Hepatitis E virus infection prevalence among men who have sex with men involved in a hepatitis A virus outbreak in Italy

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Background. The routes of hepatitis E virus (HEV) transmission have still not been fully clarified. Here, we evaluated the possibility of sexual transmission of HEV, which remains a highly disputed issue.

Materials and methods. Hepatitis E virus sexual transmission risk was assessed by comparing the prevalence of HEV infection in a sample of 196 Italian men who have sex with men (MSM) involved in a multi-country hepatitis A virus (HAV) outbreak, and in 3,912 Italian male blood donors selected from the same regions and provinces as the MSM. Selection of study of participants was motivated by the fact that HEV prevalence among Italian blood donors has been found to vary enormously between different geographical areas.

Results. Anti-HEV IgG prevalence was 14.8% and 5.6% in blood donors and MSM, respectively. Adjusted anti-HEV IgG prevalence was significantly lower in MSM than in blood donors (odds ratio [OR], 0.40; 95% confidence interval [CI]: 0.22-0.75; p<0.01), among residents in northern (OR, 0.45; 95% CI: 0.37-0.55; p<0.01) and southern (OR, 0.45; 95% CI: 0.35-0.58; p <0.01) Italy than among residents in Central Italy, while the prevalence was significantly higher in participants over 50 years of age than in those under 50 years of age (OR, 1.83; 95% CI: 1.48-2.27; p<0.01).

Discussion. Our findings suggest that sexual intercourse does not have a relevant role in HEV transmission. In particular, sexual transmission of HEV is unlikely to influence the prevalence of HEV infection at population level.

Keywords: hepatitis A virus, hepatitis E virus, men who have sex with men, prevalence, sexual transmission.

Introduction

Hepatitis E virus (HEV) has long been known to be endemic in developing countries where infections by genotypes 1 and 2 are usually transmitted feco-orally by contaminated water, causing both outbreaks and sporadic cases[1,2]. Conversely, in developed countries, autochthonous HEV infection is mostly caused by genotypes 3 and 4 and is mainly a zoonosis acquired by eating contaminated raw or undercooked meat (including organs) from infected animals (pigs, wild boars, deer, and rabbits) or by contact with them[1-4]. Feco-oral transmission of these genotypes may also occur by ingestion of vegetables, shellfish, and water contaminated by waste from infected animals and humans[1-3], while transmission by blood transfusion or transplanted solid organs has been well documented[4].

Studies conducted among the general population and blood donors in Europe found variable spatial anti-HEV seroprevalence levels, with important variation even within the same countries[5-13]. The great variability in prevalence was also confirmed by comparing studies using the same anti-HEV assay[7] and has ultimately been linked to the variation in several human, animal and environmental HEV infection-associated factors. Among these are dietary habits (e.g. eating raw or undercooked pork, wild boar and game meat or organs), presence of animal reservoirs (e.g. density of pig and wild boar populations, density of pig farms, types of pig farming), and some environmental conditions, such as the contamination of soil and watercourses by animal waste[1-3,5-14]. Dietary habits, in particular, have been recognised as some of the most important risk factors for HEV infection in some hyperendemic areas in Europe, such as the Abruzzo region in Central Italy and in the Midi-Pyrénées region in Southern France, where very high prevalence and incidence rates have been detected

either in blood donors or in transplant recipients[6-10,14,15]. However, also in these regions, dietary habits alone, although important, seem insufficient to explain such a spread of infection. Thus, a possible and more important role of other less evident transmission sources has been suggested[1,3,8,10,14]. Indeed, in developed countries, HEV transmission routes have still not been fully clarified and, in particular, the possibility of transmission through sexual intercourse needs to be clearly determined[1,3]. Various studies have assessed this, but the role of sexual intercourse in transmission of HEV is still not clear[16-25].

Hepatitis A virus (HAV) and HEV are both single-stranded RNA viruses, which have similar incubation periods and are transmissible via the fecal-oral route. Men who have sex with men (MSM) are at high-risk of hepatitis A virus (HAV) infection due to a high number of different sexual partners and sexual behaviour considered at risk. It is thought that, among MSM, HAV transmission mainly occurs *via* the fecal-oral route, through person-to-person contact, particularly sexual contact (i.e. oro-anal, digito-anal, and genito-oral sexual practices)[26].

