A nationwide serosurvey of hepatitis E virus antibodies in the general population of Portugal

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Introduction

Hepatitis E virus (HEV) is today widely recognized as a serious public health issue in industrialized countries.¹ ² Infection by HEV was in the past mistakenly considered rare and restricted to travellers returning from endemic developing countries.³ Over the past decades published data has definitely refuted that concept. It is now known that infection by HEV genotype 3 is autochthonous in Europe and of zoonotic origin, with swine as the main reservoir. Direct contact with pigs, as well as consumption of undercooked pork meat and sausages are the most important routes of transmission.³ ⁴ ⁵ In Europe, human HEV genotype 3 infections are often subclinical or asymptomatic, but a range of human illness associated to this genotype has been reported, including fulminant liver failure or chronic liver disease, as well as extra-hepatic manifestations.³ ⁶ ⁷ Epidemiological studies in several European countries have reported highly different HEV seroprevalences (ranging from 0.6% to 52.5%) between and within countries.⁸ Besides geographical differences, this wide range of seroprevalences has also been linked to demographic characteristics, namely age, sex and region.

Methods

Ethics approval

This study was approved by a regional ethics commission (Comissão de Ética para a Saúde do Centro Hospitalar de São João; Reference number: 99/2015) and the participants provided informed consent.

Study population

The sample size was calculated according to previously described methods.¹² Briefly, a sample size of 1656 was calculated assuming an a priori 50% anti-HEV seroprevalence (yielding the highest possible sample size), a confidence in the estimate of 95%, a maximum allowable error in the prevalence of 3%, and a Portuguese population size of Portugal of 10 541 840 (based on the latest Portuguese census data).¹³ A stratified random sampling design with all 30 NUTS III regions (Nomenclature of Territorial Units for Statistics; level III) of Portugal as a stratification level was set up in order to provide a representative sample. On the basis of the census data, the stratified distribution in each regional stratum was proportionally sampled by 10 age group (0–9, 10–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70+) and by gender.¹³ Sera were collected between July 2015 and February 2016 from attendees (or their legal representatives) of Clinical Analysis Laboratories from all NUTS III regions. Attendees were randomly selected based on the required age group and gender until the necessary number of samples was reached in each region. This process provided an accurate representation of the target population in our sample by region, 10-year age groups and gender.
Serological analysis

For the detection of anti-HEV IgG and anti-HEV IgM antibodies in sera the Mikrogen commercial enzyme immunoassays recomWell HEV IgG and recomWell HEV IgM (versions 2015, Mikrogen, Germany) were used and samples were considered positive according to the manufacturer’s instructions.

Statistical analysis

Sex and regional differences in prevalence were evaluated by Chi-square. Age group differences were evaluated by Chi-square for trend analysis. A \( P \)-values < 0.05 was considered significant. All statistical analyses were performed using the Statistical Software Package SPSS 20.0 (SPSS Inc., Chicago, IL).

Results

Descriptive analysis of the sampled population (\( n = 1656 \)) is given in table 1. The sample included a similar number of males and females, 813 (49.1%) and 843 (50.9%), respectively (male: female ratio of 1:1). Serum samples were obtained from all age groups being the youngest participant 3 months-old and the eldest 99-years-old. The 70+ year group was the stratum with the highest number of individuals (17.9%), while the 0–9 year group included the lowest number (9.9%). Concerning geographical distribution, Lisbon was the region with highest number of individuals included (13.6%) and the region with lowest was Pinhal Interior Sul (1.9%).

Of the 1656 serum samples studied, 270 tested positive for anti-HEV IgG giving an overall anti-HEV IgG seroprevalence of 16.3% (95% confidence interval: 14.6–18.2) (table 1).

No differences (\( P = 0.593 \)) in anti-HEV IgG seropositivity were observed between genders with females presenting 17.9% and males 14.6% (table 1).

When analyzing age, a significant increase (\( P < 0.05 \)) of anti-HEV IgG seroprevalence was detected, from the minimum of 0.6% in the 0–9 years age group to the maximum of 30.1% in the 70+ years age group (table 1, figure 1). No significant differences (\( P > 0.05 \)) in anti-HEV IgG seropositivity were found by gender within each age group.

