Epidemiol. Infect. (2017), **145**, 3370–3374. © Cambridge University Press 2017
doi:10.1017/S0950268817002412

Hepatitis E virus (HEV): seroprevalence and HEV RNA detection in subjects attending a sexually transmitted infection clinic in Brussels, Belgium

N. DAUBY[1]*, V. SUIN[2], M. JACQUES[2], M. ABADY[2], S. VAN DEN WIJNGAERT[3], M. DELFORGE[1], S. DE WIT[1] AND A. LIBOIS[1]

[1] *Department of Infectious Diseases, CHU Saint-Pierre, Université Libre de Bruxelles (ULB), Brussels, Belgium*
[2] *National Reference Center for Viral Hepatitis, Communicable and Infectious Diseases, Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium*
[3] *LHUB-ULB, Brussels, Belgium*

Received 4 April 2017; Final revision 8 September 2017; Accepted 4 October 2017; first published online 6 November 2017

SUMMARY

Men who have sex with men (MSM) have an increased incidence of pathogens transmitted by the oro-fecal route. Hepatitis E virus (HEV) is an emerging cause of acute hepatitis and fecal shedding is observed during primary infection. We investigated whether MSM are at increased risk of HEV infection. Subjects who attended a sexually transmitted infection clinic in Brussels and had an HIV test performed between 1 June 2014 and 15 January 2016 were identified. A total of 576 samples were retrospectively screened for both total HEV IgG and HEV RNA. Samples positive for IgG were tested for IgM. MSM proportion was 31·1% (179/576). Overall HEV IgG prevalence was 9·03% (52/576) and was identical in MSM and heterosexual subjects. Among the IgG positive samples, 2/52 (3·84%) samples (both women) were positive for anti-HEV IgM. No sample was positive for HEV RNA. Age over 35 was the only risk factor significantly associated with HEV seropositivity (OR 2·07; 95% CI 1·16–3·67). In conclusion, MSM were not found to have an increased prevalence of HEV as previously reported in other European countries suggesting distinct dynamics of HEV infection in this group across Europe and increased age was associated with a higher risk of seropositivity.

Key words: Hepatitis E, serology, viral hepatitis, epidemiology.

INTRODUCTION

Men who have sex with men (MSM) have an increased incidence of pathogens transmitted by the oro-fecal route such as *Shigella* and Hepatitis A virus (HAV) [1]. Notably, outbreaks of HAV infections are currently ongoing in Western-Europe [2, 3].

Hepatitis E virus (HEV), like HAV, is transmitted via the oro-fecal route. In developing countries, the main route of transmission of the virus is in contaminated drinking water while in high-income countries, HEV is considered as a zoonosis transmitted by consumption of contaminated food products. In these countries, the most frequent HEV genotype is genotype 3, and emerging evidence suggests that this genotype is also transmitted by other routes such as transfusion or contaminated water [4, 5]. A recent study performed in the Netherlands indicates that HEV is now the main cause of acute viral hepatitis [6]. In Belgium, although HEV infection is well

* Author for correspondence: N. Dauby, Department of Infectious Diseases, CHU Saint-Pierre, Rue Haute, 322, Brussels 1000, Belgium.
(Email: Nicolas_dauby@stpierre-bru.be)

documented in pig and wild boar populations [7, 8], data about HEV seroprevalence in humans are limited. In a 2011 study performed on 100 randomly selected samples in a hospital of the northern part of the country, seroprevalence was found to be 14% [9]. A recent study performed on limited numbers of hospitalized patients with liver diseases and suspicion of HEV infection found an IgG seroprevalence of 15–20%, depending on the ELISA assay [10].

Acute HEV infection in immunocompetent subjects is generally asymptomatic [11] and is associated with fecal shedding of infective viral particles during approximately 30 days [12, 13].

Recent studies in Europe suggest that HEV seroprevalence is higher in MSM and is increasing in the recent years [14, 15]. Transmission of HEV within MSM population could lead to future outbreaks. It is thus critical to determine whether MSM are at increased risk of HEV as they could benefit from vaccination. Although only available in China, an HEV vaccine has demonstrated high efficacy and to provide long-term protection against HEV infection [16].

In the present study, we assessed the seroprevalence of HEV infection in an urban population attending a sexually transmitted infection (STI) clinic and assessed the risk factors associated with HEV seropositivity.

