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Retrospective Study Evaluating Seroprevalence of Hepatitis E Virus in Blood Donors and in Swine Veterinarians in Italy (2004)

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Running Head: Hepatitis E seroprevalence in blood donors and veterinarians

Impacts

- Hepatitis E is an emerging disease, with an increasing number of cases reported in industrialized countries linked to zoonotic transmission.
- The risk of infection in exposed workers was evaluated by detecting anti-HEV antibodies in a group of swine veterinarians, compared with a group of blood donors.
- HEV IgG seroprevalence was 9.64% (8/83; 95% CI: 4.25–18.11) in veterinarians and 8.82% (15/170; 95% CI: 5.02–14.13) in blood donors, indicating no significant difference between the two groups. Three blood donors were positive for IgM.

Keywords: hepatitis E virus; blood donors; veterinarians; seroprevalence; anti-HEV IgG; anti-HEV IgM

Summary:

Hepatitis E is an emerging viral disease in developed countries, with sporadic cases occasionally linked to the consumption of raw or undercooked pork, wild boar or deer meat. Cases due to transfusion or transplantation have also been reported. In developed countries, hepatitis E is considered a zoonosis and pig is the main reservoir. In the last few years, several studies conducted in Europe reported variable seroprevalence rates among the general population, ranging between 0.26% and 52.5%. A higher seroprevalence was described among workers who come in contact

with pigs. The aim of this retrospective study was to evaluate the seroprevalence of anti-HEV IgG and IgM antibodies in blood donors (170) and in pig veterinarians (83). Archival sera were collected in Italy in 2004. The observed seroprevalence was 9.64% and 8.82% in veterinarians and blood donors, respectively. Overall, only three sera from blood donors were positive for IgM, but no HEV-RNA was detected.

Introduction

Hepatitis E is an acute human disease caused by the hepatitis E virus (HEV). It is usually self-limiting, but it can become chronic in immunosuppressed patients. The fatality rate is 1% but can increase to 25% among infected pregnant women (Kamar et al., 2014). The HEV genome (approximately 7.2 kb) contains three open-reading frames (ORF): ORF1 encodes for the non-structural proteins, ORF2 for the capsid protein and ORF3 for a small multifunctional phosphoprotein. HEV is classified in the family of Hepeviridae, recently divided into the two genera Piscihepevirus and Orthohepevirus (Smith et al., 2014). The latter includes the Orthohepevirus A species, which is known to infect humans and several mammalian species (Smith et al., 2014), classified into four genotypes and one serotype (Aggarwal and Naik, 2009). Genotypes 1 and 2, transmitted by the faecal–oral route, infect only humans and cause waterborne outbreaks in developing countries and sporadic cases in travellers from endemic areas. Genotype 3 and 4 infect both humans and animals and circulate in developed countries (Ruggeri et al., 2013). These two latter genotypes are considered zoonotic and several data, including sporadic and clustered human cases, suggest that the transmission of HEV to humans from swine, wild boar and deer is in some cases related to the consumption of raw or undercooked meat (Pavio et al., 2015). Genotype 4, firstly described only in Asia, was recently detected in Europe in both pigs and humans (Hakze-van der Honing et al., 2011; Garbuglia et al., 2013; Monne et al., 2015). In developed countries of Europe, US and Japan, the genotype 3 is the most frequently detected, infecting pigs, wild boar, deer and some novel reservoirs (e.g. rabbits) (Smith et al., 2014). In Europe, several studies have

shown that pig farmers, swine veterinarians and other swine workers have higher risk of HEV exposure (Bouwknegt et al., 2008; Krumbholz et al., 2012; Chaussade et al., 2013). Moreover, HEV infections transmitted by transfusion and by transplantation have been documented in several countries (Dreier and Juhl, 2014). Blood donors can be infected by HEV asymptomatically, as indicated by studies reporting HEV-RNA in plasma (Gallian et al., 2014).

In Europe, the prevalence of anti-HEV antibodies among blood donors was reported to vary remarkably, ranging from 0.26% in Greece (Stefanidis et al., 2004) up to 52.5% in the south-west of France (Mansuy et al., 2011).

In Italy, genotype 3 HEV caused autochthonous sporadic cases (La Rosa et al., 2011) and was frequently detected in pigs and wild boar (Ruggeri et al., 2013). However, few epidemiological studies regarding HEV seroprevalence in blood donors (Scotto et al., 2012, 2014) or veterinarians (Vulcano et al., 2007) have been conducted. This study aimed to evaluate the presence of anti-HEV IgG and IgM antibodies in the sera of 83 veterinarians working with pigs and 170 blood donors, collected in Italy in 2004.

Materials and Methods

Serum samples

Eighty-three sera were collected from a group of swine veterinarians, attending the 30th meeting (2004) of the Italian Society of Pathology and Breeding of Pigs (SIPAS). By interviewing, the enrolled veterinarians declared to work in northern and central Italy.

To avoid selection biases related to the year of sampling and geographical origin, a second group of 170 sera was randomly selected among blood donors, obtained from the Unit of Molecular Epidemiology (Siena, Central Italy), also collected in 2004. Sera were made anonymous, and the only information available was on age and sex of enrolled subjects (Table 1).

