

Seroprevalence in blood donors reveals widespread, multi-source exposure to hepatitis E virus, southern France, October 2011

J M Mansuy (mansuy.jm@chu-toulouse.fr)¹, K Sauné^{1,2}, H Rech³, F Abravanel^{1,2,4}, C Mengelle¹, S L'Homme^{1,2,4}, F Destruel³, N Kamar^{4,5}, J Izopet^{1,2,4}

1. Department of Virology, CHU Purpan, Toulouse, France

2. Medicine University Purpan, Toulouse, France

3. Pyrénées Méditerranée French Blood Service, Toulouse, France

4. Department of Physiopathology, INSERM U1043, Toulouse, France

5. Department of Nephrology, Dialysis and Organ Transplantation, CHU Rangueil, Toulouse, France

Citation style for this article:

Mansuy JM, Sauné K, Rech H, Abravanel F, Mengelle C, L'Homme S, Destruel F, Kamar N, Izopet J. Seroprevalence in blood donors reveals widespread, multi-source exposure to hepatitis E virus, southern France, October 2011. *Euro Surveill.* 2015;20(19):pii=21127. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21127>

Article submitted on 30 April 2014 / published on 14 May 2015

The apparent seroprevalence of hepatitis E Virus (HEV) varies greatly among developed countries depending on the geographical area and the sensitivity of immunoassays. We used a validated assay to determine the prevalence of HEV IgG and IgM antibodies among 3,353 blood donors living in southern France, who gave blood during the two first weeks of October 2011 and participated in the study. Demographic and epidemiological information was collected using a specific questionnaire. We also screened 591 samples for HEV RNA. Overall IgG seroprevalence was 39.1% and varied from 20% to 71.3% depending on the geographical area ($p < 0.001$) while IgM seroprevalence was 3.31%. Anti-HEV IgG was significantly correlated with increasing age ($p < 0.001$), eating uncooked pork liver sausages ($p < 0.001$), offal ($p = 0.003$), or mussels ($p = 0.02$). Anti-HEV IgM was associated with being male ($p = 0.01$) and eating uncooked pork liver sausages ($p = 0.02$). HEV RNA was detected in one of the 99 anti-HEV IgM-positive samples, but in none of the 492 anti-HEV IgM-negative samples. HEV is hyperendemic in southern France. Dietary and culinary habits alone cannot explain the epidemiology of HEV in this region, indicating that other modes of contamination should be investigated.

Introduction

Hepatitis E virus (HEV) is a non-enveloped single-stranded, positive-sense RNA virus, a member of the Hepeviridae family, genus Hepevirus [1]. At least four genotypes of HEV are recognised. Genotypes 1 and 2 are restricted to humans and are prevalent in developing countries in Asia and Africa, where hepatitis E is a waterborne disease associated with sporadic infections and large epidemics linked to drinking water contaminated with faeces. Genotypes 3 and 4 are transmitted

zoonotically and are prevalent in many industrialised countries in Asia, Europe and North America. HEV has been detected in a range of animals including pigs, wild boar, deer and rabbits and the concept of zoonosis is supported by the similarities of the sequences of human HEV strains and HEV from animals [2].

About half of HEV-infected patients in developing countries show symptoms of acute hepatitis. The patients most at risk of death are pregnant women (third trimester) and those with chronic liver disease [2]. Most HEV infections occurring in developed countries are asymptomatic. Only patients with chronic liver diseases are at great risk of fulminant hepatitis. An HEV infection can also lead to chronic hepatitis in 60% of immunosuppressed patients, which can rapidly progress to cirrhosis [2,3].

Reports of autochthonous infections in areas with good sanitation, particularly in France are becoming more frequent [4], while imported cases among travellers returning from developing countries are less frequent. These autochthonous infections are most often of genotype 3 and involve transmission through contaminated food. However transmission through occupational exposure to animals, particularly pigs [5] and through infected blood products has been reported [6]. The HEV seroprevalence among blood donors or the general population in high income countries varies widely, from 1.9% [7] to 52.5% [8]. This is partly due to variations in the sensitivity of the assays used for detection [9].

Several lines of evidence suggest that HEV transmission is frequent in southern France where locally acquired HEV infections have been documented [4] and chronic hepatitis E described in immunosuppressed

TABLE 1

Prevalence of hepatitis E virus antibodies depending on regions and respective administrative areas, southern France, October 2011

Administrative regions and areas (ID number)	Population density (inhabitants per km ²)	Anti-HEV (n)			Seroprevalence	
		IgG positive	IgM positive	Tested	IgG % (95% CI)	IgM % (95% CI)
Midi-Pyrénées	64.3	787	71	1,897	41.5 (39.9–43.1)	3.7 (2.9–4.6)
Ariège (09)	30.9	67	3	94	71.3 (62.1–80.5)	3.2 (0–6.7)
Aveyron & Lot (12 & 46)	32.3	40	6	172	23.2 (16.9–29.5)	3.5 (0.7–6.2)
Gers (30)	29.9	64	9	171	37.4 (30.1–44.6)	5.3 (1.9–8.6)
Haute Garonne (31)	195.1	360	33	777	46.3 (42.8–49.8)	4.3 (2.2–6.3)
Hautes Pyrénées (65)	51.4	77	5	234	32.9 (26.9–38.9)	2.1 (0.3–4.0)
Tarn (81)	65.0	96	9	263	36.5 (30.7–42.3)	3.4 (1.2–5.6)
Tarn et Garonne (82)	64.4	83	6	186	44.6 (37.5–51.7)	3.2 (0.7–5.8)
Languedoc-Roussillon	97.2	523	40	1,456	35.9 (33.4–38.4)	2.7 (1.9–3.5)
Aude (11)	57.7	124	8	191	64.9 (58.1–71.7)	4.2 (1.3–7.0)
Gard & Lozère (30 & 48)	71.8	54	5	246	21.9 (16.7–27.1)	2.0 (0.2–3.7)
Hérault (34)	169.1	212	18	656	32.3 (28.7–25.9)	2.7 (1.5–4.0)
Pyrénées Orientales (66)	108.3	133	8	363	36.6 (31.6–41.6)	2.2 (0.7–3.7)

CI: confidence interval; HEV: hepatitis E virus; ID: identity.

patients [3]. The incidence of HEV in transplant recipients reached 3.2/100 person-years in south-western France between 2004 and 2009 [10]. Previous serological studies on blood donors living in the same area found a high seroprevalence (52.5%), particularly in rural areas [8].