METHODS

The STI clinic of Saint-Pierre University Hospital is located in downtown Brussels and offers STI screening and treatment. Data that are prospectively collected include: sexual orientation, ethnic background, STI diagnosis (syphilis, *Chlamydia trachomatis* infection, *Neisseria gonorrhea* infection). Serum samples are stored at −20 °C at the laboratory. Subjects who attended the STI clinic of CHU Saint-Pierre and had an HIV test performed between 1 June 2014 and 15 January 2016 were identified retrospectively. Samples kept at −20 °C were screened for HEV IgG using the WANTA IgG ELISA kit (Wantai Diagnostics, Beijing, China). Positive samples for IgG were tested for IgM using the WANTAI IgM ELISA kit. HEV RNA detection was performed on all samples using the RealStar® HEV RT-PCR Kit (Altona Diagnostics, Hamburg, Germany). HEV serological and molecular detection were performed at the National Reference Center of Viral Hepatitis (Scientific Institute of Public Health, Brussels).

Risk factors for HEV seropositivity were analyzed using Fischer's Exact Test. Continuous variables were compared using Mann–Whitney non-parametric test. Statistical analyses were performed using SAS statistical software (version 9.4; SAS Institute, Cary, NC, USA) and Graphpad Prism 5.0 (GraphPad Software, San Diego California USA).

The study was approved by CHU Saint-Pierre's local ethic committee (reference AK/15-06-71/4526).

RESULTS

In total 799 subjects had at least one visit with an HIV test performed at the STI clinic within the selected time frame. A total of 576 samples were available with sufficient volume to perform serological analyses and polymerase chain reaction (PCR). The majority of subjects were male (79·7%) and of European origin (85·6%) of which 53% were Belgian. MSM proportion was 31·1%. As shown in Table 1, MSM had a higher rate of previous STI diagnosis (Syphilis, *Chlamydia trachomatis* or *Neisseria gonorrhea*) and a higher prevalence rate of HIV infection. Overall, HEV seroprevalence was 9·03% with 52/576 samples being positive for anti-HEV IgG. There was no difference in HEV prevalence between MSM and heterosexual subjects. Among the IgG positive samples, 2/52 (3·84%) were also positive for anti-HEV IgM. The 2 subjects with positive IgM were young women of European origin. All 576 samples were tested by RT-PCR and none were found to be positive. No HIV-HEV co-infection was observed. Risk factors for HEV seropositivity are presented in Table 2. Only age >35 years was significantly associated with HEV seropositivity.

DISCUSSION

HEV infection is considered as an emerging cause of acute hepatitis in Europe and is mainly caused by genotype 3 [6]. It is well established that genotype 3 HEV infection is a zoonosis; farm pigs are one of the main reservoir and consumption of pork meat is a major risk factor [4]. In humans, the prolonged shedding of infective particles following acute infection, and possibly in asymptomatic subjects, could favor transmission through the oro-fecal route following sexual activities [12, 13]. The ongoing outbreak of HAV infection in Europe [2, 3] illustrates the potential of oro-fecal transmission of pathogens in the MSM population.

However, so far, person-to-person transmission through the oro-fecal route has only been described with genotype 1 HEV. In an outbreak in Uganda in

https://doi.org/10.1017/S0950268817002412 Published online by Cambridge University Press

Table 1. *Population characteristics according to sexual orientation*

n (%)	MSM *n* = 179	Non-MSM *n* = 397	*P* value
Age (years), median (IQR25–75%)	31 (27–38)	31 (26–39)	0·33
HEV IgG positive	16 (8·9)	36 (9·1)	1
HEV IgM positive	0/16 (0)	2/36 (5·5)	1
HEV RNA	0 (0)	0 (0)	NA
HIV positive	4 (2·23)	1 (0·25)	0·034
⩾1 STI diagnosis before contact	19 (10·6)	4 (1)	<0·0001

Table 2. *Risk factors for HEV seropositivity*

N (%)	HEV IgG − *n* = 524	HEV IgG + *N* = 52	Odds Ratio (95% CI)	*P* value
Age >35 years	189 (36·1)	28 (53·9)	2·07 (1·16–3·67)	0·016
Male sex	414 (79)	45 (86·1)	1·70 (0·75–3·89)	0·28
MSM	163 (31·1)	16 (30·8)	0·98 (0·53–1·82)	1
Foreigner (non-Belgian)	242 (46·2)	29 (55·8)	1·45 (0·82–2·60)	0·19

a HEV-naive population, during which HEV was not detected in drinking water, the identified risk factors for ⩾2 cases in the affected households were being in contact with a jaundiced person, attending a funeral of a jaundiced person and lack of hand wash after defecation [17]. The attack rate was high in a household with at least one case (56·7%). In Europe, although HEV outbreaks have been reported, no evidence of person-to-person transmission has been demonstrated so far [4, 13].

