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Article

Seroprevalence Study of Anti-HEV IgG among Different Adult Populations in Corsica, France, 2019

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Abstract: Hepatitis E virus (HEV) is a major cause of acute hepatitis worldwide. In France, hyperendemic areas including Corsica have an anti-HEV Immunoglobulin G (IgG) prevalence higher than 50%. The aim of this study was to determine the seroprevalence of anti-HEV IgG in three adult populations in Corsica and the risk factors associated with antibody detection. Between 2017 and 2019, a total of 930 individuals, including 467 blood donors, 393 students or university staff members and 70 patients from general practice, were tested for the presence of anti-HEV IgG using the Wantai HEV IgG enzyme immunoassay kit and filled a questionnaire. The association between seropositivity and potential risk factors was tested with univariate and multivariate analyses. Out of the 930 samples, 52.3% (486/930) were seropositive—54.4% (254/467) among blood donors, 47.6% (187/393) among university students and 64.3% (45/70) among patients of general practice. Three main risk factors were identified: (i) skinning and butchering (Adjusted Odds Ratio aOR = 2.76, 95% confidence interval [95% CI] [1.51–5.37]; p -value $< 10^{-3}$), (ii) consumption of a local pork live raw sausage (fittonu) (aOR = 1.95 95% CI [1.45–2.64]; p -value = 10^{-5}), and (iii) increasing age (p -value = 0.003). Seropositivity rates between the different populations were homogeneous after age stratification. This cross-sectional study indicates a high anti-HEV IgG seroprevalence in the Corsican adult population, not significantly different between women and men and increasing with age. This serosurvey also showed homogeneity regarding the exposure to HEV among three different types of populations. Finally, we confirmed the endemicity of Corsica with respect to HEV and identified a strong association between consumption of figatellu/fittonu and the practice of skinning and butchering with the detection of anti-HEV IgG.

Keywords: seroprevalence; hepatitis E; IgG; risk factor; corsica; hyperendemic; hepatitis; France

1. Introduction

Hepatitis E virus (HEV) possesses a single-stranded positive-sense RNA genome of approximately 7.5 kb which contains three separate open reading frames (ORFs) [1,2]. This virus belongs to family *Hepeviridae* (genus *Orthohepevirus*) [3]. Viral strains infecting humans are classified into five genotypes (HEV-1 to HEV-4 and HEV-7) [4], but belong to a single serotype.

Genotypes HEV-3 and 4 are zoonotic pathogens infecting domestic (e.g., pigs) and wild animal species (e.g., boars, deers) which constitute the animal reservoir [5]. They are mainly detected in industrialized countries and are responsible for sporadic and autochthonous cases [1,6]. In Europe, the majority of cases is due to HEV-3 genotype and HEV is the most common cause of acute viral hepatitis [7].

A recent meta-analysis describing the HEV seroprevalence in industrialized countries, based on studies using the Wantai HEV Immunoglobulin G (IgG) enzyme immunoassay, reported an overall seroprevalence of 19% [14–25%], with great heterogeneity between countries and regions (<5% in New Zealand to >50% among French regions) [8].

The largest seroprevalence study conducted in France on 10,569 blood donors reported a 22.4% (95% [CI] 21.6–23.2%) rate with significant geographical differences and the identification of hyperendemic areas (prevalence >50%) in southern and northeastern regions [9]. The presence of anti-HEV IgG was associated with increasing age and the consumption of pork/pork liver sausages/wild game meat/offal/oysters. Conversely, drinking bottled water was associated with a lower rate of anti-HEV IgG [9]. A national report on the surveillance of HEV in France has highlighted a sharp increase (9 to 2292) in the number of autochthonous cases reported to the public health authorities between 2002 and 2016 [10]. This apparent increase is likely due (i) to improved diagnostic tests and (ii) to better awareness among physicians and in the general population, resulting in increased testing rather than a true epidemic situation. However, seroprevalence data (rates >50%), the hospitalization rate per 100,000 inhabitants, and the total number of prescribed serological tests underline the hyperendemicity of HEV in southern France.

In Corsica, a French Mediterranean island, the seroprevalence was estimated at 62% [9]. The main risk factor observed in Corsica seems to be the consumption of raw pig liver sausage (figatellu [plural: figatelli]; small liver in the Corsican language) which is traditionally eaten grilled. Indeed, grouped cases of HEV have been described and related to its consumption [11,12]. Pavio et al. (2014) described the presence of HEV RNA in 30% of tested figatelli. In Corsica, other behaviors may correlate with higher exposure to HEV such as the frequent practice of hunting, the consumption of food products derived from the porcine reservoir (figatellu and fittonu) and the existence of important rural areas.

Except for specific groups of patients (immunodepressed patients, transplanted patients, etc.), the population of blood donors is the largest group for which HEV seroprevalence studies have been performed [13,14]. Here, we recruited two additional populations to evaluate whether exposure to HEV was similar or different in other adult populations.

There is a lack of in-depth information about the actual impact of transmission route links with the porcine reservoir and other alternative sources of contamination (including environmental sources) on the epidemiology of HEV in Corsica. The main objective of this study was to improve the knowledge about the epidemiology of HEV in Corsica island, using a seroprevalence study including a large cohort of adults consisting of blood donors, general practitioner patients and staff and students of the University of Corsica. The present study gives new insights into the epidemiology of HEV in an endemic area of metropolitan France.

2. Materials and Methods

2.1. Ethics

The study received approval from the medical and scientific direction of the French Public Transfusion service (Établissement Français du Sang: EFS) and from the ad hoc ethics committee (Comité de Protection des Personnes #2016-A01000-51, 11 January 2017). The questionnaire and all data collected were validated by the data protection officer of the University of Corsica (UCPP). All participants were included on a voluntary non-remunerated basis. They were informed that samples will be used for seroprevalence studies by a letter of information and they signed a consent form.

2.2. Study Populations

Participants were included in the study if they declared living in Corsica for at least six months, at enrolment.

Population A Blood donors (BD): Voluntary blood donors accepted for classical donation according to the national requirements and agreeing to complete the questionnaire were included from 11 March 2019 to 15 April 2019.

Population B University of Corsica Pascal Paoli (UCPP): Students and personnel of the UCPP were included from January 2017 to January 2019 on the different campuses of the UCPP.

Population C Patient from General Practice (PGP): Patients from general practice >18 years old were recruited by General Practitioners (GPs) from June 2017 to September 2017. All participants were informed about the study by letter or during a face-to-face discussion with a member of the health staff.