In the present study, we determined the risk of sexual transmission of HEV by comparing infection prevalence among Italian MSM involved in a multi-country European outbreak of acute hepatitis A (AHA) with that among a sample of Italian male blood donors.

Materials and methods

Study population

The study population consisted of two distinct groups of individuals: 1) a group with high-risk sexual behaviour, represented by Italian MSM involved in a large European multi-country AHA outbreak[26]; and 2) a group of male blood donors, representing the "unexposed" control group[9].

The high-risk sexual behaviour group consisted of 196 Italian MSM (median age: 38 years; range: 19-79 years) diagnosed with AHA and harbouring one of the three distinct HAV genotype IA strains (VRD_521_2016 [n= 135], RIVM-HAV16–090 [n= 54] and V16–25801 [n= 7]), the isolation of which had served to confirm an outbreak case. These 196 AHA outbreak cases had all been diagnosed in Italian hospitals between November 2016 and September 2017 (115 cases in 5 regions of Northern Italy [Emilia-Romagna, Piedmont, Trentino-South Tyrol, and Veneto], 60 cases in 4 regions of Central Italy [Abruzzo, Lazio, Tuscany and Umbria], and 21 cases in 3 regions of Southern Italy [Basilicata, Calabria and Campania]). They all underwent molecular characterisation at the laboratory of the National Institute of Health (*Istituto Superiore di Sanità*), Rome, Italy. AHA cases that occurred in Italy during the aforementioned time period and that were not epidemiologically linked to the outbreak strain cases, or AHA cases that occurred in men aged under 18 years of age or in women were all excluded from this study.

The unexposed control group consisted of 3,912 male blood donors (median age: 44 years; range: 18-70 years) selected on the basis of their geographical area of residence from an original group of 6,835 male donors who had participated in a nationwide Italian HEV prevalence survey carried out in 2015-2016[9]. HEV prevalence in Italian blood donors varies significantly according to area of residence[9]. Given this, in the present study, male blood donors were selected from the same regions and, whenever possible, also from the same provinces within each region as the MSM.

All study participants gave signed informed consent. The study protocol conformed to the requirements set out in the Declaration of Helsinki and was approved by the Ethics Committee of the Istituto Superiore di Sanità.

Data collection

Data on age, province of residence and date of HEV tests were available for all participants. Data on date of AHA onset and diagnosis and HAV infecting strains were also available for all MSM.

Additional useful information was extrapolated from reports on the multi-country outbreak in the EU/EEA that had been elaborated by the European Centre for Disease Prevention and Control (ECDC) investigation team[26,27]. Overall, 46%, 65% and 42% of male AHA cases who harboured the outbreak strains VRD_521_2016, RIVM-HAV16-090 and V16-25801 gave information on sexual preferences, respectively, and 85%, 80% and 87% of them identified themselves as MSM, respectively[26,27]. In addition, 4.3% of all confirmed outbreak cases were reported as human immunodeficiency virus (HIV)-positive[26].

Hepatitis E virus serological assays

All serum samples were tested for anti-HEV immunoglobulin [Ig] G and M antibodies using commercial enzyme-linked immunosorbent assay (ELISA) kits (Wantai, Biologic Pharmacy Enterprise, Beijing, People's Republic of China) according to the manufacturer's instructions. Both the IgG and IgM anti-HEV assays use recombinant antigen expressed from the ORF2 region.

Detection and quantitation of hepatitis E virus RNA

Extracted RNA (100 µL of serum equivalent) was reverse transcribed and HEV RNA amplified using the RealStar HEV RT-PCR kit, version 1.0 (Altona Diagnostics, Hamburg, Germany). This kit includes primers and a probe targeting ORF3 region of the HEV genome. The sensitivity, reported as 95% limit of detection, was assessed to be 50 IU/mL of HEV RNA.

Statistical analysis

Seroprevalence was expressed as percentage of positive samples out of total tested. Ninety-five percent confidence intervals (95% CI) were calculated using binomial distribution. Seroprevalence was stratified by MSM/blood donors, geographical area and age-group (i.e., <40, 40-49, ≥50 years old; cut-offs chosen as an approximate correspondence to the tertile values of the studied population). Unadjusted differences in seroprevalences were compared in terms of statistical significance using the binomial test. To evaluate simultaneously the association of these three characteristics with the HEV seropositivity, we performed multiple logistic regression to estimate adjusted odds ratios of being HEV-positive.