In the present study, no evidence of higher previous exposure to HEV in MSM as compared with heterosexual men & women was found. When comparing with recent European studies assessing the risk of HEV seropositivity in MSM populations (summarized in Table 3), our findings contrast with the results of two recent studies. In an Italian study performed between 2002 and 2011 on 1116 serums from subjects who underwent HIV testing, HEV seroprevalence was found to be higher after a multivariate analysis in MSM and foreign-born subjects with odds ratio almost two times higher in MSM (5% *vs.* 8·2%) [14]. In a retrospective 3-year study performed in various sexual health clinics in the UK, Payne *et al.* analyzed 422 serums of MSM and heterosexual males. They found a higher prevalence of previous HEV infection in both HIV-infected and HIV-uninfected MSM as compared with heterosexuals (7·5%, 10·4% and 3·5% respectively) [15]. Despite these findings, other studies performed in European countries did not detect an association between sexual orientation and HEV seropositivity. In a large Spanish prospective study that included more than 600 HIV-infected subjects, MSM were neither found to have a higher seropositivity rate at inclusion nor to have a higher risk of seroconversion during the 2 years of follow up [18]. In another study performed in the UK in 138 HIV-infected subjects, HEV seropositivity was only associated with pork meat consumption. Sexual orientation was not found to be associated with higher seroprevalence of HEV [19].

This study shows that age over 35 years was significantly associated with increased HEV seropositivity. Increased age is a known risk factor for HEV infection, likely reflecting cumulative risk for infection [20].

The major strength of our study is that HEV RNA testing was performed on all 576 samples. Indeed, use of serological test only to diagnosis HEV infection can lead to underdiagnosis. In a recent study performed on large numbers of blood donors in England, out of 79 donations positive for HEV RNA, 56 (71%) were negative for both HEV IgM and IgG using the most sensitive ELISA test (Wantai) [21].

Our study has some limitations. First, the limited time frame of data collection from which the analyses were performed does not allow us to detect a time-varying increase in seroprevalence as suggested in the UK [15]. Secondly, the sexual behavior (number

https://doi.org/10.1017/S0950268817002412 Published online by Cambridge University Press

Table 3. *Summary of the studies that assessed HEV seropositivity in MSM subjects in Europe*

Study	Years	Country	Assay	Population	Samples (*n*)	Prevalence in MSM as compared with controls	Odds ratio for HEV seropositivity in MSM (95% CI)
Payne *et al.* [15]	2008	UK	Wantai	HIV-negative men	422	10·4% in MSM *vs.* 3·5% in heterosexuals	3·1
Lanini *et al.* [14]	2002–2011	Italy	Dia. pro	Subjects that underwent HIV testing and counseling	1116	Not reported	1·9 (1·03–3·5)
Keane *et al.* [19]	2009–2010	UK	Wantai	HIV-infected subjects	138	Not reported	0·36 (0·09–1·34)
Pineda *et al.* [18]	2009	Spain	Wantai	HIV-infected subjects	613	Not reported	1·55 (0·86–2·78)
Present study	2014–2016	Belgium	Wantai	Subjects attending a STI clinic with a HIV test performed	576	8·9% in MSM *vs.* 9·1% in heterosexuals	0·98 (0·53–1·82)

of partners and unprotected anal intercourse) could not be correlated with HEV seroprevalence due to the retrospective design. However, the MSM subjects in this study had evidence of risky sexual behaviors; they had a higher HIV prevalence and a higher number of previous STI diagnosis.

Considering the ongoing outbreak of HAV infection in Europe [2, 3], HAV should be the first diagnosis in case of acute hepatitis in MSM. However, HEV infection should be considered in the differential diagnosis according to the local epidemiology in light of a recent Dutch study indicating that HEV is now the leading cause of acute hepatitis in this country [6].