We therefore determined the prevalence of anti-HEV IgG and IgM among 3,353 blood donors from two administrative regions of southern France and studied potential contributing factors such as age, sex, as well as eating habits, hobbies, place of residence, profession, travel history, and type of dwelling. We also looked for HEV RNA in IgM-positive samples and in donors randomly selected from among those that were anti-HEV IgM-negative.

Methods

Ethics statement

All the blood donors had completed the national medical questionnaire and had been interviewed before blood collection to ensure that they fulfilled the criteria for blood donation and to eliminate anyone with temporary or permanent contra-indications. The donors gave their informed consent for the study. Information and approval documents were printed in duplicate and signed by both the donor and medical staff. One copy was given to the donor, the other is kept by the staff for 10 years.

The study was approved by the Toulouse University Hospital Research Ethics Committee.

Setting

The two administrative regions of this study are located in the south of France and represent 13.4% of the French metropolitan area. The Midi-Pyrénées region (MP) is the largest region in metropolitan France (45,348 km²), while Languedoc-Roussillon (LR) is the eighth largest (27,376 km²). Their combined population is 5,615,339 inhabitants (8.4% of the French population). LR's eastern border is the Mediterranean coast. The population density in MP (64.3 per km²) is lower than in LR (97.2 per km²). Both these densities are lower than the mean (114 inhabitants/km²) in metropolitan France.

The three main rivers that flow through the two regions arise in the Pyrenees and are the Garonne, the Aude and the Ariège.

Both regions are divided into administrative areas: Eight areas in MP and five in LR. All the areas in LR are close to four MP areas.

Population

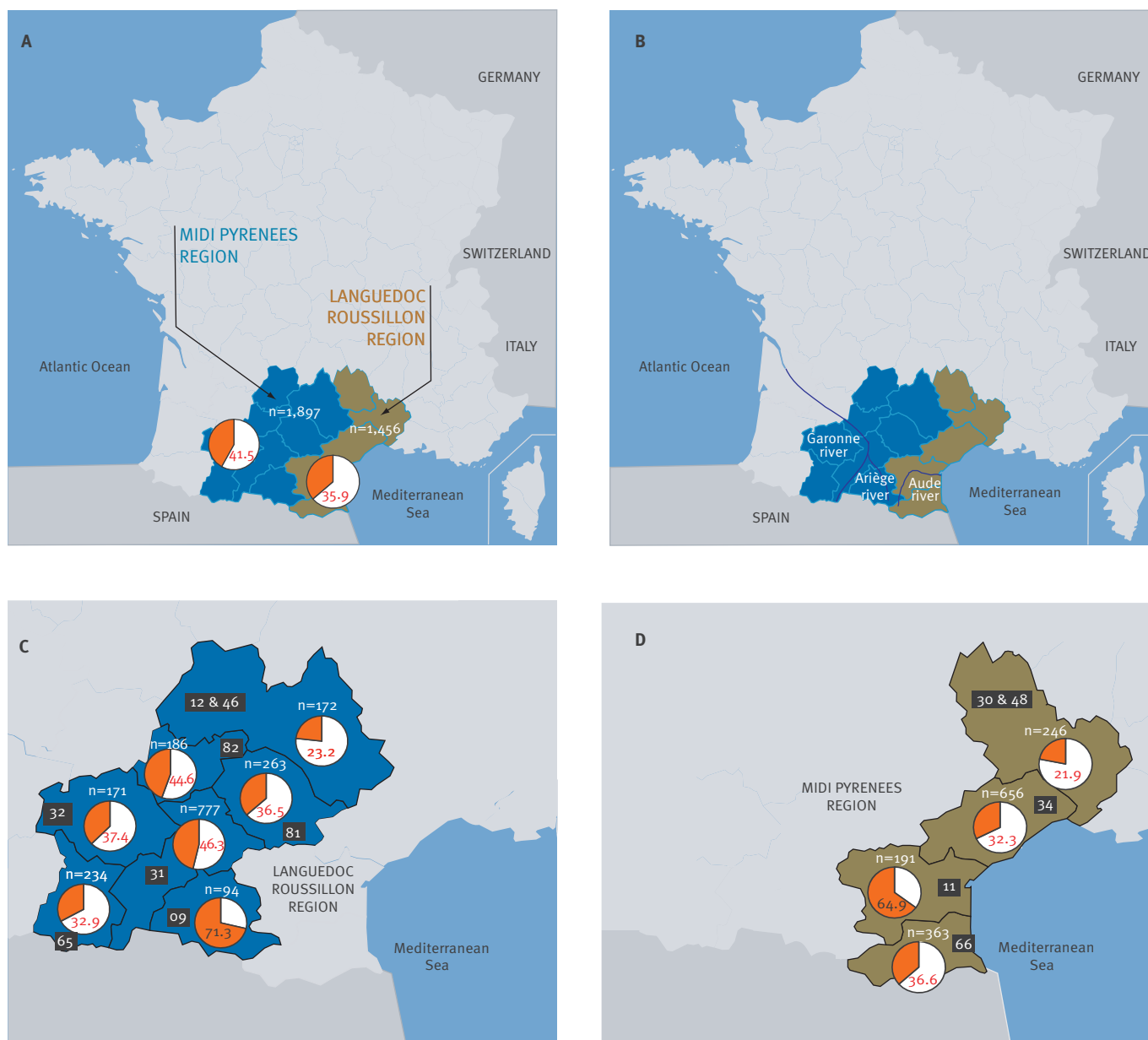
The study was carried out on blood samples from 3,353 unpaid donors, 1,897 from MP and 1,456 from LR. As stipulated by French law, they were all aged between 18 and 70 years.