2.3. Questionnaire

The questionnaire contained information about socio-demographical variables (age, gender, educational level, professional activities, type of dwelling, and sewage disposal), clinical factors (presence of chronic diseases, transplantation, blood transfusion, immunosuppression, and a past HEV infection during the life of the individual), contact with animals (pets and/or domestic farm animals), the consumption of meat (big game, little game, pork, beef, poultry, giblets, and pork liver), derived meat products (figatellu, fittonu, pâté/terrine, and sausages), fish and shellfish (seafood), organic fruits and vegetables or personal vegetable garden, wild berries and the source of drinking water (bottled, tap, mountain water sources, and fountains). We also recorded the type of cooking levels (raw, rare, medium, and well cooked) (items listed in Appendix A). Only the UCPP and PGP populations were asked as to clinical factors (presence of chronic diseases, transplantation, blood transfusion, immunosuppression, and a past HEV infection during the life of the individual).

The survey was conducted in the presence of knowledgeable medical personnel to ensure the accuracy of data collection.

2.4. Blood Samples and Laboratory Methods

2.4.1. Blood Samples

The blood samples obtained from blood donors corresponded to EDTA (Ethylenediamine tetraacetic acid) tubes collected systematically during the standard protocol. Samples from the UCPP and PGP groups were from capillary blood and were obtained using a safety lancet on a cleansed puncture finger that was collected into 0.8 mL tubes containing a coagulation activator and serum separator; these tubes were centrifuged at 6000 rpm for 15 min and the resulting serum was stored at $-20\text{ }^{\circ}\text{C}$ until processed for serology.

2.4.2. Anti-HEV IgG Detection

Serum samples were analyzed for the presence of anti-HEV IgG (EFS Provence-Alpes-Côtes-d'Azur & Corse, Marseille, and Laboratoire de Virologie Université de Corse) using the Wantai HEV IgG enzyme immunoassay kit (Wantai Biologic Pharmacy Enterprise, Beijing, PRC). The assay is based on a recombinant antigen corresponding to open reading frame 2 [15], the analytical and clinical performances of which were evaluated recently with a specificity and sensitivity of 97.96% and 99.60%, respectively [16]. Analyses were performed according to the manufacturer's instructions. For each sample, the ratio (sample OD/cutoff OD) was calculated and values ≥ 1 were positive. This assay was chosen in order to be in line with our previous work and to compare our results with the main French serosurvey [9] and the majority of European seroprevalence studies. In addition, this test is used by the National Reference Center for Hepatitis E in France.

2.5. Statistical Analysis

2.5.1. Sample Size

The sample size was calculated according to previously described methods [17]. A sample size of 384 was calculated assuming an a priori 50% anti-HEV seroprevalence, a confidence in the estimate of 95%, a maximum allowable error in the prevalence of 5%, and a Corsican population size of 330,455 habitants (based on the latest French census data).

2.5.2. Seroprevalence and Epidemiological Factors Analysis

Descriptive statistics were performed for all variables. Continuous data were reported as medians with interquartile ranges (IQRs). All categorical data were reported as percentages.

HEV seroprevalence (IgG) and its 95% exact binomial confidence intervals (CIs) were estimated for each population and overall. Frequencies were compared using the χ^2 test or Fisher's exact test (p -value < 0.05).

Associations between explanatory variables (socio-demographic, lifestyle factors and eating habits) and having anti-HEV IgG were tested in univariate analyses for each population and overall. All variables with a p -value below 0.2 were included in the multivariate analyses using an unconditional logistic regression model. Statistical significance was set at a p -value < 0.05. We also performed a logistic regression model with a random effect at the population level, taking into account that the people included came from different subpopulations. We used R packages (questionr, stats and lme4-package) and function glmer and glm. All statistical analyses were performed using the R program [18].

3. Results

A total of 930 individuals were included in the study: 467 BD, 393 UCPP, and 70 PGP (Figure 1). The characteristics of the three groups are presented in Table A1.

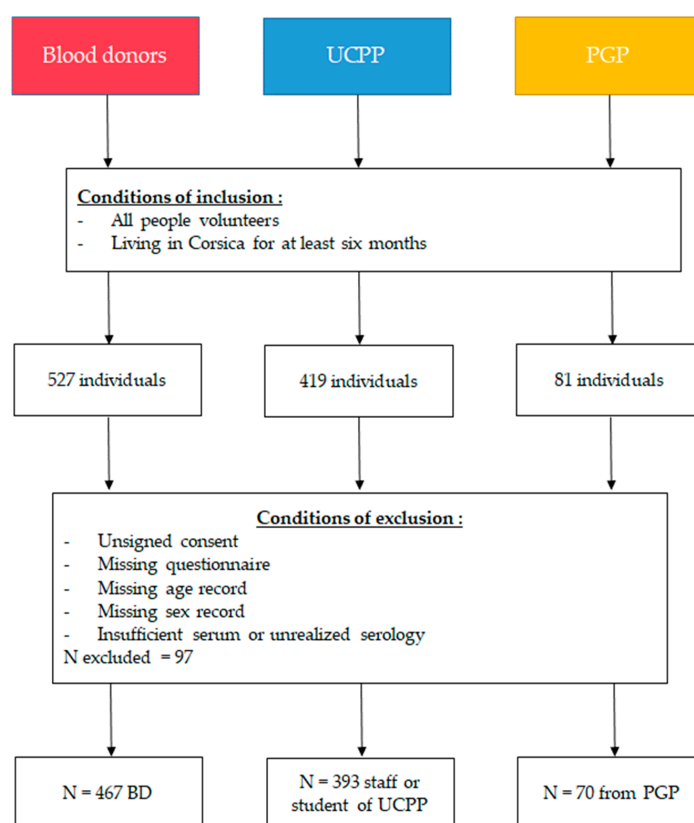


Figure 1. Flowchart for the inclusion and exclusion of studied populations.

The overall median age was 32 years (IQR: 22–49). The median age of BD was 38 years (IQR: 28–52), 24 years (IQR: 20–35) for UCPP and 55 years (IQR: 44–67) for GP. Age and gender distributions differed significantly when we compared the three groups.

Among the PGP and UCPP groups, no participant was declared to have knowledge of previous infection due to HEV (0/463).

3.1. Anti-HEV IgG Seroprevalence

Anti-HEV IgG were detected in 54.4% ($n = 254$) (IC 95% [49.8–58.9]) of the BD group, in 47.6% ($n = 187$) (IC 95% [42.6–52.5]) of the UCPP group and in 64.3% ($n = 45$) (IC 95% [53.1–75.5]) of the PGP group (Table 1). Prevalence differed significantly among the three populations (p -value = 0.015).

Males had higher rates compared with females in each of the three groups, but significantly higher only in BD and globally (p -value = 0.00169 and 0.00009, respectively) (Table 1).

Seroprevalence according to age groups and to population groups is presented in Figure 2. Among the UCPP and PGP populations, there was no significant difference between the different age groups (same letter). In BD, seroprevalences of the youngest age group (18–27) and the oldest (58–70) were significantly different (p -value < 0.05 illustrated by letters a and c). Globally, the seroprevalence of the youngest age group (18–27) was significantly different compared with all other age groups. Between the 28 to 57 years age groups, there was a plateau with observed rates that were very close to each other (54.2% to 57.7%; letter b in common), followed by two higher seroprevalences of 69.1% and 72.2% for the oldest age groups (58–70 and > 70 years) (Figure 2). For a given age group, the seroprevalence rates are not significantly different between the three groups (all p -values > 0.05).