Detection of anti-HEV antibodies by ELISA

Anti-HEV IgG antibodies were detected by the commercial ELISA Kit IgG antibodies anti-hepatitis E virus (Bio- Chain Institute, Hayward, CA, USA), following the manufacturer's instructions. The test is based on detection of synthetic ORF2 and ORF3 HEV antigens. As specified by the manufacturer, the cut-off value (COV) was determined as the optical density (OD) mean value of the negative control plus 0.1. All sera with OD under 0.14 (COV) were considered to be negative.

Detection of anti-HEV IgG and IgM antibodies by Western blotting

The HEV capsid protein (lacking the first 111 amino acids, rD111ORF2) from a genotype 3 swine HEV strain was expressed by baculovirus in Sf9 insect cells and used as antigen in Western blotting (WB), as previously described (Ponterio et al., 2014).

After pre-incubation with a crude protein extract from uninfected Sf9 cells (2 h at 4°C), serum samples (1:100) were incubated with the HEV capsid protein.

The membranes were stained with two different antihuman secondary antibodies, anti-whole IgG (H+L chains) (1:5000; KPL Inc., Gaithersburg, MD, USA) or anti-IgM (l-chain specific) (1:5000; Sigma, Saint Louis, MO, US) conjugated with alkaline phosphatase. The serum from a mouse immunized with the swine HEV capsid protein (rD111ORF2) was used as positive control (1:1000) and stained with anti-mouse IgG (SIGMA) conjugated with alkaline phosphatase.

Detection of HEV genome

Viral RNA was extracted from 100 ll of sera using QIAamp Viral RNA Mini Kit (Qiagen, Redwood City, CA, USA) according to the manufacturer's instruction. The HEV genome detection was conducted by Real-time RT-PCR using RNA Ultrasense One-Step Quantitative RT-PCR System (Life Technologies, Carlsbad, CA, USA) as described by Jothikumar et al. (2006).

Statistical analysis

For calculation of seroprevalence, a human serum was defined as positive if confirmed in at least one of the tests used (ELISA and/or WB IgG). Because of their professional contacts with pigs, veterinarians were considered as the exposed group in comparison to blood donors (not exposed). A multivariable logistic regression model was fitted to examine the association between HEV seroprevalence and sex, age (≥43 versus <43 years) and source of sample (veterinarians versus blood donations). The model was based on the simultaneous entry of all variables, and its efficacy was assessed based on the likelihood ratio and the Hosmer– Lemeshow statistic. The odds ratio (OR) and 95% confidence intervals (95% CI) were calculated from the final multivariable logistic regression model. Agreement among ELISA and WB IgG results was evaluated using Cohen's Kappa coefficient. All statistical analyses were performed using the software SPSS 23.0 (IBM SPSS Statistics, New York, NY, USA).

Results

The presence of anti-HEV antibodies was tested by ELISA and Western blotting, and a human serum was defined as positive if confirmed by at least one of the tests used (ELISA or WB IgG). The seroprevalence was 9.64% (8/83; 95% CI: 4.25–18.11) for veterinarians and 8.82% (15/170; 95% CI: 5.02–14.13) for blood donors.

Within veterinarians group, one serum was positive for anti-HEV IgG by ELISA (1.20%; 95% CI: 0.03–6.53) and 7 by WB (8.43%; 95% CI: 3.46–16.61). They were neither positive in both tests simultaneously nor positive for IgM by WB (Table 2).

Three sera of blood donors were positive for anti-HEV IgG antibodies only in ELISA (1.76%; 95% CI: 0.37–5.07), 11 only in WB (6.47%; 95% CI: 3.27–11.28) and one in both tests (0.59%; 95% CI: 0.01–3.23). The agreement between ELISA and WB was poor (proportion of positive agreement = 0.08; proportion of negative agreement = 0.95; K = 0.054; 95% CI: 0.00–0.21; P = 0.284). Furthermore, three sera of 15 were positive for IgM antibodies (20.00%; 95% CI: 4.33–48.09)

(Table 2). The sera resulting positive for IgM were also tested for the presence of viral RNA, with negative results.

Statistical analysis was performed to evaluate possible correlations between presence of anti-HEV antibodies, professional contact with pigs, sex and age data.

No significant difference in OR related to group class (OR = 1.32; 95% CI = 0.48–3.66), sex class (OR = 1.32; 95% CI = 0.49–3.55) or age class (OR = 2.06; 95% CI = 0.81–5.21) was detected (Table 3).

Discussion

Although our study has a limited power (30%, with an expected seroprevalence of 5% and a ratio of swine veterinarians to blood donors equal to 2), comparison between the two groups revealed no statistically significant difference in HEV seroprevalence comparing veterinarians (9.64%; 8/83) and blood donors (8.82%; 15/170). However, it should also be taken into account that no information is available on contact of blood donors with pigs or other HEV reservoirs, which cannot be excluded and could have partially influenced the results of the study.

