

Original Article

Outbreak of acute hepatitis A associated with men who have sex with men (MSM) in North Sardinia 2017-2018

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Abstract

Introduction: We describe an outbreak of hepatitis A among men who have sex with men (MSM) which evolved in Sassari (Italy), between January 2017 and December 2018, close to a contextual of large concurrent hepatitis A outbreak reported in Europe and recently, in Italy.

Methodology: HAV RNA detection and molecular characterization was performed from serum samples and/or stool by RT - PCR of VP1/2A junction region that ranges from nt. 2,873 to nt. 3,376. The phylogenetic correlation of the circulating hepatitis A strains was assessed by sequencing method according to the HAVNET protocol.

Results: 10 Acute Hepatitis A virus (AHA)-positive cases, 8 of which were among men who have sex with men (MSM) were identified. All patients were tested at the time of hospitalization for the presence of anti-HIV antibodies, only two MSM resulted co-infected by HIV. No differences were observed in median age (37 years vs. 41 years, P-value = 0.14), severity or duration of hospitalization between seropositive and HIV-negative men. Phylogenetic analysis was conducted in 2 cases and revealed two distinct sequences of genotype IA linking to clusters recognized in MSM in other European countries in 2016.

Conclusion: Our study reported a recent increase of notified hepatitis A cases attributable to cases in the European interconnectedness of MSM in Sassari, a small locality. Hepatitis A vaccination for MSM in Sardinia region is recommended; however, our data emphasize the need of hepatitis A screening and vaccination not only for MSM with occasional partners but also for those in monogamous relationships.

Key words: hepatitis A virus; MSM; AHA; risk factors.

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Introduction

Hepatitis A virus (HAV) is one of the most common causes of acute hepatitis worldwide [1]. The disease has a generally self-limiting and benign course. In general, the infection takes place by the fecal-oral route, either by direct contact with an HAV-infected person or through the consumption of water or not cooked enough foods, especially shellfish, raised in water contaminated by sewage containing the virus [2-3]. Epidemiological conditions around the world exist from country to country and even from region to region, based on the degree of socio-economic development [4].

In Italy the incidence is relatively low, not exceeding 10-15 cases per 100,000 citizens/year.

However, it should be considered that the infection is often asymptomatic and it is estimated that over 90% of the Italian population over 60 years of age, has anti-HAV IgG class antibodies, a testify to the enormous spread of this viral agent [5]. Nevertheless, travelers who get exposed to infected water in endemic areas, and men who have unprotected sex with men (MSM), remain at increased risk of HAV infection. Currently, HAV endemicity in European Union (EU) countries can be categorized as low (HAV seroprevalence < 50% by age 15 and ≥ 50% by age 30) or very low (< 50% by age 30 years) [6]. In these settings, the World Health Organization recommends providing HAV vaccination to high-risk groups (e.g., travellers going to higher

endemic countries, MSM, or injecting drug users) [7]. Low endemic areas in Europe resulted susceptible at HAV outbreaks [8], as reported in outbreaks detected in the past decade among travelers [9-11], in community [10], or causing by food [12]. Since June 2016, a significant number of EU Countries have been reporting hepatitis A mostly affecting men who have sex with men (MSM). Outbreak-confirmed cases provide an underestimation of the true extent of the outbreak. This is because information on genomic sequencing is available only for a percentage of positive samples for hepatitis A, since sampling strategies country by country is different and during the dissimilar phases of the epidemic it is not possible to establish a precise estimate. Clusters of the outbreak were initially described in England, Netherlands and Germany, and they primarily involved MSM. Three HAV genotype IA strains (VRD_521_2016, RIVM-HAV16-090 and V16-25801) were considered responsible for the outbreak in this group of individuals [13-15]. Some studies suggested different clinical course and disease severity when hepatitis A occurs in MSM or HIV-infected patients [16]. In the current study, we presented the demographics, risk factors, comorbidities and laboratory results of hospitalized cases of acute hepatitis A in Sardinian patients' afferent to Infectious Diseases Unit during 2017–2018. The Sardinian HAV isolates were analyzed by molecular pattern assessment to investigate the viral gene flow of HAV in Sassari (Italy).