3.2. Risk Factors Associated with Anti-HEV IgG Seroprevalence

3.2.1. Univariate Analysis

Results of univariate analyses and seroprevalences by variable are presented in Table 1.

In the BD group, fourteen variables were significantly associated with higher seropositivity rates (p -value < 0.05): age groups, male (OR = 1.81 [1.25–2.64]), breeding (OR = 2.79 [1.15–7.78]), skinning and butchering (OR = 3.05 [1.07–10.88]), fountain water (OR = 1.58 [1.08–2.33]), mountain spring waters (OR = 1.76 [1.2–2.6]), big wild game (OR = 1.8 [1.23–2.65]), pork (OR = 2.95 [1.36–6.92]), sausages and pâtés (OR = 2.47 [1.37–4.62]), liver (OR = 1.82 [1.2–2.77]), figatellu (OR = 4.14 [2.37–7.53]), fittonu (OR = 2.6 [1.76–3.86]), offal (OR = 1.86 [1.25–2.78]) and wild berries (OR = 1.57 [1.07–2.31]).

In the UCPP group, the exposure for ten variables presented significantly higher seroprevalences: hunting (OR = 2.24 [1.17–4.45]), skinning and butchering (OR = 2.73 [1.37–5.75]), big wild game (OR = 1.84 [1.19–2.88]), pork (OR = 3.26 [1.35–9.09]), sausages and pâtés (OR = 2.6 [1.11–6.78]), liver (OR = 1.56 [1.01–2.42]), figatellu (OR = 2.71 [1.53–4.98]), fittonu (OR = 2.24 [1.47–3.42]), offal (OR = 1.78 [1.15–2.76]) and shellfish (OR = 1.98 [1.17–3.45]).

In the PGP group, eight variables were significantly associated with a higher anti-HEV IgG detection rate: hunting (OR = INF), skinning and butchering (OR = INF), tap water (OR = 3.66 [1.14–12.58]), mountain spring waters (OR = 2.89 [1.01–9.21]), big wild game (OR = 3.14 [1.1–9.25]), fittonu (OR = 4.08 [1.46–12.47]), beef (OR = 8.38 [1.15–169.46]) and wild berries (OR = 3.38 [1.21–10.26]).

Only three variables were significantly associated with anti-HEV IgG in the three populations: skinning/butchering, big wild game, and consumption of fittonu.

The mean increase in seroprevalence for the practice of skinning and butchering is +25% (the increases ranged between +16% and +40% according to population) and +23% for consumption of fittonu (the increases ranged between +20% and +31% according to population). People who reported eating big game had a higher seroprevalence of 20% compared to those who did not eat it (45.1% vs. 59.7% among BD; 36.6% vs. 51.5% among UCPP and 45.5% vs. 72.3% among PGP).

Cooking types (overall population values in the Table A2) and clinical factors (data not shown) were not associated with anti-HEV IgG-seropositivity (p -value > 0.05) overall and among each population.

Table 1. Seroprevalence and factors associated with anti-Hepatitis E virus (HEV) IgG detection (univariate analysis). Significant *p*-values < 0.05 are followed by an asterisk.

| INF: Infinite NA: Missing Values | | BD | | | | UCPP | | | | | |
|-------------------------------------|-----------|----------|----------|------|-------------------|-----------------|----------|----------|------|-------------------|-----------------|
| Parameters | Variables | <i>n</i> | <i>n</i> | % | OR [95% CI] | <i>p</i> -Value | <i>n</i> | <i>n</i> | % | OR [95% CI] | <i>p</i> -Value |
| Gender | Female | 266 | 128 | 48.1 | Reference | 0.00169 * | 244 | 107 | 43.9 | Reference | 0.05807 |
| | Male | 201 | 126 | 62.7 | 1.81 [1.25–2.64] | | 149 | 80 | 53.7 | 1.48 [0.99–2.24] | |
| Age groups | 18–27 | 126 | 55 | 43.7 | Reference | 0.00633 * | 242 | 102 | 42.1 | Reference | 0.07139 |
| | 28–37 | 101 | 58 | 57.4 | 1.74 [1.03–2.97] | | 67 | 35 | 52.2 | 1.5 [0.87–2.59] | |
| | 38–47 | 83 | 40 | 48.2 | 1.2 [0.69–2.1] | | 35 | 21 | 60.0 | 2.06 [1.01–4.32] | |
| | 48–57 | 84 | 52 | 61.9 | 2.1 [1.2–3.71] | | 34 | 18 | 52.9 | 1.54 [0.75–3.2] | |
| | 58–70 | 73 | 49 | 67.1 | 2.64 [1.46–4.87] | | 11 | 8 | 72.7 | 3.66 [1.03–17.02] | |
| | 70 | | | NA | | | 4 | 3 | 75.0 | 4.12 [0.52–83.9] | |
| Hunting | No | 447 | 240 | 53.7 | 2.01 [0.79–5.77] | 0.14486 | 350 | 159 | 45.4 | 2.24 [1.17–4.45] | 0.01428 * |
| | Yes | 20 | 14 | 70.0 | | | 43 | 28 | 65.1 | | |
| Breeding | No | 442 | 235 | 53.2 | 2.79 [1.15–7.78] | 0.02165 * | 366 | 173 | 47.3 | 1.2 [0.55–2.66] | 0.64555 |
| | Yes | 25 | 19 | 76.0 | | | 27 | 14 | 51.9 | | |
| Skinning and butchering | No | 449 | 240 | 53.5 | 3.05 [1.07–10.88] | 0.03549 * | 354 | 160 | 45.2 | 2.73 [1.37–5.75] | 0.00403 * |
| | Yes | 18 | 14 | 77.8 | | | 39 | 27 | 69.2 | | |
| Contact with wastewater | No | 450 | 243 | 54.0 | 1.56 [0.58–4.6] | 0.37978 | 383 | 181 | 47.3 | 1.67 [0.47–6.64] | 0.42524 |
| | Yes | 17 | 11 | 64.7 | | | 10 | 6 | 60.0 | | |
| Tap water | No | 41 | 23 | 56.1 | 0.92 [0.47–1.74] | 0.78775 | 25 | 11 | 44.0 | 1.16 [0.52–2.69] | 0.71415 |
| | Yes | 410 | 221 | 53.9 | | | 360 | 172 | 47.8 | | |
| Bottled water | No | 15 | 10 | 66.7 | 0.57 [0.17–1.63] | 0.29725 | 10 | 5 | 50.0 | 0.88 [0.24–3.19] | 0.835 |
| | Yes | 440 | 234 | 53.2 | | | 375 | 175 | 46.7 | | |
| Water fountains in the villages | No | 213 | 102 | 47.9 | 1.58 [1.08–2.33] | 0.01895 * | 168 | 69 | 41.1 | 1.51 [1–2.29] | 0.05103 |
| | Yes | 211 | 125 | 59.2 | | | 199 | 102 | 51.3 | | |
| Mountain spring waters | No | 207 | 96 | 46.4 | 1.76 [1.2–2.6] | 0.00401 * | 182 | 86 | 47.3 | 0.96 [0.64–1.45] | 0.83939 |
| | Yes | 212 | 128 | 60.4 | | | 184 | 85 | 46.2 | | |
| Little wild game | No | 295 | 150 | 50.8 | 1.45 [0.97–2.18] | 0.06953 | 227 | 100 | 44.1 | 1.34 [0.89–2.02] | 0.16436 |
| | Yes | 145 | 87 | 60.0 | | | 154 | 79 | 51.3 | | |
| Big wild game | No | 175 | 79 | 45.1 | 1.8 [1.23–2.65] | 0.00264 * | 123 | 45 | 36.6 | 1.84 [1.19–2.88] | 0.00591 * |
| | Yes | 268 | 160 | 59.7 | | | 260 | 134 | 51.5 | | |
| Pork | No | 30 | 9 | 30.0 | 2.95 [1.36–6.92] | 0.00579 * | 26 | 6 | 23.1 | 3.26 [1.35–9.09] | 0.0075 * |
| | Yes | 421 | 235 | 55.8 | | | 356 | 176 | 49.4 | | |