Blood samples

Blood samples were randomly collected in all the administrative areas of the LR and MP regions during the two first weeks of October 2011 to determine anti-HEV IgG and IgM seroprevalences. The blood from groups of donors such as students and the military were discarded to avoid donors having the same risks of exposure.

FIGURE 1

Location of the Midi-Pyrénées and Languedoc-Roussillon regions, as well as main rivers, and anti-hepatitis E IgG prevalences in the regions, France, October 2011 (n = 3,353 blood donor samples)



HEV: hepatitis E virus.

White numbers on a black background identify administrative areas. Midi-Pyrénées region: 9, Ariège; 12 and 46, Aveyron and Lot; 31, Haute Garonne; 32, Gers; 46, Lot; 65, Hautes Pyrénées; 81, Tarn; 82, Tarn et Garonne. Languedoc-Roussillon region: 11, Aude; 30 and 48, Gard and Lozère; 34, Hérault; 66, Pyrénées Orientales.

Global anti-HEV IgG prevalences in the regions (panel A) and the administrative areas (panel C and D). The totals of donors tested and the anti-HEV IgG prevalence are shown for the regions and areas.

Pie charts: red parts are the proportion of anti-HEV IgG positive donors; prevalences (percentages) are indicated in the charts.

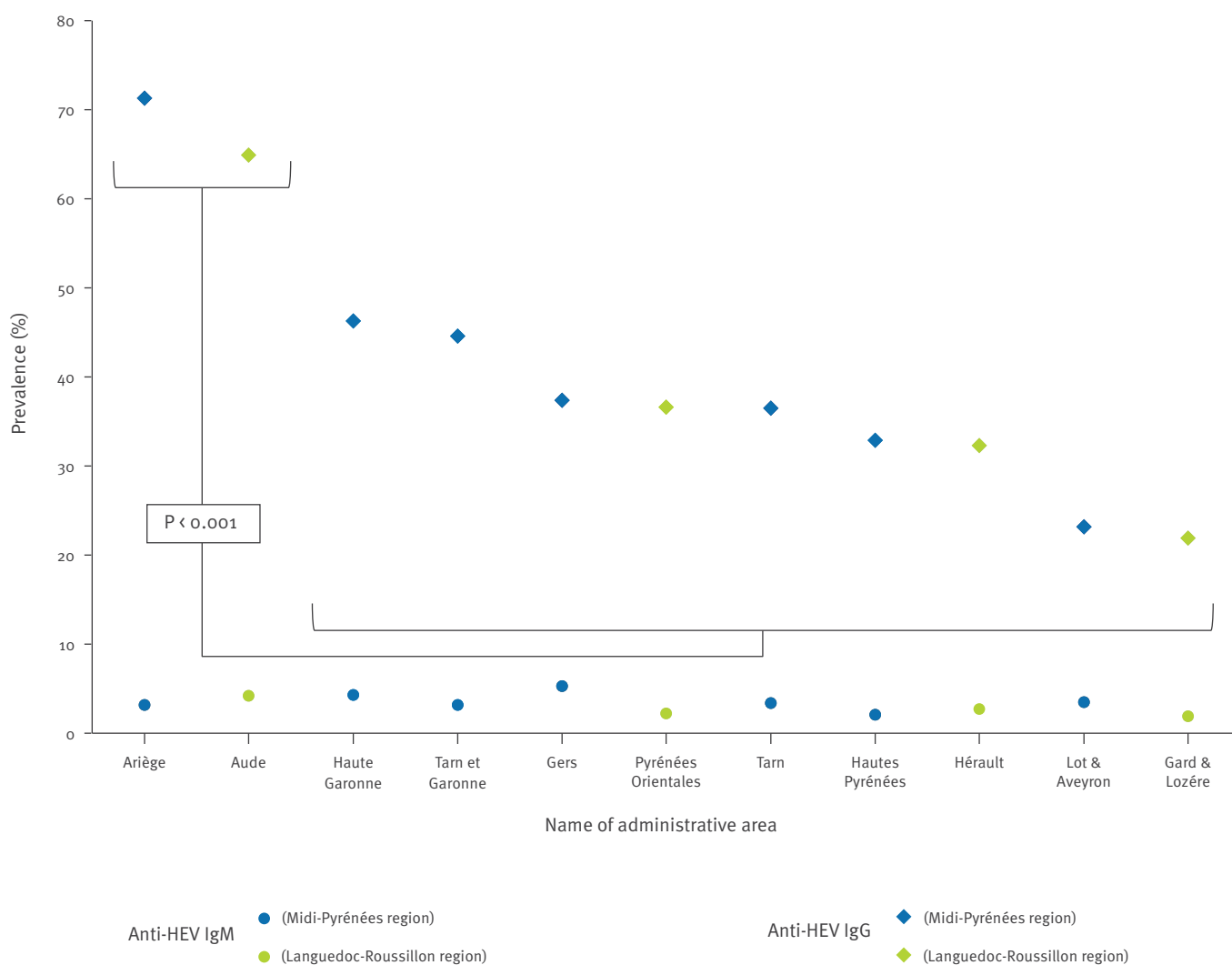
All anti-HEV IgM-positive sera except those that were too small (<100 µL) were tested for HEV RNA. A group of 492 randomly selected sera from MP donors who tested negative for anti-HEV IgM were also tested for HEV RNA.

