

Title:

**Hepatitis A epidemic in men who have sex with men (MSM) in Milan, Italy**

Running title:

Hepatitis A epidemic in MSM in Milan

**ABSTRACT**

**BACKGROUND AND AIM OF THE WORK:**

Hepatitis A is an infectious disease characterized by fecal-oral transmission; however, a rise in sexually-transmitted cases has been observed, particularly among “men who have sex with men”. In Europe, a Hepatitis A epidemic occurred among men who have sex with men between 2016 and 2018. The aim of this study is to describe this Hepatitis A epidemic in the city of Milan and to analyze the incidence of Sexually Transmitted Diseases co-infection among Hepatitis A cases.

**METHODS:**

Hepatitis A cases were traced and identified. Epidemiological data were collected and Hepatitis A vaccination was investigated. Cases were georeferenced, calculating incidence rates for each Milan Municipality. Viral genotypic analysis was carried out.

**RESULTS:**

353 cases were reported in Milan. Incidence rates resulted significantly higher in males (RR 18.1 CI95% 11.5 – 28.4). 70 cases reported foreign travel. 172 cases reported “Men who have Sex with Men” behaviour. Genotypic analysis revealed correlation with strains of the European “Men who have Sex with Men” epidemic. Georeferencing showed asymmetric case distribution. Only 12 cases reported Hepatitis A vaccination. The Relative Risk for syphilis infection among Hepatitis A cases was 133.9 (95% CI 81.7 – 219.7) and 29.7 (95%CI 9.5 – 92.7) for gonorrhoea.

## **CONCLUSIONS:**

Most genotyped cases (93.7%) correlated to the European Hepatitis A epidemic among Men who have Sex with Men. Georeferencing showed a greater incidence of Hepatitis A cases in areas characterized by the homosexual community. The higher incidence of Sexually Transmitted Diseases co-infection in Hepatitis A cases correlated to clusters responsible for the European Hepatitis A epidemic, suggests increased sexual promiscuity among Men who have Sex with Men. These data support the need for Hepatitis A vaccination programs and sensitization of Men who have Sex with Men to the adoption of safe sexual practices.

## **BACKGROUND E SCOPO DEL LAVORO:**

L'epatite A è caratterizzata da trasmissione feco-orale, tuttavia è stato osservato un aumento dei casi a trasmissione sessuale, particolarmente tra la categoria dei maschi che praticavano sesso con altri maschi. Tra il 2016 ed il 2018, si è verificata in Europa un'epidemia di Epatite A tra maschi che praticavano sesso con altri maschi. Scopo del presente studio è descrivere l'epidemia di Epatite A nella città di Milano tra i maschi che praticavano sesso con altri maschi nella città di Milano e analizzare l'incidenza della co-infezione da IST tra i casi di EA.

## **METODI:**

I casi sono stati georeferenziati e l'incidenza per singolo municipio di Milano è stata calcolata, dato che di tutti i pazienti erano disponibili i dati anagrafici ed epidemiologici. Inoltre è stata effettuata un'analisi genotipica virale.

## **RISULTATI:**

Sono stati notificati 353 casi di Epatite A, con incidenza maggiore nei maschi (RR 18,1 IC 95% 11,5 - 28,4); 70 casi hanno riferito un viaggio all'estero e 172 un comportamento da "Maschi che

praticano Sesso con altri Mzchi". La distribuzione era asimmetrica e l'analisi genotipica ha rivelato una correlazione con l'epidemia europea. 12 casi sono risultati vaccinati contro l'Epatite A. Il Rischio Relativo di sifilide tra i casi di Epatite A era 133,9 (IC 95% 81,7 - 219,7) e 29,7 (IC 95% 9,5 - 92,7) per la gonorrea.

## **CONCLUSIONI:**

I risultati confermano un'elevata similitudine (93.7%) con l'epidemia di Epatite A europea e dimostrano una maggiore incidenza in aree caratterizzate dalla presenza di comunità omosessuali. La maggiore incidenza di co-infezione con Infezioni Sessualmente Trasmesse tra i casi di Epatite A conferma un'aumentata promiscuità sessuale tra i Maschi che praticano Sesso con altri Maschi. Questi dati confermano la necessità di instaurare programmi di vaccinazione contro l'Epatite A e di sensibilizzare i Maschi che praticano Sesso con altri Maschi all'adozione di pratiche sessuali sicure.