Table 1. Cont.

| | | | | | | | | | | | | |
|---------------------------------|-----------|----------|-----------------------|-------|-------------------|---------------------------|----------|-----------------------|------|------------------|-----------------|--|
| Sausages and pâtés | No | 52 | 18 | 34.6 | 2.47 [1.37–4.62] | 0.00263 * | 26 | 7 | 26.9 | 2.6 [1.11–6.78] | 0.027 * | |
| | Yes | 388 | 220 | 56.7 | | | 358 | 175 | 48.9 | | | |
| Liver | No | 301 | 147 | 48.8 | 1.82 [1.2–2.77] | 0.00463 * | 255 | 110 | 43.1 | 1.56 [1.01–2.42] | 0.04589 * | |
| | Yes | 134 | 85 | 63.4 | | | 120 | 65 | 54.2 | | | |
| Figatellu | No | 69 | 18 | 26.1 | 4.14 [2.37–7.53] | 2.46 × 10 ⁻⁸ * | 65 | 18 | 27.7 | 2.71 [1.53–4.98] | 0.00049 * | |
| | Yes | 374 | 222 | 59.4 | | | 322 | 164 | 50.9 | | | |
| Fittonu | No | 218 | 94 | 43.1 | 2.6 [1.76–3.86] | 1.82 × 10 ⁻⁶ * | 175 | 64 | 36.6 | 2.24 [1.47–3.42] | 0.00015 * | |
| | Yes | 211 | 140 | 66.4 | | | 190 | 107 | 56.3 | | | |
| Offal | No | 281 | 136 | 48.4 | 1.86 [1.25–2.78] | 0.00213 * | 247 | 103 | 41.7 | 1.78 [1.15–2.76] | 0.009 * | |
| | Yes | 159 | 101 | 63.5 | | | 125 | 70 | 56.0 | | | |
| Beef | No | 16 | 8 | 50.0 | 1.18 [0.43–3.27] | 0.74187 | 15 | 9 | 60.0 | 0.58 [0.19–1.64] | 0.3031 | |
| | Yes | 430 | 233 | 54.2 | | | 368 | 171 | 46.5 | | | |
| Poultry | No | 16 | 8 | 50.0 | 1.18 [0.43–3.28] | 0.73897 | 19 | 9 | 47.4 | 0.99 [0.39–2.56] | 0.99112 | |
| | Yes | 437 | 237 | 54.2 | | | 362 | 171 | 47.2 | | | |
| Shellfish | No | 76 | 38 | 50.0 | 1.2 [0.73–1.97] | 0.47138 | 71 | 24 | 33.8 | 1.98 [1.17–3.45] | 0.01107 * | |
| | Yes | 376 | 205 | 54.5 | | | 314 | 158 | 50.3 | | | |
| Fish | No | 22 | 10 | 45.5 | 1 [0.41–2.36] | 0.99845 | 31 | 10 | 32.3 | 1.98 [0.93–4.51] | 0.07726 | |
| | Yes | 431 | 235 | 54.5 | | | 352 | 171 | 48.6 | | | |
| Organic fruits and vegetables | No | 62 | 27 | 43.5 | 1.64 [0.96–2.84] | 0.07122 | 30 | 12 | 40.0 | 1.4 [0.66–3.07] | 0.38191 | |
| | Yes | 383 | 214 | 55.9 | | | 348 | 168 | 48.3 | | | |
| Wild berries | No | 234 | 115 | 49.1 | 1.57 [1.07–2.31] | 0.02001 * | 194 | 84 | 43.3 | 1.32 [0.88–1.99] | 0.17687 | |
| | Yes | 199 | 120 | 60.3 | | | 179 | 90 | 50.3 | | | |
| Total | | 467 | 254 | 54.4 | | | | 393 | 187 | 47.6 | | |
| | | PGP | | | | | Overall | | | | | |
| Parameters | Variables | <i>n</i> | Anti-HEV IgG Positive | % | OR [95% CI] | <i>p</i> -Value | <i>n</i> | Anti-HEV IgG Positive | % | OR [95% CI] | <i>p</i> -Value | |
| Gender | Female | 34 | 20 | 58.8 | Reference | 0.35362 | 544 | 255 | 46.9 | Reference | 0.00009 * | |
| | Male | 36 | 25 | 69.4 | 1.59 [0.6–4.33] | | 386 | 231 | 59.8 | 1.69 [1.3–2.2] | | |
| Age groups | 18–27 | 6 | 3 | 50.0 | Reference | 0.40753 | 374 | 160 | 42.8 | Reference | 0.00001 * | |
| | 28–37 | 5 | 3 | 60.0 | 1.5 [0.13–19.11] | | 173 | 96 | 55.5 | 1.67 [1.16–2.4] | | |
| | 38–47 | 13 | 10 | 76.9 | 3.33 [0.43–29.22] | | 131 | 71 | 54.2 | 1.58 [1.06–2.37] | | |
| | 48–57 | 19 | 9 | 47.4 | 0.9 [0.13–5.99] | | 137 | 79 | 57.7 | 1.82 [1.23–2.71] | | |
| | 58–70 | 13 | 10 | 76.9 | 3.33 [0.43–29.22] | | 97 | 67 | 69.1 | 2.99 [1.87–4.87] | | |
| Hunting | No | 64 | 39 | 60.9 | INF | 0.01785 * | 861 | 438 | 50.9 | 2.21 [1.32–3.82] | 0.0024 * | |
| | Yes | 6 | 6 | 100.0 | | | 69 | 48 | 69.6 | | | |
| Breeding | No | 65 | 41 | 63.1 | 2.34 [0.32–47.23] | 0.42697 | 873 | 449 | 51.4 | 1.75 [1.01–3.11] | 0.04641 * | |
| | Yes | 5 | 4 | 80.0 | | | 57 | 37 | 64.9 | | | |
| Skinning and butchering | No | 61 | 36 | 59.0 | INF | 0.00322 * | 864 | 436 | 50.5 | 3.07 [1.76–5.64] | 0.00005 * | |
| | Yes | 9 | 9 | 100.0 | | | 66 | 50 | 75.8 | | | |
| Contact with wastewater | No | 67 | 42 | 62.7 | INF | 0.09878 | 900 | 466 | 51.8 | 1.86 [0.88–4.19] | 0.1043 | |
| | Yes | 3 | 3 | 100.0 | | | 30 | 20 | 66.7 | | | |
| Tap water | No | 15 | 6 | 40.0 | 3.66 [1.14–12.58] | 0.02965 * | 81 | 40 | 49.4 | 1.13 [0.71–1.78] | 0.60849 | |
| | Yes | 55 | 39 | 70.9 | | | 825 | 432 | 52.4 | | | |
| Bottled water | No | 2 | 2 | 100.0 | INF | 0.17973 | 27 | 17 | 63.0 | 0.62 [0.27–1.34] | 0.22479 | |
| | Yes | 68 | 43 | 63.2 | | | 883 | 452 | 51.2 | | | |
| Water fountains in the villages | No | 35 | 21 | 60.0 | 1.39 [0.52–3.8] | 0.50842 | 416 | 192 | 46.2 | 1.5 [1.15–1.97] | 0.00289 * | |
| | Yes | 34 | 23 | 67.6 | | | 444 | 250 | 56.3 | | | |
| Mountain spring waters | No | 42 | 23 | 54.8 | 2.89 [1.01–9.21] | 0.04808 * | 431 | 205 | 47.6 | 1.36 [1.04–1.79] | 0.02331 * | |