Laboratory methods

Sera were tested for anti-HEV IgG and IgM using two commercial enzyme immunoassays (Wantai Pharmaceutical Enterprise, Beijing, PRC). These assays use antigens encoded by a structural region of open reading frame (ORF)-2 from a Chinese isolate of genotype 1 HEV and were performed according to the manufacturer's instructions. Serum samples that gave an absorbance value greater than the cutoff value were

FIGURE 2

Anti-hepatitis E virus IgG and IgM seroprevalences in each administrative area of the Languedoc-Roussillon and Midi-Pyrénées regions, France, October 2011 (n = 3,353 blood donor samples)



HEV: hepatitis E virus.

The prevalences of anti-HEV IgG significantly differed between Ariège and Aude taken together, and all the other administrative areas considered ($p < 0.001$).

considered to be positive for HEV antibodies. These enzyme-linked immunosorbent assays (ELISAs) have been previously evaluated; their analytical and clinical performances were excellent [11,12]. The limit of detection of the IgG assay was as high as 0.25 World Health Organization (WHO) units/mL [11].

Nucleic acids were extracted using Cobas AmpliPrep-Total Isolation kits on a Cobas Ampliprep sSystem instrument (Roche Molecular Systems, Inc, Branchburg NJ US). HEV RNA was detected by amplifying a 70-base fragment from ORF3 [13] using a Light Cycler 480 instrument (Roche Molecular Systems). The limit of detection was 60 international units (IU)/mL.

Questionnaire

Each donor completed a structured, specific questionnaire to document demographic data and putative

risk factors. It covered demographics, type of dwelling (apartment, house, farm, institution) and its waste water system (main sewer, septic tank), professional and recreational activities and travel history. Information about frequent contact with pets and/or domestic farm animals was also recorded, as were the donor's eating habits, including if garden fruit and vegetables were consumed. The questions concerned how frequently meat and other products were consumed; the products comprised ham, sausages, pâté, shellfish and fish, uncooked and unpeeled vegetables. The way in which meat or meat-based products were cooked as well as which of such items were eaten uncooked were also recorded. Last, we recorded the source of drinking water (bottled mineral water, tap water, untreated private well).

TABLE 2

Identifying factors associated with anti-hepatitis E virus IgG and IgM seropositivity, Languedoc-Roussillon and Midi-Pyrénées regions, France, October 2011 (n = 3,353 blood donors)

Factors	Anti-HEV IgG				Anti-HEV IgM			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age >45 years (median age)	1.59 (1.31–1.92)	<0.001	1.46 (1.16–1.82)	<0.001	0.49 (0.28–0.86)	0.04	0.52 (0.29–0.92)	0.02
Male sex	1.32 (1.15–1.52)	<0.001	1.19 (0.96–1.47)	0.12	1.37 (0.94–2.02)	0.1	1.69 (1.1–2.6)	0.01
Region MP or LR ^a	1.27 (1.10–1.46)	<0.001	1.59 (1.26–2.00)	<0.001	1.41 (0.95–2.01)	0.09	1.32 (0.79–2.21)	0.29
Administrative area	1.16 (1.13–1.19)	<0.001	1.16 (1.12–1.19)	<0.001	0.91 (0.60–1.40)	0.6	– ^a	–
Eating uncooked pork liver sausage	2.44 (2.09–2.86)	<0.001	2.17 (1.73–2.72)	<0.001	1.71 (1.13–2.59)	0.01	1.88 (1.10–3.23)	0.02
Offal consumption	1.99 (1.69–2.35)	<0.001	1.45 (1.14–1.85)	0.003	1.60 (0.99–2.58)	0.05	1.54 (0.85–2.79)	0.16
Rabbit meat consumption	1.40 (1.16–1.68)	<0.001	1.27 (0.95–1.66)	0.1	1.22 (0.72–2.08)	0.4	– ^a	–
Game meat consumption	1.36 (1.17–1.59)	<0.001	1.12 (0.89–1.40)	0.35	0.95 (0.63–1.44)	0.82	– ^a	–
Oyster consumption	1.80 (1.48–2.17)	<0.001	1.16 (0.80–1.67)	0.44	0.85 (0.56–1.30)	0.46	– ^a	–
Mussel consumption	1.45 (1.14–1.83)	0.002	1.38 (1.07–1.79)	0.02	1.24 (0.64–2.41)	0.5	– ^a	–
Drinking untreated water	1.35 (1.13–1.63)	<0.01	1.27 (0.99–1.63)	0.052	1.34 (0.80–2.25)	0.27	– ^a	–
Consuming items grown in garden	1.10 (1.04–1.17)	<0.01	1.09 (0.99–1.18)	0.053	1.03 (0.87–1.23)	0.7	– ^a	–

CI: confidence interval; HEV: hepatitis E virus; LR: Languedoc-Roussillon; MP: Midi-Pyrénées; OR: odds ratio.

Each variable statistically significant at $p \leq 0.1$ in univariate analysis was included into the logistic regression model.

^a The variables of $p > 0.1$ were excluded from the multivariate analysis.