**Key words:** hepatitis A, epidemic, men who have sex with men

## **BACKGROUND**

Hepatitis A (HA) is an infectious disease characterized by fecal-oral transmission; however, in recent years, a constant increase in the number of sexually-transmitted cases has been observed, particularly among "men who have sex with men" (MSM) (1,2). In Europe, a HA epidemic mostly affecting the MSM category occurred between the second half of 2016 and the beginning of 2018 (3). The epidemic began in three European countries during events involving several MSM who then imported the infection to their Country (4). During the same period, the number of HA reports was higher than in previous years even in Italy, particularly within the Lombardy Region where the highest number of cases was recorded: 778 or 22.7% out of the 3,426 cases at national level (5). The aim of this study is to epidemiologically and molecularly describe the HA epidemic

that occurred in the city of Milan (Lombardy, Northern Italy) in 2017, focusing on MSM population. In addition, other sexually-transmitted disease (STD) reports were evaluated, namely syphilis and gonorrhoea, in order to calculate the relative risk (RR) of STD in HA cases, compared to the general population in Milan during the same year.

## **METHODS**

### ***Case definition and epidemiological investigation***

HA cases were defined as all patients living in the city of Milan, with a confirmed HA diagnosis, and reported symptoms onset between January 1<sup>st</sup> and December 31<sup>st</sup> 2017. According to the Commission Implementing Decision (EU) 2018/945 of June 22<sup>nd</sup> 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions (6), a confirmed case is identified as any person meeting clinical and laboratory criteria. The clinical criteria include any person with a discrete onset of symptoms (for example, fatigue, abdominal pain, loss of appetite, intermittent nausea and vomiting) and at least one of the following: fever, jaundice or elevated serum aminotransferase levels. The laboratory criteria include the finding of at least one of the following: detection of hepatitis A virus (HAV) nucleic acid in serum or stool, HAV specific antibody response.

Personal and epidemiological data including gender, age, address, travel abroad in previous months, MSM sexual behaviour and other HA risk factors were available for all cases. Furthermore, HA vaccination was investigated via a record linkage to the regional vaccination registry.

Cases' home addresses were georeferenced using QGIS software, mapping case distribution in the city of Milan by Municipality (n=9). Thus age- and gender-specific incidence rates were calculated overall and for each Municipality.

### ***Genotypic characterization and phylogenetic analysis***

A serum sample for each HA case was sent to the regional reference laboratory (Department of Biomedical Sciences for Health, University of Milan, Italy) for HAV genotypic characterization and phylogenetic analysis. Briefly, following nucleic acid extraction and amplification of a genomic fragment (394 nt) in the VP1-2A region, amplicons were subjected to direct sequencing (7). Using the ClustalW program implemented in the BioEdit sequence alignment editor (version 7.2.3), studied sequences were aligned with reference viral genotypes along with the three epidemic genotype IA isolates (VRD-521-2016, RIVM-HAV16-090 and V16-25801) associated with the European multi-country HA outbreak in MSM. Phylogenetic analysis was carried out using MEGA6 bioinformatics software. A phylogenetic tree was generated by means of the Neighbor-Joining method and the Kimura 2-parameter model. Nucleotide identity between studied sequences and reference viral strains for the respective epidemic cluster was computed using the Sequence Identity Matrix tool implemented in the BioEdit software. Overall, 206 HAV sequences from as many HA cases of the Milan 2017 epidemic were analyzed.

## **RESULTS**

### ***Epidemiological investigation***

Between January 1<sup>st</sup> and December 31<sup>st</sup> 2017 a total of 353 HA cases were reported in the city of Milan, equal to 45.4% of all cases in the Lombardy region (8). As reported in Figure 1, the peak epidemic occurred in March. Among observed cases, 333 (94.3%) were male with a median age of 35 years (mean 36 years; mode 29 years), whereas 20 cases (5.7%) were female with a median age of 42 years (mean 37 years; mode 43 years). Regarding risk factors, 32 cases (30 males and 2 females) reported travel in Europe and 38 cases (35 males and 3 females) reported travel outside the EU; 172 cases (48.7%) reported homosexual behavior (MSM).