Table 1. Cont.

| | | | | | | | | | | | |
|--|-----|----|----|------|--------------------|-----------|-----|-----|------|------------------|--------------------------|
| | Yes | 27 | 21 | 77.8 | | | 423 | 234 | 55.3 | | |
| Little wild game | No | 46 | 27 | 58.7 | 1.99 [0.69–6.38] | 0.20866 | 568 | 277 | 48.8 | 1.38 [1.05–1.82] | 0.02055 * |
| | Yes | 23 | 17 | 73.9 | | | 322 | 183 | 56.8 | | |
| Big wild game | No | 22 | 10 | 45.5 | 3.14 [1.1–9.25] | 0.03188 | 320 | 134 | 41.9 | 1.84 [1.4–2.43] | 0.00001 * |
| | Yes | 47 | 34 | 72.3 | | | 575 | 328 | 57.0 | | |
| Pork | No | 1 | 0 | 0.0 | INF | 0.14871 | 57 | 15 | 26.3 | 3.27 [1.83–6.18] | 0.00004 * |
| | Yes | 69 | 45 | 65.2 | | | 846 | 456 | 53.9 | | |
| Sausages and pâtés | No | 5 | 2 | 40.0 | 2.93 [0.45–23.52] | 0.25158 | 83 | 27 | 32.5 | 2.44 [1.52–3.99] | 0.00017 * |
| | Yes | 65 | 43 | 66.2 | | | 811 | 438 | 54.0 | | |
| Liver | No | 48 | 28 | 58.3 | 2.29 [0.75–7.92] | 0.1474 | 604 | 285 | 47.2 | 1.7 [1.28–2.28] | 0.00028 * |
| | Yes | 21 | 16 | 76.2 | | | 275 | 166 | 60.4 | | |
| Figatellu | No | 6 | 2 | 33.3 | 4.1 [0.74–31.28] | 0.1066 | 140 | 38 | 27.1 | 3.48 [2.35–5.24] | 1.88 × 10 ⁻¹⁰ |
| | Yes | 64 | 43 | 67.2 | | | 760 | 429 | 56.4 | | |
| Fittonu | No | 35 | 17 | 48.6 | 4.08 [1.46–12.47] | 0.00694 * | 428 | 175 | 40.9 | 2.46 [1.87–3.24] | 8.41 × 10 ⁻¹¹ |
| | Yes | 34 | 27 | 79.4 | | | 435 | 274 | 63.0 | | |
| Offal | No | 39 | 26 | 66.7 | 0.75 [0.28–2.02] | 0.56837 | 567 | 265 | 46.7 | 1.72 [1.3–2.28] | 0.00012 * |
| | Yes | 30 | 18 | 60.0 | | | 314 | 189 | 60.2 | | |
| Beef | No | 5 | 1 | 20.0 | 8.38 [1.15–169.46] | 0.0349 * | 36 | 18 | 50.0 | 1.08 [0.55–2.11] | 0.82208 |
| | Yes | 65 | 44 | 67.7 | | | 863 | 448 | 51.9 | | |
| Poultry | No | 2 | 1 | 50.0 | 1.83 [0.07–47.76] | 0.67522 | 37 | 18 | 48.6 | 1.15 [0.59–2.24] | 0.67789 |
| | Yes | 68 | 44 | 64.7 | | | 867 | 452 | 52.1 | | |
| Shellfish | No | 15 | 7 | 46.7 | 2.49 [0.77–8.21] | 0.12501 | 162 | 69 | 42.6 | 1.57 [1.11–2.21] | 0.00988 * |
| | Yes | 54 | 37 | 68.5 | | | 744 | 400 | 53.8 | | |
| Fish | No | 3 | 1 | 33.3 | 3.83 [0.35–84.93] | 0.26558 | 56 | 21 | 37.5 | 1.61 [0.94–2.83] | 0.08474 |
| | Yes | 67 | 44 | 65.7 | | | 850 | 450 | 52.9 | | |
| Organic fruits and vegetables or personal vegetable garden | No | 11 | 8 | 72.7 | 0.61 [0.12–2.38] | 0.49248 | 103 | 47 | 45.6 | 1.34 [0.89–2.03] | 0.1605 |
| | Yes | 58 | 36 | 62.1 | | | 789 | 418 | 53.0 | | |
| Wild berries | No | 37 | 19 | 51.4 | 3.38 [1.21–10.26] | 0.01937 * | 465 | 218 | 46.9 | 1.52 [1.17–1.99] | 0.00203 * |
| | Yes | 32 | 25 | 78.1 | | | 410 | 235 | 57.3 | | |
| Total | | 70 | 45 | 64.3 | | | 930 | 486 | 52.3 | | |