In previous studies conducted in Italy, different results have been obtained including either no significant difference in anti-HEV seroprevalence between pig breeders and the general population (Vulcano et al., 2007), or higher seroprevalence in workers in contact with farm animals (Tabibi et al., 2013). Beside the different number of subjects enrolled in the studies conducted (Tabibi et al., 2013; analysed 13 non-breeders) and the different test used, these results confirm that other risk factors should be also evaluated such as consuming of pork meat and travelling in endemic area. In Italy, a high prevalence of genotype 3 (HEV-RNA up to 50% and anti-HEV ≥ 80) was reported in pigs (Di Bartolo et al., 2011), which contrasts with the relative low seroprevalence found in this study among veterinarians who work with pigs. This could be linked either to a high level of hygienic standard for workers or to a low efficiency of transmission of the genotype 3 HEV viruses circulating in the animal population.

In this study, an anti-HEV antibody seroprevalence of 8.82% (15/170) was detected in blood donors, among which three anti-HEV IgM positive individuals were identified. None of them were positive for HEV-RNA. The 8.82% seroprevalence observed in our work was similar to a previous study conducted in Italy reporting a prevalence of 9.1% (Puttini et al., 2015). However, other investigations conducted in Italy reported lower anti-HEV seroprevalence rates, varying from 1.3 up to 5.0% among blood donors (Masia et al., 2009; Scotto et al., 2012, 2014; Puttini et al., 2015) and from 2.7 to 2.9% for the general population (De Donno et al., 2003; Scotto et al., 2014). In Europe, the seroprevalence reported among blood donors varied from 0.26% in Greece (Stefanidis et al., 2004) up to 52.5% in France (Mansuy et al., 2011).

Nevertheless, in Germany, Sweden and France, the seroprevalence was 6.8%, 9.3% and 10.7%, respectively, (Mansuy et al., 2004; Olsen et al., 2006; Juhl et al., 2014) closer to mean value obtained in our present study. However, the comparison of seroprevalence is difficult because it may largely depend on the assay used, the geographical region of the study and the cohort enrolled (Hartl et al., 2016). In fact, at least partially different performances of the tests used in different studies have been reported (Tabibi et al., 2013). Nevertheless, our current investigation indicates that healthy individuals not exposed professionally can be infected asymptomatically. Therefore, blood and derivate products might be contaminated with HEV and represent a possible source for transmitting the virus.

In the present study, the commercial ELISA and the in house Western blotting tests used showed poor concordance (K = 0.054); in fact, only one serum was positive in both tests. The 18 sera resulting positive only by WB might recognize epitopes in the capsid protein exposed following denaturation that are hidden in the experimental conditions of the ELISA. Furthermore, the presence of anti-HEV IgA cannot be excluded because they are only detectable by the secondary antibodies used in WB, but not by that included in the ELISA.

Conversely, four sera were positive in ELISA and negative in WB. This result may be partially explained by the presence of anti-ORF3 antibodies. In fact, the commercial ELISA kit used is based on both ORF2 and ORF3 antigens, while the WB includes only the ORF2 protein.

ELISA is more suitable for rapid screening of high numbers of sera, but the use of more than one test is advisable. Certainly, a major issue in HEV diagnoses is linked to the tests used, because sensitivity and specificity of the commercial assays used for HEV antibody detection are not yet well established (Lin et al., 2000; Thiry et al., 2014).

In conclusion, surveillance of HEV in blood donors should be implemented because transmission of infections through blood and derivatives has been reported in Europe (Dreier and Juhl, 2014). Further surveillance studies should be conducted to understand if the risk of exposure in some worker categories is still underestimated.

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Table 1. Age and sex distribution of veterinarians and blood donors tested for HEV exposure status

Group		Sex (%)		Age (years)	
	No. of sera	Males	Females	Range	Median
Veterinarians	83	64 (77.1)	19 (22.9)	24-76	41
Blood donors	170	59 (34.7)	111 (65.3)	31-63	44
Total	253	123 (48.6)	130 (51.4)	24-76	43

Table 2. Anti-HEV IgG serum antibodies in veterinarians and blood donors

	No. of positive/ total tested (%)	
Test	**Veterinarians**	**Blood donors**
ELISA	1/83 (1.2)	3/170 (1.76)
WB and ELISA	0/83	1/170 (0.59)
WB	7/83 (8.43)	11[a]/170 (6.47)
Total[a]	8/83 (9.64)	15/170 (8.82)

[a]three out of 11 samples were also positive for IgM by WB

Table 3. The results of multivariable logistic regression analysis of the association between HEV exposure status and age, sex, and sample source (i.e. veterinarians *vs*. blood donors

Risk factor		HEV positive/total (%)	OR	95% CI	P
Group	Veterinarians	8/83 (9.64)	1.32	0.48-3.66	0.594
	Blood donors	15/170 (8.82)	Referent	-	-
Sex	Male	11/123 (8.94)	1.32	0.49-3.55	0.577
	Female	12/130 (9.23)	Referent	-	-
Age	≥43 years	15/129 (11.63)	2.06	0.81-5.21	0.128
	<43 years	8/124 (6.45)	Referent	-	-