Seroprevalence of hepatitis E virus among blood donors in northern Italy (Sondrio, Lombardy) determined by three different assays

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Introduction

Viral hepatitis E infection is caused by hepatitis E virus (HEV), a small non-enveloped virus with a single-stranded positive-sense RNA. A single serotype and four major mammalian genotypes with several HEV subtypes have been described: genotype 1 is most prevalent in Asia and Africa, genotype 2 is usually detected in Mexico and Africa, genotype 3 is distributed globally and genotype 4 is found particularly in Asia, although it has also recently been isolated in Europe. All four genotypes infect humans, but genotypes 3 and 4 have a zoonotic reservoir.

For a long time, viral hepatitis E was suspected to be endemic only in developing countries where sporadic cases and large outbreaks transmitted by the faecal-oral route and associated with contamination of water supplies have been reported. In non-endemic areas, such as European countries, hepatitis E was mainly considered an infection associated with travel in endemic areas (imported disease). Recently, growing numbers of HEV-infected patients with no history of travel abroad have been identified in industrialised countries. These autochthonous cases have a zoonotic origin and may occur through consumption of raw or undercooked contaminated meat and meat products or through exposure to infected animals[1]. Although HEV is spread mainly by zoonotic and food-borne routes, it can also be transmitted parenterally via blood. The recent detection of HEV viraemic blood donors may indicate a threat to the safety of the blood supply[2-4].

Hepatitis E is usually an acute asymptomatic and self-limiting infection in industrialised countries. However, in the last years an increasing number of chronic HEV infections in immunocompromised hosts and some cases of transfusion-transmitted HEV infections, with a broad variety of outcomes, have been described[2].

Despite an apparently low number of symptomatic cases, probably due to the subclinical course of the infection, a wide variability in the prevalence of HEV antibodies (anti-HEV) among the general population and blood donors has been reported. In fact, in developed countries the seroprevalence rates are sometimes higher than expected and appear very variable not only from country to country, but also in the same geographical area and study population. The different sensitivities and specificities of the serological assays employed in the various studies contribute to this variability[5].

Studies carried out in Italy have shown the presence of clinical autochthonous hepatitis E cases, with the number of cases possibly being underestimated[6-8]. Data concerning the prevalence of anti-HEV in Italy are limited and the results differ considerably depending on the type of population, geographical area and serological assays used in the studies[9-12].

The purpose of our research was to assess the prevalence of anti-HEV among blood donors in northern Italy (Sondrio, Lombardy). In order to do this we used three different immunoenzymatic assays.

Materials and methods

Plasma samples were collected from 685 volunteer blood donors who attended the Department of Transfusion Medicine and Haematology in Sondrio (northern Italy). For each donor, demographic data regarding gender, age and place of birth were recorded. All collected plasma samples were tested for the presence of anti-HEV with three different, commercially available enzyme-linked immunosorbent assays (ELISA): (i) HEV IgG Dia.Pro (Diagnostic BioProbes Srl, Milan, Italy), (ii) HEV IgG Wantai (Biological Pharmacy Enterprise Co., Beijing, China) and (iii) HEV Ab Version Ultra Dia.Pro (Diagnostic BioProbes Srl).

The first assay uses HEV-specific synthetic antigens encoding for conservative and immunodominant determinants derived from ORF2 and ORF3 of all the four human HEV genotypes; the second uses HEV recombinant antigens derived from ORF2 and ORF3 and able to cross-neutralise human HEV genotypes 1, 2 and 3. The last assay is a new ELISA for the detection of total HEV antibodies using HEV-specific recombinant virus-like particles derived from HEV-RNA ORF2 and bearing immunodominant regions of the four viral strains. All tests were performed and the results

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interpreted according to the manufacturers' instructions.

Comparisons between frequencies were performed using the chi-squared test; p values <0.05 were considered statistically significant.

Results

The 685 blood donors studied were all Italian and about three-quarters were male (72% male, 28% female). Their median age at the time of blood donation was 48 years (range, 19-68 years).