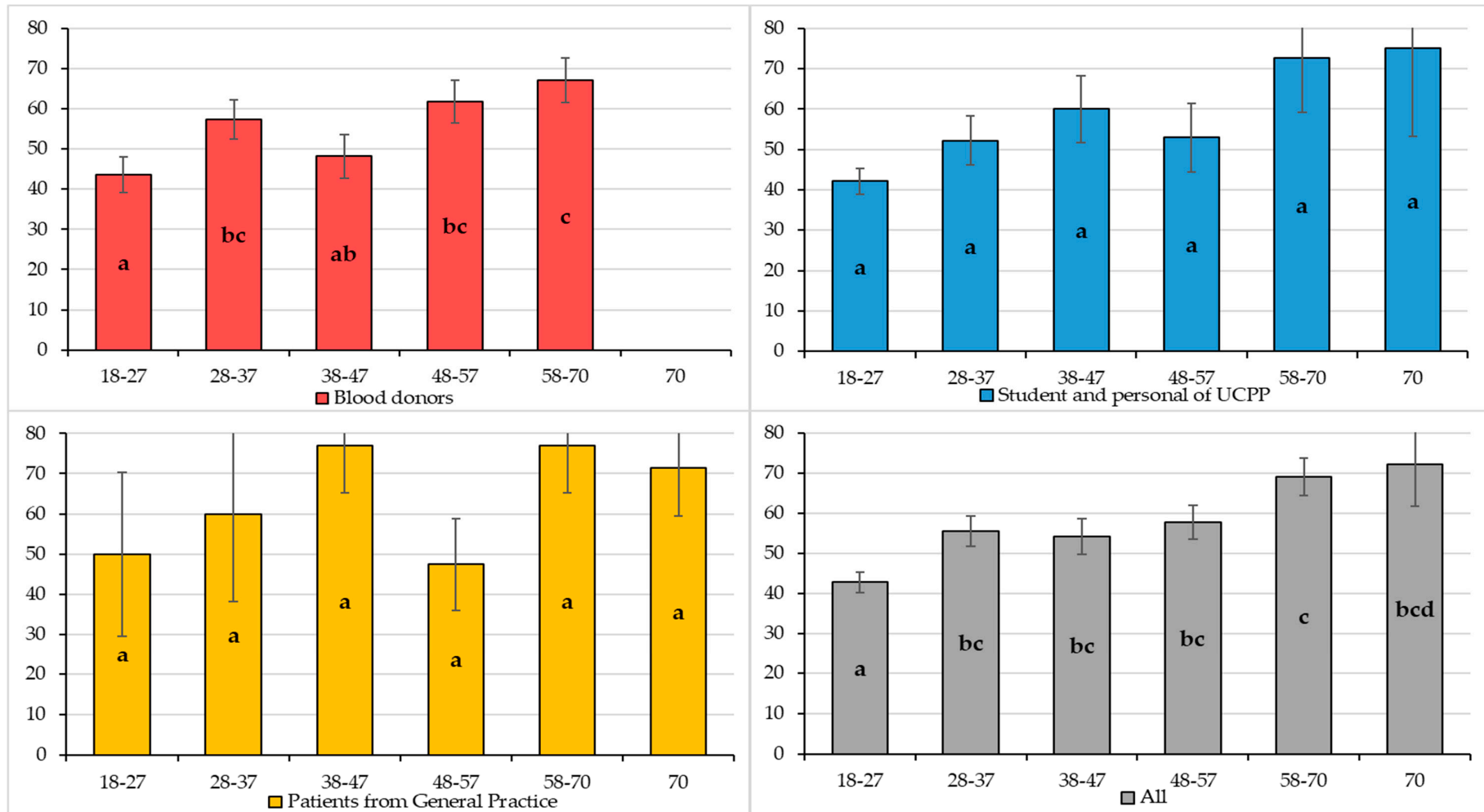


Figure 2. Graphs of the different seroprevalences of anti-HEV IgG (%) by age group and populations. The different lowercase letter (a, b, c, and d) indicates a significant difference ($p < 0.05$) between age groups of a given population. For example: among blood donors, the group 18–27 is significantly different from that of 58–70 (“a” vs. “c”), but not from that of 38–47 (presence of the letter a). The black bars correspond to the standard deviation of each proportion.

3.2.2. Multivariate Analysis

In multivariate analysis (Table 2), “skinning and butchering” and “consumption of fittonu” remained independent predictors for anti-HEV IgG seropositivity in each population. Male and consumption of figatellu were associated with anti-HEV IgG detection only in the BD population (p -value = 0.03 and $< 10^{-3}$ respectively).

In overall multivariate analyses (glm and lmer) “skinning and butchering” (aOR = 2.76; 95% CI 1.51–5.37), “consumption of fittonu” (aOR = 1.95; 95% CI 1.45–2.64) and of “figatellu” (aOR = 2.22; 95% CI, 1.45–3.45) were associated with anti-HEV detection. Increasing age was also significantly associated with anti-HEV IgG detection in the overall multivariate analysis (p -value = 0.003) (Table 2).

3.2.3. Discussion

In France, seroprevalence studies were conducted in blood donors and allowed the identification of different risk factors such as increasing age, hunting, consumption of pork liver sausages (figatellu), game meat offal and oysters [9,19,20]. Hyperendemic areas were identified in southwestern France (Occitanie), southeastern France (Provence-Alpes-Cotes-d’Azur), and Corsica. Our study is the first to address Corsican populations other than blood donors, using a specific questionnaire and a large regional sampling.

A total of 52.2% of 930 individuals were positive for the presence of anti-HEV IgG. Interestingly, results of a very similar order of magnitude were observed in each of the three groups. Although the three groups were different regarding age, sex, and socio-demographic data, they seem to be fairly homogeneous with regard to HEV. Indeed, for a given age group, no significant differences in seroprevalences were observed.

We observed an age-related increase in anti-HEV IgG in the 18–27 group (42.8%) and the older than 70 group (73.7%), reflecting a cumulative life-time exposure in agreement with previous studies performed in European populations [9,13,21–23]. This age-related increased rate could be associated with differences in dietary habits and other behaviors such as rare or absence of consumption of uncooked meat products, a shorter exposure time and lack of hunting and skinning/butchering in the younger group. Repeated exposure leading to infection/reinfection might also play a role, although it appears that a single infection leads to long-life immunity [24], and that there is no clear data about the possibility of immunity acquisition through repeated contacts with HEV without systemic infection.

In the present study, although we recorded higher rates in men (59.8%) vs. women (46.9%), the difference was not statistically significant in the multivariate analysis. This is in line with previous studies performed in French blood donors living in the hyperendemic regions in southern France [25] and in other industrialized countries [22,26,27]. Overall, these results could suggest that exposure to HEV is not directly related to gender but rather to individual behavior (differences in dietary habits and other behaviors such as a different frequency of consumption of meat products, and lack of hunting or other practices in contact with the animal reservoir).