Methodology

Patients

All adult patients diagnosed with AHA at the Infectious Diseases Hospital, Department of Medical, Surgical, and Experimental Sciences University of Sassari, Italy, between 01/02/2017 and 30/10/2018 were included in the study (n = 10). A case of acute hepatitis A was defined as an individual with acute

hepatitis symptoms and the presence of immunoglobulin M antibodies to HAV (anti-HAV IgM). The date of anti-HAV IgM-positivity was considered as the date of diagnosis as part of standard care from hospitalized patients. Liver function tests – measuring serum levels of aminotransferases, alanine phosphatase, gamma-glutamyltransferase activity and total bilirubin using the Laboratory system – were performed for every patient in the study. Information about possible exposure to fecal/oral risk factors (shellfish consumption, well water consumption, travel in endemic areas, contact with a person with symptoms of hepatitis A and on sexual orientation) were collected

at hospital admission, as a part of the clinical examination. The study was conducted in compliance with the Helsinki Declaration and was approved by the Internal Committee Group of University Hospital in Sassari. Informed consent for the inclusion in the study and collection samples was obtained before patient discharge.

Microbiological investigation

At the time of admission to the hospital, in detail, we analyzed 7 serum and 3 stool samples. The serum 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, Marcy l'Etoile, France). All the 10 patients were also tested in admission by anti-HBc IgM antibody (VIDAS HBc IgM; BioMérieux, Marcy l'Etoile, France), anti-HCV IgG antibody (ETI-AB-HCVK-4; DiaSorin, Saluggia, Italy), and HIV virus (VIDAS HIV DUO Quick BioMérieux, Marcy l'Etoile, France). In the additional preliminary step, 2.0 ± 0.1 grams fecal specimens were carefully suspended in 2.0 ± 0.1 mL of phosphate-buffered-saline (PBS) (pH 7.4). The suspension was clarified by centrifugation for 20 minutes at $4,000 \times g$ at 4°C . The supernatant was filtered using a $0.22 \mu\text{m}$ syringe filter (Millipore, Bedford, MA, USA), and 500 μL of eluate was then used for HAV nucleic acid purification extraction system (NucliSENS® miniMag® - BioMérieux Corporate, BioMérieux, Durham, NC, USA). The remaining procedures are common to stool and serum. The RNA was recovered in 100 μL elution buffer and viral fragment spanning the VP1/2A junction of HAV genome was amplified by real time PCR (ISO 15216-1:2017). We completed typing HAV strains by a molecular diagnostic typing protocol in according to reference laboratory for hepatitis A at the National Institute of Public Health and Environment (RIVM) [17]. Genotype was assigned by phylogenetic analysis of a dataset HAV reference sequences of genotypes and subgenotypes downloaded from public database (<https://www.ncbi.nlm.nih.gov/pubmed/>). Maximum Likelihood phylogenetic trees were constructed with bootstrapping on 1000 replicates using MEGA X program (v10.1) (MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Trees were rendered using FigTree (v1.4.4) (<http://tree.bio.ed.ac.uk/software/figtree/>). BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to retrieve sequences showing high similarity and isolated in Europe from 2016 to 2018. The phylogenetic relationship with sequences from Italy and different

European countries were evaluated by means of the MrBayes program using a general-time reversible (GTR) model of nucleotide substitution, a proportion of invariant sites, and gamma distributed rates among sites [18]. ModelTest v. 3.6 [19] was used to select the simplest evolutionary model that adequately fitted the sequence data. A Markov Chain Monte Carlo (MCMC) search was made for 5.0×10^6 generations using tree sampling every 100th generation and a burn-in fraction of 50%. Statistical support for specific clades was obtained by calculating the posterior probability of each monophyletic clade, and a posterior consensus tree was generated after a 50% burn-in.