Seventy of the 685 blood donors (10.2%) tested positive for anti-HEV immunoglobulins G (IgG) with assay 1 while 67 (9.8%) tested positive with assay 2 (p=n.s.). The prevalence of anti-HEV detected by assay 3 was 119/685 (17.4%), which was significantly higher than the prevalences determined by the other two assays (p<0.001). Among the 119 plasma samples found to be positive by assay 3, 58 were positive by both the other two tests, 17 were positive by assay 1 or 2, while 44 were positive only according to assay 3. A small proportion (11/685, 1.6%) of samples resulted equivocal when tested by assay 2 and/or 3 (Table I).

Overall, the concordance among the three ELISA was 89.9% (58 positive and 558 negative results), corresponding to a prevalence rate of 8.5% (58/685). The concordance between at least two assays was 99.7% (616 concordant results with all tests and 67 with two tests). In detail, considering test-by-test agreement, the concordance was 96.8% (663/685) between assays 1 and 2, 91.5% (627/685) between assays 1 and 3 and 91.2% (625/685) between assays 2 and 3 (Figure 1).

As illustrated in Figure 2, we observed an age-related difference in the prevalence of anti-HEV with all three tests employed. The lowest anti-HEV prevalence values were found in the youngest age groups while the highest prevalence rates were recorded in the oldest age groups. No significant difference in anti-HEV prevalence was observed between genders.

Discussion

The detailed picture of the global spread of HEV infection is still unclear. In developed countries the number of clinical cases of viral hepatitis E is limited, probably because infection with this virus is frequently asymptomatic; however, the anti-HEV seroprevalence in the general population and blood donors is very variable and sometimes higher than expected. Indeed, studies conducted in Europe have found anti-HEV prevalence rates ranging from 0.6% to 52.5%. Such broad ranges have been observed not only between countries but also in the same country and sometimes in the same geographical area, in the same study population and study period. This variability could be explained in

Table I - Anti-HEV results obtained by three different ELISA used to determine the prevalence among blood donors.

Anti-HEV result	Assay 1 n (%)	Assay 2 n (%)	Assay 3 n (%)
Positive	70 (10.2)	67 (9.8)	119 (17.4)
Negative	615 (89.8)	612 (89.3)	560 (81.7)
Equivocal	0 (0)	6 * (0.9)	6 ** (0.9)
Total	685 (100)	685 (100)	685 (100)

* 4/6 positive by assay 1 and assay 3; ** 4/6 negative by assay 1 and assay 2.
HEV: hepatitis E virus.