Interestingly, none of the 463 participants and, more specifically, none of the 232 participants with anti-HEV IgG (50.2%) reported a known previous infection with HEV. This suggests that asymptomatic cases or cases of infection for which the patients are not seeking medical check-up might be much larger than the 50–80% commonly reported for genotype 3 [28–30]. This is specifically important in hyperendemic regions where efforts should be exerted for better awareness of Hepatitis E and for a more systematic strategy of testing compared to what is currently done. The risk factors described in our study together with those reported in the scientific literature should be used to define “the at-risk population” which merit to be tested for the presence of viral RNA and for anti-HEV IgM whenever clinical manifestations are coherent with acute infection with HEV.

Table 2. Multivariate analysis in the three populations and overall.

| Parameters | Variables | BD | | UCPP | | PGP | | Overall Glm | | Overall Lmer Fixed Subpopulations | |
|-------------------------|-----------|-------------------|---------|------------------|---------|----------------------|---------|-------------------|---------|-----------------------------------|---------|
| | | aOR [95% CI] | p-Value | aOR [95% CI] | p-Value | aOR [95% CI] | p-Value | aOR [95% CI] | p-Value | aOR [95% CI] | p-Value |
| Gender | Male | 1.58 [1.05–2.39] | 0.02973 | | | | | NS | | | |
| | Figatellu | 2.82 [1.54–5.34] | 0.00065 | NS | | NS | | 2.22 [1.45–3.45] | 0.00023 | 1.77 [1.04–3.01] | 0.035 |
| | Fittonu | 1.97 [1.29–3.03] | 0.00183 | 2.14 [1.4–3.28] | 0.00039 | 4.35 [1.48–13.92] | 0.00696 | 1.95 [1.45–2.64] | 0.00001 | 1.95 [1.38–2.74] | 0.00013 |
| Skinning and butchering | 18–27 | 3.52 [1.09–15.83] | 0.03451 | 2.43 [1.16–5.38] | 0.01764 | 7.88 e6 [0–INF] | 0.00294 | 2.76 [1.51–5.37] | 0.00077 | 3.45 [1.37–8.71] | 0.0087 |
| | 28–37 | | | | | | | Reference | | | |
| Age groups | 38–47 | | | | | | | 1.61 [1.09–2.38] | | | |
| | 48–57 | NS | | NS | | NS | | 1.52 [0.98–2.37] | 0.00272 | NS | |
| | 58–70 | | | | | | | 1.54 [1–2.38] | | | |
| | 70 | | | | | | | 2.44 [1.45–4.19] | | | |
| | | | | | | | | 3.46 [1.17–12.65] | | | |

NS: Non-significant value; INF: infinite value.

An independent factor associated with anti-HEV IgG seropositivity was to practice skinning or butchering. In Corsica, traditionally, hunter or breeder family members commonly engage in such activities, leading to HEV exposure in a distinct manner compared to food or water intake. This practice was strongly associated with HEV IgG positivity in our study. HEV infection can occur during the evisceration of an infected animal—through contact with its blood or feces. In a similar manner, higher rates in seroprevalence studies were identified in butchers and slaughterhouse workers compared with the general population [31]. The presence of HEV RNA in wild boar and swine bile, liver, sera, and faces [11,32,33] is in line with a higher risk of exposure and strengthens the need for protective gloves during the disemboweling of wild boars [34].

Here, we observed that participants reporting eating specific types of meat such as figatellu were significantly associated with higher HEV IgG seropositivity as previously described [35–37]. Our study was the first to examine the consumption of fittonu, a dried pork liver sausage that is not cooked before eating (in contrast with figatellu). In the Netherlands, traditional Dutch dry raw sausages called “cervelaat”, “fijnkost”, “salami” and “salametti” were also associated with higher seroprevalence [38]. Interestingly, anti-HEV IgG rates were in range from 27% to 31% according to three recent Dutch studies [8,22,38,39]. The same situation is observed in Poland with seroprevalence between 44% and 50% and where Polish dry sausage known as “Kabanos” are very popular [8].

A cooking temperature of 71 °C for twenty minutes is required to inactivate HEV [40,41]. The virus stays viable after heating at 56 °C for one hour and remains infectious up to 60 °C [41,42]. Although figatellu is usually roasted, it remains strongly associated with HEV infection; cooking does not appear to have a significant impact on seroprevalence in our study (p -value = 0.87). This could be explained by the fact that figatellu is also (i) either eaten without being roasted or (ii) eaten after the necessary cooking times and temperatures have not been respected; (iii) last, pre-roasting handling of the raw figatellu might be a risk [43]. In this regard, washing hands after product handling or wearing gloves during disemboweling must be recommended.

Meat products were not statistically associated with HEV seropositivity but showed higher rates (>55%) (game, offal, liver, pork). These meat products have been frequently associated with higher seroprevalence or HEV RNA detection [44–47].

HEV is increasingly found in the environment [48]. In our study, higher anti-HEV IgG rates were associated with consumption of fountain waters in villages and waters of natural springs (mountain hiking), and seafood. In Corsica, the Regional Health Agency (Agence Régionale de la Santé) has carried out a study on the quality of drinking water, and many counties reported unsatisfactory bacteriological results although HEV was not tested [49]. As a non-enveloped virus, HEV transmission through water consumption (such as hepatitis A virus and other picornaviruses) must be taken into consideration as an important route of infection. Many studies have identified the presence of HEV in running water. In Italy, HEV was detected in river water [50]. Irrigation water is also involved, and the virus was detected in fruits and vegetables in several European countries [51–54] or associated with higher seroprevalence in Turkey [55].

HEV was repeatedly detected in seafood in the United Kingdom, Spain and Japan [56–59] or epidemiologically associated with higher anti-HEV IgG rates in population with frequent consumption of seafood and shellfish [9]. In these studies, strains belonging to genotype 3 (swine and human strains) were identified, suggesting the existence of an epidemiologic cycle between the different animal reservoir, environment, and human cases.

This study has several limitations. First, we investigated anti-HEV IgG seroprevalence in the adult population only. Data for children are scarce, and the risk factors remain to be thoroughly evaluated [60]. Second, the sample issued from PGP was small in size with respect to the samples issued from blood donors and university. Third, we cannot exclude that other risk factors that seem to play a minor role in our study may be more prominent when increasing the sample size. Finally, the number of individuals included who were older than 70 years was small compared to other age groups, which may underestimate the overall seroprevalence calculated.

This cross-sectional study indicates an anti-HEV IgG seroprevalence > 50% in the Corsican adult population, similar between women and men and increasing with age. This serosurvey also showed homogeneity regarding the exposure to HEV among three different types of populations. Finally, we identified a strong association between consumption of figatellu/fittonu and the practice of skinning and butchering, with the detection of anti-HEV IgG among the three populations studied. These results provide relevant information for control and preventive strategies and concrete advice to risk groups. Surface, irrigation, or consumption water could be a potential source for exposure. A study on the presence of the virus in surface waters or bivalve molluscan shellfish as an indicator of water pollution (or food products) could be carried out to better understand the epidemiology of the virus in Corsica.