Statistical analysis

Data collected by the questionnaires were verified and digitised. They were analysed using Stata version 9.2 (StataCorp LP, College Station, TX, US). Demographic and life style factors associated with HEV antibodies were evaluated using univariate analyses. Chi-squared and Fisher's exact tests were used to analyse categorical variables when appropriate. Quantitative variables are expressed as means (\pm standard deviation (SD)) and compared using Student's *t*-test or the Mann-Whitney U test; others are given as medians (25th–75th percentile). Variables with a p value ≤ 0.10 by univariate analysis were entered into a multivariate, backwards, stepwise logistic regression analysis to identify variables independently associated with HEV seroprevalence. The odds ratios (OR) for all variables were calculated by univariate and multivariate logistic regression. Statistical significance was set at $p < 0.05$.

Results

Samples from 3,353 blood donors (1,897 from MP and 1,456 from LR) were analysed for HEV antibodies. There were 1,744 (52%) males and the median age was 42.9 ± 13.8 years.

Anti-hepatitis E virus IgG and IgM seroprevalences in Languedoc-Roussillon and Midi-Pyrénées

A total of 1,311 blood donors were IgG-positive, corresponding to an overall seroprevalence of 39.1%. (95% confidence interval (CI): 37.4–40.7). Seroprevalence

varied from one administrative area to another: it was highest in Ariège with 71.3% (95% CI: 62.1–81.5) and lowest in Gard and Lozère with 21.9% (95% CI: 16.7–27.1). Population densities and seroprevalences by regions and areas which were not statistically associated are shown in Table 1.

The locations of the administrative regions and respective areas within France, the courses of the main rivers, and the anti-HEV IgG seroprevalences are shown in Figure 1.

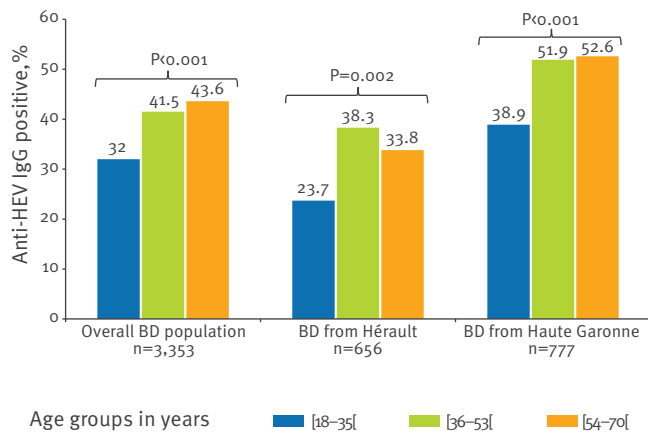
We identified four categories of areas according to the seroprevalence of anti-HEV IgG. The anti-HEV IgG prevalences were significantly different from each other. The first includes areas in which the seroprevalence was over 50% (Ariège and Aude); the prevalences in the other areas were 40 to 50% (Haute Garonne and Tarn et Garonne), 30 to 40% (Gers, Pyrénées Orientales, Tarn, Hautes Pyrénées), and below 30% (Lot and Aveyron and Gard and Lozère) (Figure 2).

Factors associated with anti-hepatitis E virus IgG or IgM seroprevalence

Several characteristics were associated with the presence of anti-HEV IgG (Table 2). Multivariate analysis identified five independent factors associated with the presence of anti-HEV IgG. These included eating uncooked pork liver sausages, offal and mussels. It is noteworthy however, that the prevalence of anti-HEV IgG among donors who declared that they had never

FIGURE 3

Prevalence of anti-hepatitis E virus IgG in donors by age group, among all blood donors in the study and among donors from the most populated areas (Haute Garonne and Hérault), southern France, October 2011



BD: blood donor; HEV: hepatitis E virus.

eaten uncooked pork liver sausage, offal or mussels was 18.1% (95% CI: 12.3–23.9). The other factors linked to the presence of IgG were the region of residence (MP or LR), as well as increasing age (Figure 3).

We found 111 (3.3%; 95% CI: 2.7–3.9) sera that were anti-HEV IgM-positive. The IgM seroprevalence in MP was 3.7% (95% CI: 2.9–4.6), whereas it was 2.7% (95% CI: 1.9–3.6) in LR (Table 1). Multivariate analysis (Table 2) showed that being male was associated with a higher seroprevalence (adjusted OR: 1.69; 95% CI: 1.10–2.60), as was eating uncooked pork liver sausage (adjusted OR: 1.87; 95% CI: 1.22–2.86). The prevalence of anti-HEV IgM among donors who declared that they had never eaten uncooked pork liver sausage was 2.4% (95% CI: 1.6–3).

Hepatitis E virus RNA detection

We tested for the presence of HEV RNA in two groups of sera including (i) 99 IgM-positive samples (the 12 remaining positive samples were too small) and (ii) 492 randomly selected from all IgM-negative specimens, independent of IgG status. HEV RNA was detected in only one IgM-positive sample (virus load: 630 copies/mL). Genotyping was unsuccessful due to low viraemia.

Discussion

The assay used gave an overall prevalence of HEV IgG antibodies as high as 39.1%, quite similar to those observed in China using the same assay [14]. Exposure seemed to vary from one area to another: from 20 to 71.3%. Associated factors were the consumption of uncooked pork liver sausages, offal and mussels.

There have been few serological studies using this type of sensitive assay on blood donors in Europe; those that have been done found that seroprevalence varied

widely from one country to another. The lowest prevalence was in Scotland (4.7%) in sera collected between 2004 and 2008 [15] and the highest reported was in MP in France (52.5%), where we had collected and tested 512 blood donors samples between 2003 and 2004 [8]. The figures reported for the Netherlands (27%) in 2011 and 2012, and Germany (29%) in 2010 were intermediate [9,16].