Results

Out of a total of 4,108 individuals tested for anti-HEV IgG, 590 tested positive (14.4%, 95% CI: 13.3-15.5) (Table I). Nineteen participants tested positive for anti-HEV IgM, and all of them were blood donors; 18 of these 19 were both IgM and IgG positive, while one was only IgM positive. No participants tested positive for HEV RNA.

Crude data analysis showed that the anti-HEV IgG prevalence was significantly higher in male blood donors than in MSM, in Central than in Southern and Northern Italy, and in progressively older age groups (Table I).

After adjusting for all potential co-variates/confounding variables (Table II), the risk of being anti-HEV IgG positive was significantly lower in MSM than in male blood donors, among participants residing in Northern (OR, 0.45; 95% CI: 0.37-0.55; p<0.01) and Southern (OR, 0.45; 95% CI: 0.35-0.58; p<0.01) Italy than in those residing in central Italian regions, while the risk was significantly higher in participants over 50 years of age than in those under 50 years of age (OR, 1.83; 95% CI: 1.48-2.27; p<0.01).

Discussion

In our study, no increased risk of HEV infection was found among Italian MSM involved in a multi-country HAH outbreak compared to a control group of Italian male blood donors. Indeed, anti-HEV IgG prevalence was significantly lower in MSM than in controls; whereas, as expected[9], the prevalence was significantly higher in Central Italy than in Northern and Southern Italy, and in participants over 50 years of age.

Various studies have explored the risk of HEV transmission through sexual intercourse, focusing particularly on transmission among MSM or HIV-infected individuals; these studies gave conflicting results[16-25]. The increased risk of HEV infection among MSM, co-infected or not with HIV, or of having other

Table I - Study population prevalence of anti-hepatitis E virus (HEV) IgG according to comparison group, geographic area, and age.

	N	Anti-HEV +ve	Prevalence (%)	95% CI	p-value
Group					
Blood donors	3,912	579	14.8	13.7-15.9	<0.01
MSM	196	11	5.6	3.1-9.9	
Geographical area					
Northern Italy	1,572	160	10.2	8.8-11.8	<0.01
Central Italy	1,683	343	20.4	18.5-22.4	
Southern Italy	853	87	10.2	8.3-12.4	
Age (years)					
<40	1,518	166	10.9	9.5-12.6	<0.01
40-49	1,235	168	13.6	11.8-15.6	
≥ 50	1,355	256	18.9	16.9-21.1	
Total study population	4,108	590	14.4	13.3-15.5	

N: number of participants; Anti-HEV+ve: anti-hepatitis E virus positive; CI: confidence interval; MSM: men who have sex with men.

Table II - Adjusted risk of being anti-HEV positive according to sexual exposure, area of residence, and age among study participants.

	OR	95% CI	p-value
Group			
Blood donors (ref)	1.00	-	
MSM	0.40	0.22-0.75	<0.01
Geographical area			
Central Italy (ref)	1.00	-	
Northern Italy	0.45	0.37-0.55	<0.01
Southern Italy	0.45	0.35-0.58	<0.01
Age (years)			
<40 (ref)	1.00	-	
40-49	1.24	0.99-1.57	0.06
≥50	1.83	1.48-2.27	<0.01

HEV: hepatitis E virus; OR: odds ratio; CI: confidence interval; MSM: men who have sex with men.

sexually transmitted infections (STI), already reported by Montella *et al.* in the early '90s[16], has been reaffirmed more recently by other studies[17-19]. In contrast, other authors have found no significant increased risk of HEV infection among MSM or HIV infected individuals or those attending STI clinics[20-25].