Results

Epidemiological characteristics of subjects affected by HAV are reported in Table 1. The mean age of patients was 40.2 years, confidence interval (CI): 32–46 years and ± 4.32 standard deviation (SD). Only eight subjects reported homosexual contacts and, particularly, six out eight MSM patients had unprotected sex in the eight weeks preceding the onset of symptoms. All of the serum samples were negative for anti-HBc IgM, but two of the eight MSM patients resulted also HIV-1 positive; four patients travelled abroad (all in Spain) and four cases the transmission was unknown; one positive for anti-HCV IgG (data not shown). The CD4 cell count of the two HIV- positive MSM, tested when hepatitis A had been diagnosed, was low (range: 106–406/ μ L). In the two cases of HAV/HIV coinfection, the patients reported several episodes of unprotected oral and/or anal sex with unknown partners before hospitalization. The comparison of liver functions in HIV positive and negative subjects is reported in Table 2 and all patients obtained the same treatment for their symptoms of hepatitis A. At the time of admission, the HAV/HIV patients were already undergoing antiretroviral treatment by Abacavir/Lamivudine/Dolutegravir and

Table 1. Epidemiological characteristics of patients affected by Hepatitis A virus (HAV) in Sassari (Northern Sardinia - Italy): January 2017 - December 2018 (n = 10).

Clinico-epidemiological features	Number of cases
Average age (\pm SD); (95% Confidence Interval = 3.087)	40.2 (\pm 4.32); (32-46)
Sex (Male)	10
MSM	8
Addicts	Nobody
Travelled 6 weeks before symptoms appear	4
Symptom appearance after travel / stay outside the area of residence (days)	5
HIV infection	2
Other infections (HCV/HBV)	1
Unprotected sexual contacts with extra-partners	6
Vaccination for hepatitis A	Nobody
Bivalve mollusc consumption - berries	6

SD: standard deviation; MSM: men who have sex with men (including cases suspects; self-determination).

Darunavir + Raltegravir. Microbiological assessments were negative also for: *Salmonella spp.*, *Shigella spp.* and *Campylobacter spp.* In all samples HAV-RNA was detected by real time RT-PCR, therefore, the presence of HAV was confirmed by molecular and analytical-chemical tests.

Sufficient PCR products for further analysis were not obtained by the molecular method. In contrast to the TaqMan RT-PCR results, significant inhibition of cDNA amplification was observed when we performed extracts assayed by nested RT-PCR, therefore we used only two amplicons.