## ***Georeferencing***

Georeferencing of HA cases in the city of Milan showed an asymmetric distribution, with the majority of cases concentrating in easterly city areas, particularly in Municipalities 2 and 3 (Figure 2).

The total incidence rate per 100,000 inhabitants resulted significantly higher in males of all age classes (50.8) compared to females (2.8) (RR 18.1; CI95%: 11.5 – 28.4); among males, a higher incidence rate per 100,000 inhabitants was observed in the 25-34 and 35-54 years' age classes compared to other age classes. Regarding georeferencing, a higher HA incidence rate was observed in Municipalities 2 and 3 compared to all other Municipalities, and compared to the city's total (44.0 and 46.1 per 100,000 people, respectively), particularly among males of the 25-34 years' age class (260 and 332.7 per 100,000 people, respectively). Results are shown in tables 1 and 2.

Among the 353 HA cases observed, only 12 (3.4%; 11 males and 1 female) cases reported hepatitis A vaccination. Of these, only one case was vaccinated more than one year prior to symptom onset, whereas the remaining 11 cases underwent vaccination around 10 days prior to symptom onset.

## ***Genotypic characterization and phylogenetic analysis***

Figure 3 represents the phylogenetic tree obtained by analyzing 206 VP1-2A HAV nucleotide sequences from as many HA cases (58.4% of total HA studied cases) along with reference HAV sequences from different genotypes and the three epidemic genotype IA isolates associated with the concomitant European multi-country HA outbreak in MSM. Eleven (5.3%) studied sequences were genotype IB, while the remaining 195 (94.7%) sequences belonged to genotype IA; amongst the latter, 193 (93.7%) were correlated to one of the European HA epidemic clusters in MSM. Particularly, 106 (51.5%) studied sequences showed a mean nucleotide identity of 99.8% (range:

99.3-100%) to the reference sequence VRD-521-2016; 86 (41.7%) sequences revealed a mean nucleotide identity of 100% (range: 99.6-100%) to the reference sequence RIVM-HAV 16-090; and 1 (0.5%) studied sequence was V16-25801-like with a 100% nucleotide identity to the respective reference strain.

A HAV sequence was available for 13 out of 20 (65%) female cases: nucleotide sequences correlated to a HA epidemic MSM cluster in 10 cases (76.9%; 6 cases were associated to cluster 2, and 4 cases to cluster 1). Furthermore, 2 additional female cases without a HAV sequence available were found to be epidemiologically linked with a HA male case whose HAV strain belonged to the epidemic cluster 1.

### ***Co-infection with other STDs***

In 2017, 16 syphilis and 3 gonorrhea co-infections were reported among HA cases. All subjects were male with a HAV genotype IA infection associated to one of the European MSM clusters. Since STD co-infection diagnoses only involved male subjects, to calculate the RR of STD in HA cases, the incidence of STDs among HA cases was compared to the incidence of STDs in the male population in Milan in 2017. The RR for syphilis infection among HA cases in 2017 was 133.9 (95%CI 81.7 – 219.7) times the risk in the male population in Milan. Similarly, the RR for gonorrhea infection among HA cases in 2017 was 29.7 (95%CI 9.5 – 92.7) times that of the male population in Milan.

## **DISCUSSION**

A multi-country epidemic, mainly affecting MSM, was observed in Europe since the second half of 2016 (3). Similarly, from the first few months of 2017, a significant increase in the number of HA cases was detected even in Italy. Milan was the city with the highest involvement in Lombardy, accounting for around half of the cases observed in the entire region (8). Phylogenetic analysis of

approximately 60% of HA Milan cases showed that almost all cases (93.7%) were infected by a HAV strain sharing high nucleotide identity with one of the three viral epidemic strains involved in the European HA outbreak mostly affecting MSM. As further evidence to support the link between the Milan HA epidemic and the European outbreak, it is interesting to note that 70 cases (19.8%) reported international travels, representing a potential risk factor for importing and spreading HA epidemic throughout the territory of Milan (9). Georeferencing of HA cases in the city of Milan and its nine Municipalities showed a greater incidence in two Municipalities, both characterized by a strong presence of the homosexual community and gay-friendly clubs. This fact is crucial for the organization of specific programs aimed at actively offering HA vaccination to MSM (10), perhaps even in collaboration with Lesbian, Gay, Bisexual and Transgender associations in the area. In fact, only a minority (3.4%) of individuals was previously vaccinated against HA, and nearly all (11 out of 12 subjects) were immunized only few days prior to symptom onset, probably already after exposure. This observation enhances the need to implement HA vaccination programs among MSM.