It is worthy of note that, among the above-mentioned studies, that carried out by Heil *et al.* in a Dutch sexually high-risk population from an STI clinic cohort (including MSM and other individuals having promiscuous sex and multiple partners)[21], although not finding any increased risk of HEV infection according to sexual preference, showed a significant higher anti-HEV prevalence among individuals with *Chlamydia* or gonorrhoeal infections. Equally noteworthy were the results of another study conducted among MSM attending a STI clinic in Milan, Italy[17]. However, in contrast with Heil et al.[21], this study reported an increased risk of HEV infection among MSM that was also two and three times greater in Treponema pallidum and HIV co-infected participants, respectively, than in those not co-infected[17]. It is well known that ulcerative STI (such as *Treponema pallidum* and *Chlamydia* infections) but also non-ulcerative STI (such as gonorrhoeal infections) represent an efficient portal of entry for HIV and other viruses, and may be associated with numerous other subsequent infections[28]. Interestingly, in our study the prevalence of HIV positive participants among MSM was reported to be 4.3%, whereas in studies indicating an increased risk of HEV infection among MSM- or HIV-infected individuals or those with STI, such a prevalence was significantly higher (10%, 12.4% and 33.6% in the studies by Greco *et al.*[17], Lanini *et al.*[18], and Payne *et al.*[19], respectively). It is legitimate to expect that, among participants of these studies, similar high prevalence rates could be detected also for other STI. All the above reported data would seem to suggest a role of HIV infection and of other STI in determining HEV sexual transmission risk. However, a recent study from Taiwan carried out among HIV-infected individuals during a large HAV outbreak found no increased prevalence and incidence of HEV infection; this was associated with older age but not with sexual orientation or presence of STIs[25].

Indeed, even if HAV and HEV share the fecal-oral transmission route, there is a big difference in the efficiency of their transmission through person to person contact. HEV concentration in the stool is markedly lower than that of HAV. Thus, HEV infection is less contagious than hepatitis A, and direct spread of HEV through contact with HEV-infected persons is uncommon[29]. In more detail, so far, HEV person-to-person transmission through the fecal-oral route has only been documented for genotype 1; this was among same-household contacts of more than two cases during a large HEV outbreak in Uganda[30]. This person to-person HEV transmission was ascribed to very poor household hygiene after contact with a jaundiced person or failure to wash hands after defecation. Thus, other possible transmission pathways for HEV acquisition (e.g., direct mucosal contact, blood-to-blood contact) during sexual intercourse has to be taken into consideration when examining a sexually high-risk population (i.e., having promiscuous sex and multiple partners, STI, probable traumatic sexual practices, etc.)[17-21]. Therefore, in studies carried out among individuals attending STI clinics and including MSM- and/or HIV-infected individuals (as do most of those reporting an association between MSM and HEV), the risk of HEV infection (if any), as for other possible infectious agents, is more likely linked to the participants' high-risk sexual behaviour and the co-existence of STI than to an individual's specific sexual orientation and practices. In our opinion, these considerations may go a long way in explaining the inconsistency in the results from different studies on HEV sexual transmission, particularly those carried out among MSM.

In our study, the unexposed control group consisted of male blood donors who had participated in a nationwide Italian HEV prevalence survey carried out in 2015-2016[9]. The rationale for this choice was because a male control group was needed for comparison and blood donors are generally considered to represent a group with low-risk behaviour patterns. In addition, since in Italian blood donors HEV prevalence, besides increasing with age, also varies significantly according to area of residence[9], we selected male blood donors from the same regions and, whenever possible, also from the same provinces within those regions as the MSM. In our study, an analysis of the crude data showed anti-HEV IgG prevalence was significantly higher among male blood donors than among MSM. This was mainly because, despite our efforts to balance the regional location of the study participants, the proportion of those residing in Central Italy was higher for male blood donors than for MSM (40.5 *vs* 30.6%). In addition, while among male blood donors, 65% of those residing in Central Italy were over 40 years of age, among MSM, nearly 48% of those from Central Italy were over 45 years of age. However, in our analysis, all the possible variables influencing study findings were taken into account and were adjusted for by a multiple logistic regression model.

Conclusions

The risk of HEV infection was significantly lower among MSM than among male blood donors. This suggests that sexual transmission does not have a relevant role in the spread of HEV. We cannot exclude

the possibility that HEV transmission may occur, especially in a sexually high-risk population group with STI; however, it is unlikely to influence the prevalence of HEV infection at population level.

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Authorship contributions

ES and AC contributed equally as co-first authors.

ES and ARC conceived the study. ES, ARC, AC, and RB designed the original study. ES, AC, and PP analysed the data and prepared the first draft of this manuscript. AC, RB, ST, and ARC were involved in hepatitis A viral strain isolates characterisation. All Authors contributed to the study design development, data interpretation, data analysis/consolidation, and drafting/approval of the manuscript.

Disclosure of conflicts of interest

GML is the Editor-in-Chief of Blood Transfusion and this manuscript has undergone additional external review as a result. The other Authors declare no conflicts of interest.

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