Author Contributions: Conceptualization and design of the study: L.C., P.G., T.B., X.d.L., R.C., and A.F.; Investigation: B.P.; Writing—Original Draft preparation: L.C., R.C. and A.F.; Methodology: L.C., A.F., R.C., C.S. and T.B.; Analysis: L.C., C.I., S.M., and C.S.; Writing—Review and Editing: L.C., X.d.L., P.G., R.C., S.M. and A.F.; Funding acquisition, A.F.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

List of items of the questionnaire

- Age
- Gender
- Situation
- Professional activity
- Educational level
- ZIP code
- Type of residence
- Water distribution
- Sewage disposal
- Contact with wastewater
- Consumption water
- Hunting
- Breeding
- Home at one kilometer of a farm or a breeding
- Skinning or butchering activity
- Contact with different animals
- Eating habits and type of cooking
- Clinical factors (presence of chronic diseases, transplantation, blood transfusion, immunosuppression, and a past HEV infection during the life of the individual) except for blood donors

Table A1. Description of population and repartition of individuals included by variable.

| | | Blood Donors | | Student and Personal of UCPP | | Patient of General Practice | | Overall | |
|-------------------------|------------------|--------------|------|------------------------------|------|-----------------------------|------|----------|-------|
| | | <i>n</i> | % | <i>n</i> | % | <i>n</i> | % | <i>n</i> | % |
| Gender | Men | 201 | 43.0 | 149 | 37.9 | 36 | 51.4 | 386 | 41.5 |
| | Women | 266 | 57.0 | 244 | 62.1 | 34 | 48.6 | 544 | 58.5 |
| Age groups | 18–27 | 126 | 27.0 | 242 | 61.6 | 6 | 8.6 | 374 | 40.2 |
| | 28–37 | 101 | 21.6 | 67 | 17.0 | 5 | 7.1 | 173 | 18.6 |
| | 38–47 | 83 | 17.8 | 35 | 8.9 | 13 | 18.6 | 131 | 14.1 |
| | 48–57 | 84 | 18.0 | 34 | 8.7 | 19 | 27.1 | 137 | 14.7 |
| | 58–70 | 73 | 15.6 | 11 | 2.8 | 13 | 18.6 | 97 | 10.4 |
| | 70 | | | 4 | 1.0 | 14 | 20.0 | 18 | 1.9 |
| Graduate studies level | Pre bac | 87 | 18.6 | 30 | 7.6 | 42 | 60.0 | 159 | 17.1 |
| | Bac | 145 | 31.0 | 180 | 45.8 | 8 | 11.4 | 333 | 35.8 |
| | Post bac | 232 | 49.7 | 179 | 45.5 | 20 | 28.6 | 431 | 46.3 |
| Professional activity | No | 64 | 13.7 | 112 | 28.5 | 1 | 1.4 | 177 | 19.0 |
| | Yes | 403 | 86.3 | 281 | 71.5 | 69 | 98.6 | 753 | 81.0 |
| Kind of habitation | Apartment | 179 | 38.3 | 182 | 46.3 | 23 | 32.9 | 384 | 41.3 |
| | Individual House | 284 | 60.8 | 204 | 51.9 | 47 | 67.1 | 535 | 57.5 |
| | Farm | 4 | 0.9 | 6 | 1.5 | 0 | 0.0 | 4 | 0.4 |
| Hunters | No | 447 | 95.7 | 350 | 89.1 | 64 | 91.4 | 861 | 92.6 |
| | Yes | 20 | 4.3 | 43 | 10.9 | 6 | 8.6 | 69 | 7.4 |
| Breeders | No | 442 | 94.6 | 366 | 93.1 | 65 | 92.9 | 873 | 93.9 |
| | Yes | 25 | 5.4 | 27 | 6.9 | 5 | 7.1 | 57 | 6.1 |
| Skinning and butchering | No | 449 | 96.1 | 354 | 90.1 | 61 | 87.1 | 864 | 92.9 |
| | Yes | 18 | 3.9 | 39 | 9.9 | 9 | 12.9 | 66 | 7.1 |
| Contact with wastewater | No | 450 | 96.4 | 383 | 97.5 | 67 | 95.7 | 900 | 96.8 |
| | Yes | 17 | 3.6 | 10 | 2.5 | 3 | 4.3 | 30 | 3.2 |
| Housing water | Private | 22 | 4.7 | 19 | 4.1 | 2 | 0.4 | 43 | 9.2 |
| | Collective | 429 | 91.9 | 360 | 91.6 | 69 | 98.6 | 858 | 92.3 |
| Total | | 467 | 50.2 | 393 | 42.3 | 70 | 7.5 | 930 | 100.0 |

Missing values (NA): Seven for the variable “Graduate studies level” and “kind of habitation”; 29 for housing water.

Table A2. Univariate analysis of the association between anti-HEV IgG detection and the type of cooking.

| Food Products | Cooking | OR [95% CI] | p-Value |
|--------------------|-------------|------------------|---------|
| Little wild game | Well-cooked | Reference | 0.45337 |
| | Raw | INF | |
| | Rare/Medium | 0.85 [0.51;1.4] | |
| Big wild game | Well-cooked | Reference | 0.23411 |
| | Raw | INF | |
| | Rare/Medium | 0.75 [0.5;1.15] | |
| Pork | Well-cooked | Reference | 0.2255 |
| | Raw | 0.99 [0.4;2.49] | |
| | Rare/Medium | 0.69 [0.46;1.05] | |
| Sausages and pâtés | Well-cooked | Reference | 0.81779 |
| | Raw | 0.87 [0.53;1.45] | |
| | Rare/Medium | 0.9 [0.56;1.44] | |
| Liver | Well-cooked | Reference | 0.18698 |
| | Raw | INF | |
| | Rare/Medium | 0.71 [0.38;1.34] | |
| Figatellu | Well-cooked | Reference | 0.86939 |
| | Raw | 1.18 [0.62;2.32] | |
| | Rare/Medium | 1.05 [0.67;1.64] | |
| Offal | Well-cooked | Reference | 0.17264 |
| | Rare/Medium | 0.65 [0.35;1.21] | |
| Beef | Well-cooked | Reference | 0.60331 |
| | Raw | 1.29 [0.77;2.2] | |
| | Rare/Medium | 1.01 [0.75;1.36] | |
| Poultry | Well-cooked | Reference | 0.51125 |
| | Raw | INF | |
| | Rare/Medium | 0.97 [0.66;1.41] | |
| Shellfish | Well-cooked | Reference | 0.40387 |
| | Raw | 1.24 [0.86;1.8] | |
| | Rare/Medium | 1.3 [0.79;2.14] | |
| Fish | Well-cooked | Reference | 0.7241 |
| | Raw | 1.07 [0.73;1.58] | |
| | Rare/Medium | 1.16 [0.8;1.69] | |

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