Our previous serological study among blood donors demonstrated that HEV infection is endemic in southwestern France [8]. The present study was done to complete the earlier data [8], it involved a 6.5-times larger blood donor population living in a 6.4-times larger area, covering two administrative regions in the south of France. This enabled us to demonstrate that prevalence varied from 20% to 71.3%, depending on the geographic area.

The wide range of prevalence observed clearly implies that there is more than one main risk factor for HEV contamination. Previous studies have shown that age [8], occupation [17], food consumed [18] and even hobbies [8] are linked to a high seroprevalence. We also found that the seroprevalence increased with age, in line with a cumulative lifetime exposure to the virus whatever the mode of contamination. The regional variation in HEV seroprevalence was not linked to differences in the age profile of our subjects.

The consumption of uncooked pork liver sausages is one of the modes by which people living in southern France become infected with HEV. We previously reported finding HEV-RNA in eight of 18 uncooked pork liver sausages bought in MP [8]. Eating pork liver sausage had already been implicated in autochthonous cases of hepatitis E in the south-eastern part of France [18]. This convinced the French Health Authorities to compel, in May 2009, the manufacturers of uncooked pork liver sausages to include recommendations for cooking their product to consumers. We also found that the consumption of offal was linked to hepatitis E. This correlates well with the findings of a German case–control study, that eating offal was a risk factor for Hepatitis E infection [19].

A case–control study among transplant recipients in MP found a significant association between HEV infection and the consumption of game meat [20]. The virus was detected in the livers of wild boar from the forests of the Ariège and Aude; these virus strains were very similar, genomically, to those isolated from patients suffering from hepatitis E (data not shown). It was demonstrated recently that wild boar from southern France are almost four times more likely to test positive for anti-HEV IgG than similar animals from northern France [21]. However, the present study does not associate the consumption of game meat with HEV seroprevalence after multivariate analysis.

The fourth dietary factor that we found to be linked to the presence of IgG is the consumption of mussels. This was described in a previous study on solid organ transplant recipients who developed autochthonous hepatitis E in south-western France [20]. Eating mussels was also responsible for a hepatitis E outbreak on a cruise ship [22]. The consumption of shellfish is a known risk factor for many viral infections, particularly for viral hepatitis as the shellfish act as a reservoir of HEV.

Shellfish are cultivated in estuarine waters into which may flow human or animal effluent, sewage or contaminated rivers. Bivalves filter and concentrate virus particles. As they are frequently eaten raw or lightly cooked they can pass on these particles, which explains their role in human hepatitis E epidemiology. Multivariate analysis indicated that eating oysters was not linked to the presence of anti-HEV IgG (Table 2). This could be due to differences in the way the oysters are cultivated. Further studies are needed to test this hypothesis.

The dietary and culinary habits of people living in southern France do not completely account for the epidemiology of HEV. The dietary habits of about one fifth of the blood donors who were positive for anti-HEV IgG did not appear to include any risk factors, such as uncooked pork liver sausage, offal or mussels. In the same way some donors who had never eaten pork liver sausage were positive for HEV IgM antibodies. The main parameters of the multivariate analysis explain only 10% of variability. There are probably other silent modes of contamination, probably linked to the environment, that explain the high seroprevalences in these subjects.

The epidemiology of HEV in southern France may share some characteristics with that of developing countries where HEV is responsible for frequent outbreaks linked to contaminated drinking water [2]. Autochthonous cases of hepatitis E associated with drinking water from a private source have been previously described in France [23]. In the present study, seroprevalence among blood donors living in the Ariège and Aude was found particularly high. This did not appear to be related to older age of donors in these areas, as the mean age of the donors from Ariège, where the highest IgG seroprevalence occurred, and the mean age of the donors from the other areas in the two regions considered were not different (data not shown).

Ariège and Aude have similar geological and ecological characteristics, and host the main rivers in MP and LR respectively. Water, including river water, can act as vehicles for HEV. For example, HEV has been detected in surface water in the Netherlands [24] and in Slovenia, where over 3% of the samples tested were positive [25]. Of the urban sewage samples taken in Spain, which neighbours the areas in this study, 30% were positive for HEV RNA [26].

Factories producing cold meats whose main ingredient is uncooked pork liver are located in Ariège and Aude as well as a number of pig farms. The prevalence of HEV antibodies can reach 88% in pig farms in France [27], and HEV RNA is frequently detected in porcine stool samples at all times of the breeding cycle [25]. Hence, sewage from pig farms, slaughterhouses and cold meat factories, as well as humans, can pollute the environment and potentiate virus dissemination. Wild boars, which are abundant in both areas, probably also excrete HEV for months or years, which can contaminate the areas where they live and pollute the river catchments. In this way Ariège and Aude could be considered the 'epidemiological epicentre' of the hepatitis E in the south of France because the prevalence decreased from these areas in concentric circles. River water samples should be tested for HEV RNA to confirm this hypothesis.

Of note, no association has been found between the density of the pig population and HEV antibodies in humans in north European countries as Denmark [28]. But the ecological and environmental characteristics of these pig farms are different, which probably explains why these data differ from those for our areas.

The great prevalence of anti-HEV IgM reported here (3.3%) agrees well with the prevalence of anti-HEV IgG and the incidence of hepatitis E (3.2/100 person-years) among solid organ transplant recipients from the same area [10]. This suggests that these people had asymptomatic hepatitis E in the recent past. Moreover the detection of HEV RNA in only 1% of IgM positive sera could be in line with the prolonged persistence of IgM described with the same assay [12]. As observed for IgG antibodies, anti-HEV IgM were more prevalent among consumers of uncooked pork liver sausage.

