known whether a higher load and longer duration of viremia are associated with more severe liver disease and fulminant hepatitis [30]. Some authors have reported that the morbidity of HAV infection was not significantly associated with HIV infection [28–31]. In the present outbreak, HAV infection was fatal in an HCV/HIV-coinfected patient yet non-fatal in a HCV/HBV/HIV-coinfected patient. The fatal case had a longer duration of drug addiction (25 vs. 14 years) and thus probably a longer duration of HCV/HIV infection and, consequently, more advanced chronic liver disease, which HAART could only have decelerated. Furthermore, we cannot exclude the co-existence of HAART-induced hepatotoxic damage, which is more common among HIV/HCV coinfected patients with advanced liver disease [32]. These findings suggest that a severe or fatal course of HAV infection is influenced by the presence and severity of underlying liver damage (whatever the etiology), rather than by coinfection per se with HCV, HBV, or HIV.

In the outbreak, three strains were found (one of genotype IIIA and two of genotype IB). The T16 isolate was apparently not related to the outbreak, considering that the patient was not an IDU and had eaten raw shellfish. The co-circulation of different strains of the same genotype and even of different genotypes has been reported for HAV outbreaks associated with seafood consumption [33,34] and those not associated with this factor [7,19,35,36]. The detection of different strains indicates that more than one outbreak occurred in the same area, in the same population, at nearly the same time, although the cases due to genotype IIIA occurred later than those due to genotype IB. This nearly simultaneous occurrence could probably be explained by a low HAV immunity among IDUs, poor hygienic conditions, and the co-circulation of viral strains of different origin, perhaps due to a slightly delayed introduction of one or more new strains, complicating the endemic circulation of established strains.

This is the first report of the circulation of genotype IIIA in Italy. Originating from Southeast Asia, this genotype has been reported in northern Europe among IDU communities [7,11,13] and was perhaps introduced in Europe through an initial drug contamination in Southeast Asia [7,13]. It has been suggested that the fecal contamination of drugs, which is associated with smuggling in the rectum, may be a mode of HAV transmission [4,6]. Our case-control analysis showed an association, though not significant, between being an IDU case and knowledge of other IDU cases with the same drug supplier. Several IDUs reported that their white heroin (the most commonly injected drug) was generally smuggled into the country by North Africans who hid the drug in their rectums. White heroin was first introduced in Terni in 2000, and the first cases of HAV infection among IDUs were reported in 2001. However, in

Fig. 2. Phylogenetic analysis of hepatitis A virus (HAV) isolates during an outbreak in Terni (Central Italy) in 2002-2003 and other variants based on the 266 bp region of the VP1/2A junction of HAV genome. Numbers at the branches indicate the percentages of reproducibility after 1000 bootstraps. The outbreak sequences T8, T9, T17, T19, T34, T49 and T50 belong to genotype IIIA. The sequence T2, T3, T7, T11, T16, T18, T42 and T44 belong to genotype IB.
our study, no difference was found in white heroin use between cases and controls. Furthermore, HAV was not detected in the confiscated drug samples.

Person-to-person transmission could occur through fecal-oral contact, facilitated by poor living conditions and personal hygiene. It could also occur through the percutaneous route, following fecal contamination of the drug or the injecting equipment or as a result of sharing needles or injecting equipment contaminated with the blood of a viremic patient \[10,37\]. In the outbreak, person-to-person transmission via the fecal-oral route among IDUs is likely to have played an important role, although the percutaneous route cannot be excluded. In the case-control study, contact with a jaundiced person was the only factor significantly associated with HAV infection. Furthermore, IDU cases had a lower socioeconomic status than IDU controls and may thus have lower hygienic standards (as reflected in the frequency of hand-washing) and a higher risk of fecal-oral transmission. That several HAV variants were isolated from outbreak cases, together with the other results, seems to be most consistent with the hypothesis of transmission through the fecal-oral route.

Several recent studies have demonstrated that HAV viremia lasts longer than previously believed \[16,17\]. Since the blood of HAV-infected patients is highly infectious before the onset of symptoms \[16,17\], transmission through direct or indirect sharing might not be infrequent and perhaps occurred. However, we did not find any significant differences in injecting practices between IDU cases and IDU controls.

In interpreting the results of this study, several limitations should be considered. First, because the population was relatively small, the case-control study had limited statistical power. Second, since the control group included IDUs who could have had natural HAV immunity before vaccination, the associations between infection and risk behaviors may have been underestimated. Third, the outbreak may have been larger than described, since asymptomatic and sub-clinical cases are difficult to identify. We decided to limit the analysis to symptomatic cases in part because of difficulties in obtaining blood samples from apparently healthy IDUs and their contacts.

Since July 2003, no other cases have been observed. Vaccination early in the outbreak probably contributed to containing the outbreak, as suggested by the reduction in cases in the months following vaccination \[12\]. However, short-term vaccination programs for controlling outbreaks among IDUs in non-endemic countries are unlikely to reduce the number of future infections or to prevent further outbreaks, which are often characterized by increased morbidity among this at-risk group \[12\]. In non-endemic countries such as Italy, owing to the acquisition of natural immunity later in life, the incoming cohorts of young drug users are susceptible to HAV infection. The occurrence of this outbreak stresses the need to routinely vaccinate this population group because they are at increased risk of infection and of complications, due to the high prevalence rates of HCV, HBV, and HIV infections and alcohol abuse. Educational programs are important, yet they are unlikely to have a major impact.

In conclusion, HAV infection is a public-health problem among IDUs. As in other studies, the modes of introduction and transmission of the virus were not concretely defined in our study, neither were the reasons for the infection’s abrupt emergence or the co-circulation of different strains. Poor hygiene and living conditions among IDUs seem to be the most important determinants. The high case-fatality rate stresses the role of underlying chronic liver damage as a determinant of severe and fatal HAV disease.

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