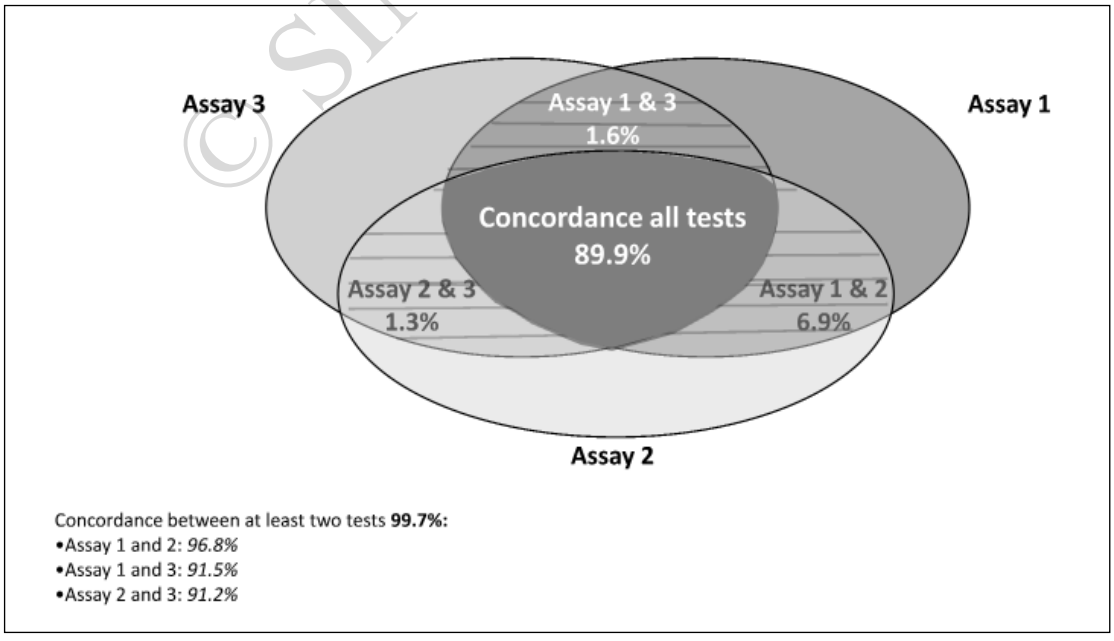

Figure 1 - Concordance among anti-HEV results obtained by three different ELISA.
HEV: hepatitis E virus.

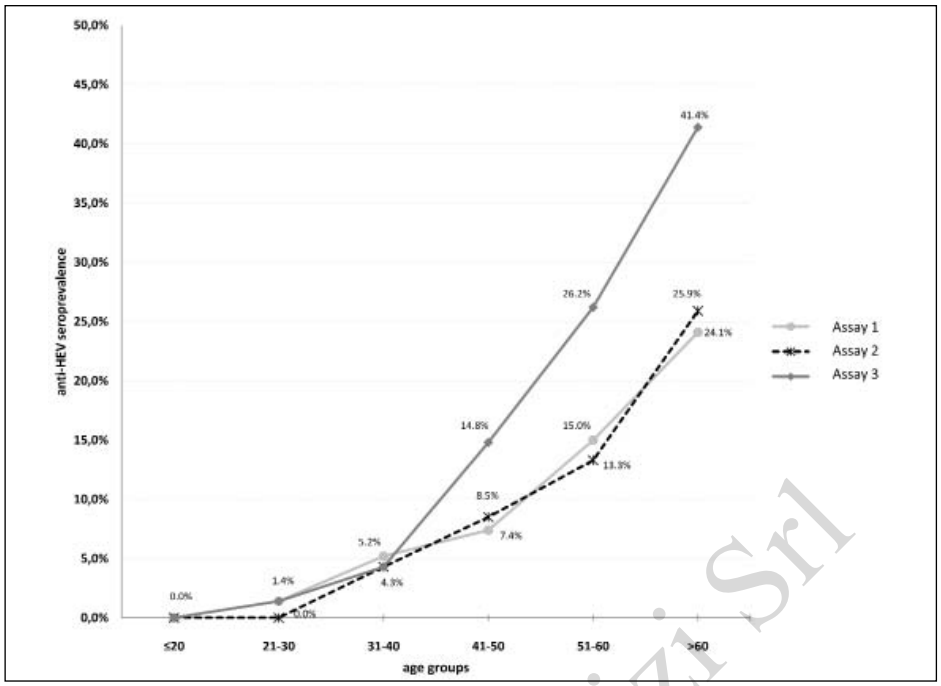

Figure 2 - Prevalence of anti-HEV, detected by three ELISA, among blood donors according to age groups.
HEV: hepatitis E virus.

part by the use of different immunoenzymatic assays, with different characteristics in terms of the antigens employed, their sensitivity and their specificity[5].

In this study, we evaluated the anti-HEV prevalence in blood donors in northern Italy (Sondrio, Lombardy) using three different assays and we obtained an overall anti-HEV prevalence of 8.5% when considering concordant results for all three tests. However, the percentages of anti-HEV positivity were different when considering the three assays individually, being 9.8%, 10.2% and 17.4%, respectively. We, therefore, confirm the variability in anti-HEV seroprevalence depending on the different ELISA used, as previously observed in Denmark (10.7% *vs* 19.8%)[13] and Spain (10.7% *vs* 19.96%)[14].