The rate at which donated blood tests positive for HEV RNA depends on the country. Figures obtained from mini-pools can vary throughout Europe: from 1:1,240 in Germany [29] to 1:7,986 in Sweden [30]. Even if the blood donor population is small, analysis of our data suggests that the incidence of silent hepatitis E among blood donors from the two south of France regions can reach or even exceed that described in Germany by Vollmer [29]. A 1:1,240 incidence adjusted to the 184,000 annual blood donations in the two regions could lead to 148 RNA-positive donations per year. But the risk of transfusion-transmitted hepatitis E infections by contaminated blood products remains unknown and transfusion-related hepatitis E seems to be rare in our area, even in multi-organ-transplanted recipients [2].

In conclusion, HEV is endemic in southern France and hyper-endemic in some areas. There appears to be a correlation between the presence of HEV antibodies and eating pork, offal and mussels. We need to look for infectious HEV particles in water from rivers and

initiate studies to determine the risk of HEV transmission through HEV-contaminated blood products.

Acknowledgments

Financial support. Agence Nationale de la Recherche France, grant PNRA HEVZOONEPI.

Conflict of interest

None declared.

Authors' contributions

Jean-Michel Mansuy: drafting the manuscript; Karine Sauné: statistical analysis; Henri Rech: blood collection; Florence Abravanel: National HEV reference centre, laboratory analyses; Catherine Mengelle: specimen and information collection; Sébastien L Homme: National HEV reference centre, laboratory analyses; François Destruel: blood collection; Nassim Kamar: supervision of the study; Jacques Izopet: National HEV reference centre; supervisor of the study.