Epidemiological and molecular analysis of HA cases involving female population allows us to suppose a spillover infection from MSM to females. Consequently, post-exposure vaccination should be promptly offered to all identified contacts (11).

A higher incidence of STD co-infection among male cases with HA infection due to a HAV strain correlated with the European MSM epidemic clusters, confirms an increased sexual promiscuity among MSM (12). However, although sexual promiscuity may have allowed the spread of the HA epidemic, the latter may have allowed to report a greater number of STDs that may have otherwise remained un-diagnosed. The possible spread of STDs during HA outbreaks underlines the need to raise awareness of MSM to adopt safe sexual practices (10).



## BIBLIOGRAPHY

1. WHO. Hepatitis A. factsheet 2019 Available at: <https://www.who.int/news-room/factsheets/detail/hepatitis-a>
2. Gervasi G, Biticchi M, Zaratti L, Franco E. Epidemics of Hepatitis A and opportunities for vaccination: a focus on the category of men who practice sex with men (MSM). *Ig Sanita Pubbl.* 2018;74(3):295–304.
3. Ndumbi P, Freidl GS, Williams CJ, et al. Hepatitis A outbreak disproportionately affecting men who have sex with men (MSM) in the European Union and European Economic Area, June 2016 to May 2017. *Euro Surveill.* 2018;23(33).
4. European Centre for Disease Prevention and Control (ECDC). Epidemiological update: hepatitis A outbreak in the EU/EEA mostly affecting men who have sex with men. [Internet]. 2018. Available from: <https://ecdc.europa.eu/en/newsevents/%0Aepidemiological-update-hepatitis-outbreak-eueeamostly-%0Aaffecting-men-who-have-sex-men-1>
5. Istituto Superiore di Sanità. Bollettino SEIEVA Epidemiologia delle epatiti virali acute in Italia Numero 2 - Aggiornamento 2017 [Internet]. Available from: <https://www.epicentro.iss.it/epatite/bollettino/Bollettino-n.2-2017.pdf>
6. European Commission. Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions [Internet]. 2018. Available from: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX%3A32018D0945&from=EN%29>
7. Bruni R, Taffon S, Equestre M, et al. Key Role of Sequencing to Trace Hepatitis A Viruses

Circulating in Italy During a Large Multi-Country European Foodborne Outbreak in 2013. 2016;11(2):e0149642.

8. Caso D, Faccini M, Formenti C, Lamberti A, Rossetti E, Senatore S. Report malattie infettive ATS Milano [Internet]. 2017. Available from: <https://www.ats-milano.it/portale/Pubblicazione-documenti/Report-sulla-prevenzione>
9. European Centre for Disease Prevention and Control (ECDC). EMIS-2010 The European Men-Who-HaveSex-With-Men Internet Survey [Internet]. 2010. Available from: <https://www.ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/EMIS-2010-european-men-who-have-sex-with-men-survey.pdf>
10. Cotter SM, Sansom S, Long T, et al. Outbreak of Hepatitis A among Men Who Have Sex with Men : Implications for Hepatitis A Vaccination Strategies. *J Infect Dis.* 2003;187(8):1235–40.
11. Friesema IHM, Sonder GJB, Petriagnani MWF, et al. Spillover of a hepatitis A outbreak among men who have sex with men ( MSM ) to the general population , the Netherlands, 2017. *Euro Surveill.* 2018;23(23).
12. Hess KL, Crepaz N, Rose C, Purcell D, Paz-bailey G. Trends in Sexual Behavior Among Men Who have Sex with Men (MSM) in High-Income Countries, 1990-2013: A Systematic Review. *AIDS Behav.* 2017;21(10):2811–34.