Given the marked variability of anti-HEV seroprevalence in blood donors, we compared our results obtained using HEV IgG Wantai with epidemiological data recorded using the same assay in the same population in other European countries. The anti-HEV seroprevalence in our study population was slightly higher than that in Austria (13.5%)[15] and Norway (14%)[16], but lower than the anti-HEV level detected in Danish (19.8%)[13], Spanish (19.96%)[14] and French (52.5%)[17] blood donors.

When comparing our current results with data collected in the same population from the same country, we noted that several studies conducted in Italy on blood donors have already revealed large differences in anti-HEV seroprevalence especially according to geographical area and different immunoenzymatic test used. A recent epidemiological study found anti-HEV rates of 7% and 4.5% using the HEV Ab Version Ultra Dia.Pro and the HEV IgG Wantai, respectively[12]. In another study on HEV seroprevalence carried out in central Italy, 49% of the tested blood donors were anti-HEV IgG-positive using the HEV IgG Wantai[11]. Lower rates have been observed in southern Italy (1.3%)[10] and Sardinia (5%)[9] using the HEV IgG Dia.Pro.

In accordance with almost all the just mentioned studies, we found no difference in anti-HEV prevalence between genders and an increase of anti-HEV positivity with age. This anti-HEV age-related trend is a typical cohort effect that could be explained by a decreased risk of HEV infection due to the improvement of sanitary conditions or by an increased risk of HEV exposure during life[18].

Our finding of 89.9% concordance among the three ELISA used in this study is quite low. This value, which cannot be compared with those of other similar reports, suggests the importance of standardising serological diagnostic tests for HEV.

In separate comparisons, the concordance between assay 3 (HEV Ab Version Ultra Dia.Pro) and assay 1 (HEV IgG Dia.Pro) or 2 (HEV IgG Wantai) was low (91.5% and 91.2%, respectively). The differences may be due to a higher sensitivity or lower specificity of the assay based on virus-like particles compared to the other two assays. A recent study comparing the HEV IgG Wantai assay to the HEV Ab Version Ultra Dia.Pro assay revealed a low concordance between the results of the

two, with a slightly higher sensitivity of the latter assay[12]. This finding could justify our anti-HEV seroprevalence rate obtained with the same assay (assay 3), which was significantly higher than the prevalences obtained with the other two anti-HEV IgG assays.

On the other hand, two recent studies carried out to assess the diagnostic performance of five commercial HEV IgG ELISA found a comparable sensitivity for the detection of anti-HEV IgG when using the HEV IgG Dia.Pro assay (assay 1) and the HEV IgG Wantai assay (assay 2)[19,20]. Norder *et al.*[20] showed that the two above-mentioned assays were the most sensitive ELISA. Moreover, in accordance with our results, they found a high concordance in anti-HEV IgG detection with the two anti-HEV IgG assays, showing a higher reactivity in anti-HEV IgG detection with the Dia. Pro assay than with the Wantai assay. This finding may explain why our anti-HEV seroprevalence was higher with the HEV-specific synthetic antigen assay than with the HEV recombinant antigen assay.

Conclusions

At present, hepatitis E is not a major public health problem in Italy. However, the prevalence of anti-HEV in blood donors in northern Italy is higher than expected, suggesting that the infection is endemic in our country. Globally, the anti-HEV seroprevalence greatly varies, depending on the geographical area considered and the population studied, as well as the assay used in anti-HEV detection. In order to clarify the epidemiology of HEV infection, a standard immunoenzymatic assay, avoiding variability in terms of sensitivity and specificity, is required. Further studies are also needed to evaluate the role of HEV transmission by blood to better understand whether HEV infection could become a real transfusion-transmitted issue.

Authorship contributions

CG, CV, ARZ and LR contributed to study design, development and interpretation of data. CG, ARZ and LR contributed to the writing of the manuscript drafts. LF collected the samples. CT carried out the serological test. All authors critically reviewed and revised the manuscript drafts, approved the final version of the manuscript and take responsibility for the integrity of the data and accuracy of data analysis.

The Authors declare no conflicts of interest.

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