References

- Meng XJ, Anderson D, Arankalle VA, Emerson SU, Harrison TJ, Jameel S, et al. Hepeviridae. In King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. *Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses*. San Diego: Elsevier Academic Press; 2012.
- Kamar N, Dalton HR, Abravanel F, Izopet J. Hepatitis E virus infection. *Clin Microbiol Rev*. 2014;27(1):116-38. <http://dx.doi.org/10.1128/CMR.00057-13> PMID:24396139</jrn>
- Kamar N, Mansuy JM, Cointault O, Selves J, Abravanel F, Danjoux M, et al. Hepatitis E virus-related cirrhosis in kidney- and kidney-pancreas-transplant recipients. *Am J Transplant*. 2008;8(8):1744-8. <http://dx.doi.org/10.1111/j.1600-6143.2008.02286.x> PMID:18557740</jrn>
- Mansuy JM, Abravanel F, Miedouge M, Mengelle C, Merviel C, Dubois M, et al. Acute hepatitis E in south-west France over a 5-year period. *J Clin Virol*. 2009;44(1):74-7. <http://dx.doi.org/10.1016/j.jcv.2008.09.010> PMID:18993112</jrn>
- Chaussade H, Rigaud E, Allix A, Carpentier A, Touzé A, Delzescaux D, et al. Hepatitis E virus seroprevalence and risk factors for individuals in working contact with animals. *J Clin Virol*. 2013;58(3):504-8. <http://dx.doi.org/10.1016/j.jcv.2013.08.030> PMID:24084601</jrn>
- Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, et al. Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. *Transfus Med*. 2006;16(2):79-83. <http://dx.doi.org/10.1111/j.1365-3148.2006.00652.x> PMID:16623913</jrn>
- Verhoef L, Koopmans M, Duizer E, Bakker J, Reimerink J, Van Pelt W. Seroprevalence of hepatitis E antibodies and risk profile of HEV seropositivity in The Netherlands, 2006-2007. *Epidemiol Infect*. 2012;140(10):1838-47. <http://dx.doi.org/10.1017/S0950268811002913> PMID:22269886</jrn>
- Mansuy JM, Bendall R, Legrand-Abravanel F, Sauné K, Miédouge M, Ellis V, et al. Hepatitis E virus antibodies in blood donors, France. *Emerg Infect Dis*. 2011;17(12):2309-12. <http://dx.doi.org/10.3201/eid1712.110371> PMID:22172156</jrn>
- Wenzel JJ, Preiss J, Schemmerer M, Huber B, Jilg W. Test performance characteristics of Anti-HEV IgG assays strongly influence hepatitis E seroprevalence estimates. *J Infect Dis*. 2013;207(3):497-500. <http://dx.doi.org/10.1093/infdis/jis688> PMID:23148290</jrn>
- Legrand-Abravanel F, Kamar N, Sandres-Saune K, Lhomme S, Mansuy JM, Muscari F, et al. Hepatitis E virus infection without reactivation in solid-organ transplant recipients, France. *Emerg Infect Dis*. 2011;17(1):30-7. <http://dx.doi.org/10.3201/eid1701.100527> PMID:2192851</jrn>
- Abravanel F, Chapuy-Regaud S, Lhomme S, Miedouge M, Peron JM, Alric L, et al. Performance of anti-HEV assays for diagnosing acute hepatitis E in immunocompromised patients. *J Clin Virol*. 2013;58(4):624-8. <http://dx.doi.org/10.1016/j.jcv.2013.10.003> PMID:24183927</jrn>
- Pas SD, Streefkerk RH, Pronk M, de Man RA, Beersma MF, Osterhaus AD, et al. Diagnostic performance of selected commercial HEV IgM and IgG ELISAs for immunocompromised and immunocompetent patients. *J Clin Virol*. 2013;58(4):629-34. <http://dx.doi.org/10.1016/j.jcv.2013.10.010> PMID:24210958</jrn>
- Abravanel F, Sandres-Saune K, Lhomme S, Dubois M, Mansuy JM, Izopet J. Genotype 3 diversity and quantification of hepatitis E virus RNA. *J Clin Microbiol*. 2012;50(3):897-902. <http://dx.doi.org/10.1128/JCM.05942-11> PMID:22205792</jrn>
- Guo QS, Yan Q, Xiong JH, Ge SX, Shih JW, Ng MH, et al. Prevalence of hepatitis E virus in Chinese blood donors. *J Clin Microbiol*. 2010;48(1):317-8. <http://dx.doi.org/10.1128/JCM.01466-09> PMID:19940058</jrn>
- Cleland A, Smith L, Crossan C, Blatchford O, Dalton HR, Scobie L, et al. Hepatitis E virus in Scottish blood donors. *Vox Sang*. 2013;105(4):283-9. <http://dx.doi.org/10.1111/vox.12056> PMID:23763589</jrn>
- Slot E, Hogema BM, Riezebos-Brilman A, Kok TM, Molier M, Zaaijer HL. Silent hepatitis E virus infection in Dutch blood donors, 2011 to 2012. *Euro Surveill*. 2013;18(31):20550. <http://dx.doi.org/10.2807/1560-7917.ES2013.18.31.20550> PMID:23929229</jrn>
- Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, et al. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol*. 2002;40(1):117-22. <http://dx.doi.org/10.1128/JCM.40.1.117-122.2002> PMID:11773103</jrn>
- Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P, et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. *J Infect Dis*. 2010;202(6):825-34. <http://dx.doi.org/10.1086/655898> PMID:20695796</jrn>
- Wichmann O, Schimanski S, Koch J, Kohler M, Rothe C, Plentz A, et al. Phylogenetic and case-control study on hepatitis E virus infection in Germany. *J Infect Dis*. 2008;198(12):1732-41. <http://dx.doi.org/10.1086/593211> PMID:18983248</jrn>
- Legrand-Abravanel F, Kamar N, Sandres-Saune K, Garrouste C, Dubois M, Mansuy JM, et al. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. *J Infect Dis*. 2010;202(6):835-44. <http://dx.doi.org/10.1086/655899> PMID:20695798</jrn>
- Carpentier A, Chaussade H, Rigaud E, Rodriguez J, Berthault C, Boué F, et al. High hepatitis E virus seroprevalence in forestry workers and in wild boars in France. *J Clin Microbiol*. 2012;50(9):2888-93. <http://dx.doi.org/10.1128/JCM.00989-12> PMID:22718947</jrn>
- Said B, Ijaz S, Kafatos G, Booth L, Thomas HL, Walsh A, et al.; Hepatitis E Incident Investigation Team. Hepatitis E outbreak on cruise ship. *Emerg Infect Dis*. 2009;15(11):1738-44. <http://dx.doi.org/10.3201/eid1511.091094> PMID:19891860</jrn>
- Renou C, Moreau X, Pariente A, Cadranet JF, Maringe E, Morin T, et al.; ANGH, France. A national survey of acute hepatitis E in France. *Aliment Pharmacol Ther*. 2008;27(11):1086-93. <http://dx.doi.org/10.1111/j.1365-2036.2008.03679.x> PMID:18346187</jrn>
- Rutjes SA, Lodder WJ, Lodder-Verschoor F, van den Berg HH, Vennema H, Duizer E, et al. Sources of hepatitis E virus genotype 3 in The Netherlands. *Emerg Infect Dis*. 2009;15(3):381-7. <http://dx.doi.org/10.3201/eid1503.071472> PMID:19239749</jrn>
- Steyer A, Naglič T, Močilnik T, Poljšak-Prijatelj M, Poljak M. Hepatitis E virus in domestic pigs and surface waters in Slovenia: prevalence and molecular characterization of a novel genotype 3 lineage. *Infect Genet Evol*. 2011;11(7):1732-7. <http://dx.doi.org/10.1016/j.meegid.2011.07.007> PMID:21802527</jrn>
- Rodriguez-Manzano J, Mjagostovich M, Hundesa A, Clemente-Casares P, Carratala A, Buti M, et al. Analysis of the evolution in the circulation of HAV and HEV in eastern Spain by testing urban sewage samples. *J Water Health*. 2010;8(2):346-54. <http://dx.doi.org/10.2166/wh.2009.042> PMID:20154397</jrn>
- Rose N, Lunazzi A, Dorenlor V, Merbah T, Eono F, Eloit M, et al. High prevalence of Hepatitis E virus in French domestic pigs. *Comp Immunol Microbiol Infect Dis*. 2011;34(5):419-27. <http://dx.doi.org/10.1016/j.cimid.2011.07.003> PMID:21872929</jrn>
- Christensen PB, Engle RE, Hjort C, Homburg KM, Vach W, Georgsen J, et al. Time trend of the prevalence of hepatitis E antibodies among farmers and blood donors: a potential zoonosis in Denmark. *Clin Infect Dis*. 2008;47(8):1026-31. <http://dx.doi.org/10.1086/591970> PMID:18781880</jrn>
- Vollmer T, Diekmann J, John R, Eberhardt M, Knabbe C, Dreier J. Novel approach for detection of hepatitis E virus infection in German blood donors. *J Clin Microbiol*. 2012;50(8):2708-13. <http://dx.doi.org/10.1128/JCM.01119-12> PMID:22675127</jrn>
- Baylis SA, Gärtner T, Nick S, Ovemyr J, Blümel J. Occurrence of hepatitis E virus RNA in plasma donations from Sweden, Germany and the United States. *Vox Sang*. 2012;103(1):89-90. <http://dx.doi.org/10.1111/j.1423-0410.2011.01583.x> PMID:22220775</jrn>