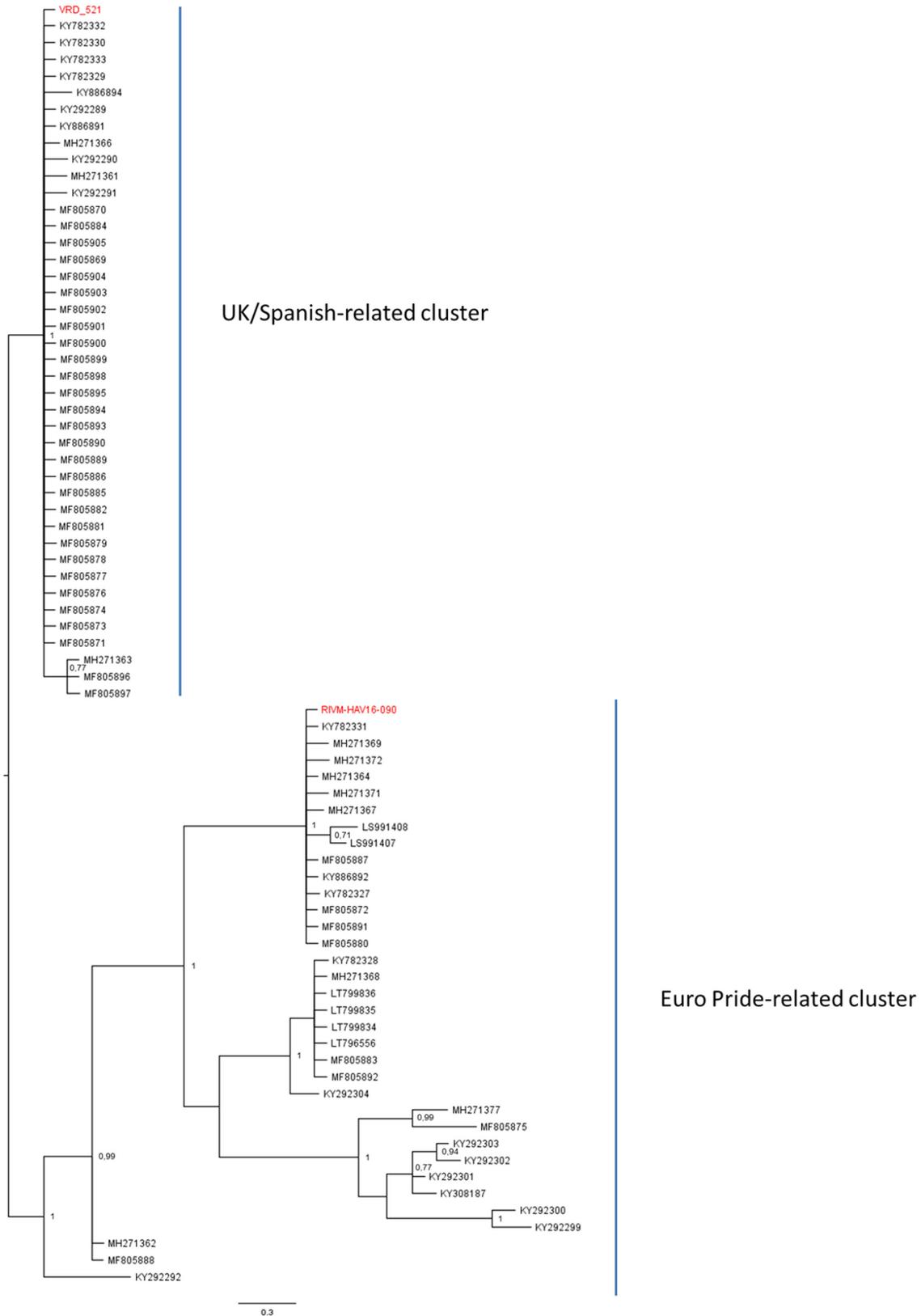
Based on phylogenetic analysis only two MSM analyzed strains resulted of genotype 1A. Bayesian analysis conducted on the dataset of European sequences obtained in MSM outbreaks highlighted that one strain was related to the EuroPride strain (Figure 1).

Table 2. Laboratory results for liver function tests on admission to hospital for HIV-positive (n = 2) and HIV-negative (n = 8) men who have sex with men with hepatitis A; Sassari – Italy (January 2017 to December 2018).

Variable	HIV-positive MSM	HIV-negative MSM
	Median (range)	Median (range)
Number of days hospitalised	10 (5–15)	8 (3–12)
AST (IU/mL)	753 (609-897)	2,143 (181-3,290)
ALT (IU/mL)	2,424 (2,039-2,809)	2616 (311-12,420)
ALP (IU/mL)	154 (153-155)	193 (103-263)
GGT (IU/mL)	234 (208-260)	297 (170-522)
Total bilirubin (μ M/L)	260 (126-393)	91 (26-227)
Number of MSM	2	6

ALT: alanine aminotransferase; ALP: alanine phosphatase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; IU: international units; MSM: men who have sex with men.

Figure 1. Bayesian tree obtained with MrBayes program of HAV sequences showing the relationships between Italian (in red) and European strains (in black). Significant statistical support for clades along the branches (posterior probability > 0.7) are reported.



The second Sardinian strain did not result related to the first and significantly clustered with strains circulating in Spain and UK; our study has highlighted that one of the eight MSM cases reported to have likely been infected Barcelona, Spain [15,16] (Table 3); however, we were not able to perform further genetic analyses due to the extremely small size of the samples.