In conclusion, this study did not find an increased prevalence of HEV seropositivity in MSM as previously reported in the UK and Italy probably reflecting a distinct dynamic of HEV infection across Europe. Moreover, in the whole cohort, no subject was found to have a detectable viremia suggesting low circulation of HEV in this urban population.

ACKNOWLEDGEMENTS

We thank Dr Sheila Cattell for English language revision. This study was supported by a grant from "Association d'aide à la recherche médicale André Vésale" to N.D.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Simms I, *et al.*** Intensified shigellosis epidemic associated with sexual transmission in men who have sex with men – Shigella flexneri and S. sonnei in England, 2004 to end of February 2015. *Eurosurveillance* 2015; **20**: 21097.
2. **Beebeejaun K, *et al.*** Outbreak of hepatitis A associated with men who have sex with men (MSM), England, July 2016 to January 2017. *Eurosurveillance* 2017; **22**: 30454.
3. **European Centre for Disease Prevention and Control.** *Hepatitis A Outbreaks in the EU/EEA Mostly Affecting Men Who Have Sex With Men – First Update, 23 February 2017*. ECDC, 2017.
4. **Sayed IM, *et al.*** Is hepatitis E virus an emerging problem in industrialized countries? *Hepatology* 2015; **62**: 1883–1892.
5. **Mansuy JM, *et al.*** A nationwide survey of hepatitis E viral infection in French blood donors: HEV seroprevalence in France. *Hepatology* 2016; **63**: 1145–1154.
6. **Doting MHE, *et al.*** The added value of hepatitis E diagnostics in determining causes of hepatitis in routine diagnostic settings in the Netherlands. *Clinical Microbiology and Infection* 2017; **23**: 667–671.
7. **Thiry D, *et al.*** Belgian wildlife as potential zoonotic reservoir of hepatitis E virus. *Transboundary and Emerging Diseases* 2017; **64**: 764–773.
8. **Thiry D, *et al.*** Estimation of hepatitis E virus (HEV) pig seroprevalence using ELISA and Western blot and comparison between human and pig HEV sequences in Belgium. *Veterinary Microbiology* 2014; **172**: 407–414.
9. **Van Hoecke F, *et al.*** Hepatitis E seroprevalence in east and west Flanders, Belgium. *Acta Gastroenterologica Belgica* 2012; **75**: 322–324.
10. **Cattoir L, *et al.*** Hepatitis E virus serology and PCR: does the methodology matter? *Archives of Virology* 2017.
11. **Kamar N, *et al.*** Hepatitis E virus infection. *Clinical Microbiology Reviews* 2014; **27**: 116–138.
12. **Takahashi M, *et al.*** Prolonged fecal shedding of hepatitis E virus (HEV) during sporadic acute hepatitis E:

https://doi.org/10.1017/S0950268817002412 Published online by Cambridge University Press

evaluation of infectivity of HEV in fecal specimens in a cell culture system. *Journal of Clinical Microbiology* 2007; **45**: 3671–3679.
13. **Nicand E, *et al.*** Viraemia and faecal shedding of HEV in symptom-free carriers. *The Lancet* 2001; **357**: 68–69.
14. **Lanini S, *et al.*** Epidemiology of HEV in the Mediterranean basin: 10-year prevalence in Italy. *BMJ Open* 2015; **5**: e007110.
15. **Payne BAI, *et al.*** Hepatitis E virus seroprevalence among men who have sex with men, United Kingdom. *Emerging Infectious Diseases* 2013; **19**: 333–335.
16. **Zhang J, *et al.*** Long-term efficacy of a hepatitis E vaccine. *New England Journal of Medicine* 2015; **372**: 914–922.
17. **Teshale EH, *et al.*** Evidence of person-to-person transmission of hepatitis E virus during a large outbreak in Northern Uganda. *Clinical Infectious Diseases* 2010; **50**: 1006–1010.
18. **Pineda JA, *et al.*** Incidence and natural history of hepatitis E virus coinfection among HIV-infected patients. *AIDS* 2014; **28**: 1931–1937.
19. **Keane F, *et al.*** Hepatitis E virus coinfection in patients with HIV infection. *HIV Medicine* 2012; **13**: 83–88.
20. **Hartl J, *et al.*** Hepatitis E seroprevalence in Europe: a meta-analysis. *Viruses* 2016; **8**: 211.
21. **Hewitt PE, *et al.*** Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. *The Lancet* 2014; **384**: 1766–1773.