The HEV seroprevalence in different regions of Portugal varied from the minimum of 8.1% in Dão-Lafões to 27.8% in Douro (figure 2), but the regional differences were not statistically significant (\( P > 0.05 \)). The most cosmopolitan regions in Portugal,
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Figure 1 Estimated prevalence of anti-HEV IgG antibodies in Portugal by age groups

Figure 2 Anti-HEV IgG seroprevalence by region of Portugal (30 NUTS III regions)

Table 1 indicating current or recent HEV infection. Therefore, antibodies were found in 8 individuals, 4 females and 4 males produce lower rates than Wantai.9,14,15 When we compare our general, seroprevalence studies performed with Mikrogen assay, it has been widely demonstrated by us.24 In general, seroprevalence studies performed with Mikrogen assay produce lower rates than Wantai.9,14,15 When we compare our results with country data from other European studies that used the Mikrogen assay, it was interesting to find that the Portuguese seroprevalence was similar to others found in Germany, namely 14.5%,16 16.8%,17 18%,18 and 17.9%.19

It was interesting to find that the seroprevalence in the Portuguese population was substantial (16.3%), comparing with the fairly low number of autochthonous hepatitis E cases that have been reported so far.8,20 In fact, most cases of HEV genotype 3 are asymptomatic and the symptomatic ones are not usually recognized because the rate of icteric illness is low and severe hepatitis is rare.3

However, the high circulation of HEV genotype 3 in both domestic pigs6 and wild boar21 in Portugal can help explain the substantial seroprevalence in the Portuguese population, which has a strong tradition of pork consumption. Moreover, we previously reported that 32% of fatteners group of pigs of Portugal (the ones close to slaughter age and about to enter the food chain for consumption) were excreting HEV in stools.9

In the present study, an increase of anti-HEV IgG seroprevalence with age (P < 0.05) was observed, reaching the maximum of 30% in the eldest. A similar increase has been reported by others and is expected by the longer period of exposure to risk factors, cumulatively occurring during the life-time exposure. Accordingly, the youngest age group (0–9-years-old) was also the one with the lowest anti-HEV IgG seroprevalence (0.6%), which is in line with a recent study in Germany suggesting that only a very small number of HEV infections occur in children.22 Similar anti-HEV IgG positivity (~1%) has been also found in recently collected sera from Portuguese children aging 0–4-years-old.23 However, the anti-HEV IgG seroprevalence in these youngest groups must be interpreted with caution since they can include infants in which the presence of maternal antibodies cannot be excluded. In fact, an efficient transplacental transport of anti-HEV IgG has been previously demonstrated by us.24

On the other hand, no significant difference was found in anti-HEV IgG seroprevalence according to gender, which seems to be the case for HEV infections throughout Europe.9

Concerning geographic distribution, no significant difference was observed regarding HEV seroprevalence, although a generally higher seropositivity was found in the most rural areas of Portugal (25–30%), the regions with the highest concentration of pigs.25 As pigs are the main reservoir for HEV, this probably explains the high seropositivity in these regions. Further, the lower seropositivity in
the archipelagos of Madeira (9%) and Azores (10%) is consistent with the lowest concentration of pigs.

Regarding anti-HEV IgM seroprevalence it was not possible to identify statistically significant differences in seropositivity regarding age, gender or region.

In summary, an overall seroprevalence of 16.3% was found in this study, the first nationwide study in Portugal. Moreover, this study provides insight in the HEV infection of the Portuguese general population and generates information on risk profiles regarding demographic data.

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Conflicts of interest: None declared.

Key points

- An overall anti-HEV IgG seroprevalence of 16.3% was found in the Portuguese population.
- This HEV seroprevalence increase with age reaching a maximum (30%) in the eldest.
- No significant differences in HEV seroprevalence were found between genders and regions of Portugal.
- The 16.3% HEV seroprevalence is not in accordance with the low number of reported hepatitis E cases in the country, possibly due to the lack of attention to autochthonous hepatitis E.

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