Discussion

Since June 2016, 16 EU countries reported 1,500 confirmed AHA cases and 1,402 genotype IA-confirmed cases, belonging to three separate clusters based on genetic sequencing of HAV. To our knowledge, 41 MSM-associated HAV outbreaks have only been reported in Italy in 2016 by European Centre for Disease Prevention and Control (ECDC). Italy is considered a low endemic country, but, in the past years, multiple HAV outbreaks in MSM occurred worldwide, probably affecting the prevalence rate in this population. Hepatitis A represents a serious problem of growing public health in the North Europe region and the most recent clinical-epidemiological investigations conducted in the Community countries confirm the information obtained in the present research. According to ECDC classification, mean annual incidence of AHA was very low between 2006 and 2015 in the Centre of Italy, i.e. about 1.77 cases per 100.000 persons-year (range 0.73 ± 3.09) [5]; in Sardinia in 2009-2017 only 24 HAV cases were recorded (adults with not a clear food-borne acquisition) [5]. We describe an outbreak of 10 patients with HAV in Sassari (Sardinia; Italy). The outbreak started in February 2017 and the last case was recorded in September 2018: a total of 8 MSM cases were reported, a considerable increase in HAV cases compared with the last 10 years; phylogenetic analysis conducted in 2 cases revealed two distinct sequences of

genotype IA linking to clusters recognized in MSM in other European countries in 2016. Our study does not provide strong evidence that a large AHA epidemic is currently ongoing in the North Sardinia, mainly involving adult men living in Sassari. The results of molecular investigation, the age and patient's behavior of most cases suggest that infections are mainly transmitted by sexual contacts between men.

Conclusion

Based on these data a greater surveillance of cases and the appropriate active immunization (vaccination) of close contacts including sexual partners are needed to reduce transmission of the virus limiting the most severe forms of the disease. Although there are no particular and specific risk conditions for the recovery of the infection in the general population, there is a real risk of spreading in the local MSM community; this is supported by the fact that the age of the 32- to 46-year-old male population does not appear to be vaccinated. Therefore, it might be warranted to recommend the use of sexual protection devices. In spite of this, our data, in line with the literature, showed a high prevalence of genotype Ia in MSM patients with acute HAV hepatitis, previously described by Sentinel Surveillance System for Acute Viral Hepatitis-SEIEVA (Italy) and contribute to outlining the HAV epidemiology in the Sardinia region in this population [14]. In addition, in Sardinia, information on HIV status is not routinely collected for hepatitis A surveillance purposes; but in the course of this study we detected one HAV infection in a HIV positive MSM who meet the case definition. Sequencing showed infection with a strain identical to the Netherlands (EuroPride strain). Indeed, asymptomatic individuals, even if they do not satisfy the case definition, can still be epidemiologically relevant and should therefore be included in future

Table 3. Characteristics of hepatitis A cases by MSM status and Sardinian strain: January 2017 to December 2018 (n = 8).

Characteristics	Men who have sex with men (MSM)		
	RIVM-HAV16-090_2016 (EuroPride)	VRD_521_2016 (UK/S)	Sequence information unknown
Number of cases	1	1	6
	30-32 (n = 0)	30-32 (n = 0)	30-32 (n = 1)
	33-35 (n = 0)	33-35 (n = 0)	33-35 (n = 0)
	36-38 (n = 0)	36-38 (n = 1)	36-38 (n = 1)
Number per 3-year age group	39-41 (n = 1)	39-41 (n = 0)	39-41 (n = 2)
	42-44 (n = 0)	42-44 (n = 0)	42-44 (n = 1)
	45-47 (n = 0)	45-47 (n = 0)	45-47 (n = 1)
Number of cases hospitalised	1	1	6
Number of patients vaccinated against hepatitis A	0	0	0
Suspected place of infection	The Netherlands (n = 1)	Spain - Barcelona (n = 1)	

MSM: men who have sex with men; UK: United Kingdom; S: Spain.

epidemiological analyses. The comparison with HAV sequences present in molecular databases showed that the RIVM-HAV16-090 strain is present in one patient, while in the other one strain VRD_521 is present, both responsible for the current hepatitis A epidemic in Europe. In the period between June 2016 and December 2017, the epidemic involved 3,813 cases, distributed in 22 European countries. Infected individuals are primarily, but not exclusively, males who have sex with males (MSM) [13-16].

Ethics, consent and permissions

We used and collected anonymous data for this study and HAV was tested on anonymized serum and stool samples, no ethical clearance was sought. Consent to publish: not applicable, there are no patient identifying data.

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