

## RESEARCH ARTICLE

# High prevalence of anti-hepatitis E virus antibodies among blood donors in central Italy, February to March 2014

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**Prevalence of anti-hepatitis E virus (HEV) antibodies is highly variable in developed countries, which seems partly due to differences in assay sensitivity. Using validated sensitive assays, we tested 313 blood donors attending a hospital transfusion unit in central Italy in January and February 2014 for anti-HEV IgG and IgM and HEV RNA. Data on HEV exposure were collected from all donors. Overall anti-HEV IgG prevalence was 49% (153/313). Eating raw dried pig-liver sausage was the only independent predictor of HEV infection (adjusted prevalence rate ratio=2.14; 95% confidence interval: 1.23–3.74). Three donors were positive for either anti-HEV IgM (n = 2; 0.6%) or HEV RNA (n = 2; 0.6%); they were completely asymptomatic, without alanine aminotransferase (ALT) abnormalities. Of the two HEV RNA-positive donors (both harbouring genotype 3), one was anti-HEV IgG- and IgM-positive, the other was anti-HEV IgG- and IgM-negative. The third donor was positive for anti-HEV IgG and IgM but HEV RNA-negative. HEV infection is therefore hyperendemic among blood donors (80% men 18–64 years-old) from central Italy and associated with local dietary habits. Nearly 1% of donors have acute or recent infection, implying potential transmission to blood recipients. Neither ALT nor anti-HEV IgM testing seems useful to prevent transfusion-transmitted HEV infection.**

## Introduction

Hepatitis E virus (HEV) is a non-enveloped single-stranded RNA virus of the genus *Hepevirus* in the *Hepeviridae* family. This family contains viruses that infect mammals, including humans, as well as birds

and fish. Four major mammalian HEV genotypes (HEV1 to 4) have been identified [1-3]. HEV, once thought to be limited to developing countries, has recently been found also in developed countries of Europe, North America and Asia-Pacific where human autochthonous cases, probably of zoonotic origin, have become prevalent [1,2].

HEV1 and HEV2 infect only humans and are endemic in developing areas of Asia, Africa and Central and South America, where faecal-oral transmission usually occurs through contaminated water and causes both outbreaks and sporadic cases. Clinical disease mainly affects young adults and is severe and associated with excess mortality in pregnant women and in patients with chronic liver disease [1,2].

HEV3 and HEV4 infect humans and various domestic and wild mammals such as pig, wild boar, deer and rodents. HEV strains infecting humans and animals in the same area are usually phylogenetically closely related, supporting zoonotic transmission [1]. HEV3 is ubiquitous, while HEV4 is mainly prevalent in Asia. These genotypes are transmitted by eating contaminated raw or undercooked meat and meat products or shellfish, and by contact with infected animals, causing autochthonous sporadic cases [1,2]. Most HEV3 or HEV4 infections are asymptomatic; clinical disease mainly affects middle-aged and elderly men with underlying illness, and HEV3 may cause chronic hepatitis in immunosuppressed patients [1-3].

In addition to hepatitis, HEV infection also appears to be associated with some extrahepatic manifestations: neurological disorders such as Guillain–Barré syndrome and neuralgic amyotrophy due to peripheral nerve involvement, haematological diseases such as haemolytic anaemia and severe thrombocytopenia, glomerulonephritis and mixed cryoglobulinaemia [4].

Epidemiological studies have reported variable and sometimes unexpectedly high prevalence rates of IgG anti-HEV antibodies in the general population in Europe, ranging from 1.1% to 16.8% in the period between 2004 and 2011, although a decline was observed for example in south-eastern Germany from 1996 to 2011 [5–9]. Rates ranging from 0.4% to 52.5% have been reported among European blood donors in the period between 1993 and 2014, with the highest value observed in the Midi-Pyrénées hyperendemic area in south-western France [10–18]. In Italy, anti-HEV IgG prevalence rates of 1.0–4.3% [19–21] and 0.7–9.1% [19,21–23] were found in the general population (in the period between 1993 and 2011) and in blood donors (in the period between 1993 and 2013), respectively. The large variations in anti-HEV prevalence in different European countries and even within the same country are probably due to several factors such as the performance of antibody assays used in the studies but also dietary habits and occupational exposure [2,10,11].

HEV can be transmitted by blood transfusion [10,24]. Transfusion-transmitted HEV infection has been reported in several countries [25–27], but its true frequency is probably underestimated because it is often asymptomatic and testing of blood donors is infrequent [1,10]. In this study we have assessed the prevalence of HEV infection among blood donors in the Abruzzo region in central Italy by using highly sensitive and validated assays, and we have examined its association with putative risk factors.

## Methods

### Study population

This study was designed to evaluate the prevalence of and the risk factors for HEV infection among voluntary, unpaid blood donors attending the blood transfusion unit of the San Salvatore Hospital in L'Aquila during February and March 2014. L'Aquila is a city of around 70,000 inhabitants in the Abruzzo region in central Italy.

Serum samples were collected from blood donors who agreed to participate in the study. Participants were administered a questionnaire collecting information on demographics and putative risk factors for HEV infection (professional and recreational activities, contact with domestic or wild animals, eating habits and travel history).

All blood donors provided written informed consent. The study protocol conformed to the Helsinki

Declaration and was approved by the Ethics Committee of San Salvatore Hospital.

### Serological assays

All serum samples were tested for anti-HEV IgG and IgM antibodies using commercial enzyme-linked immunosorbent assay (ELISA) kits (Wantai, Biologic Pharmacy Enterprise, Beijing, China). Both the IgG and IgM anti-HEV assays use recombinant antigen expressed from the ORF2 region.

### Detection and quantitation of hepatitis E virus RNA

Plasma samples from anti-HEV IgG-positive or -negative donors were assembled in minipools of 10 samples (20 µL each for a total volume of 200 µL), or fewer if a full set of 10 was not available, and total RNA was extracted by QIAamp MinElute Virus Spin kit silica columns (Qiagen, Hilden, Germany). Total HEV RNA was extracted individually from each anti-HEV IgM-positive sample.

One half of the extracted RNA was reverse transcribed and HEV RNA amplified using the RealStar HEV RT-PCR kit, version 1.0 (Altona Diagnostics, Hamburg, Germany). This kit includes primers and a probe targeting the ORF3 region of the HEV genome. The sensitivity, reported as 95% limit of detection, was assessed to be 50 IU/mL of HEV RNA. Reactive pools were deconstructed to identify HEV RNA-positive donations by individual HEV RNA testing.

An external standard curve, made from a log dilution series of a HEV RNA World Health Organization (WHO) International Standard (Paul-Ehrlich-Institute, Langen, Germany, code 6329/10) from  $5 \times 10^4$  to  $5 \times 10^1$  IU/mL was used for estimating the viral load in positive samples.

### Sequencing and phylogenetic analysis

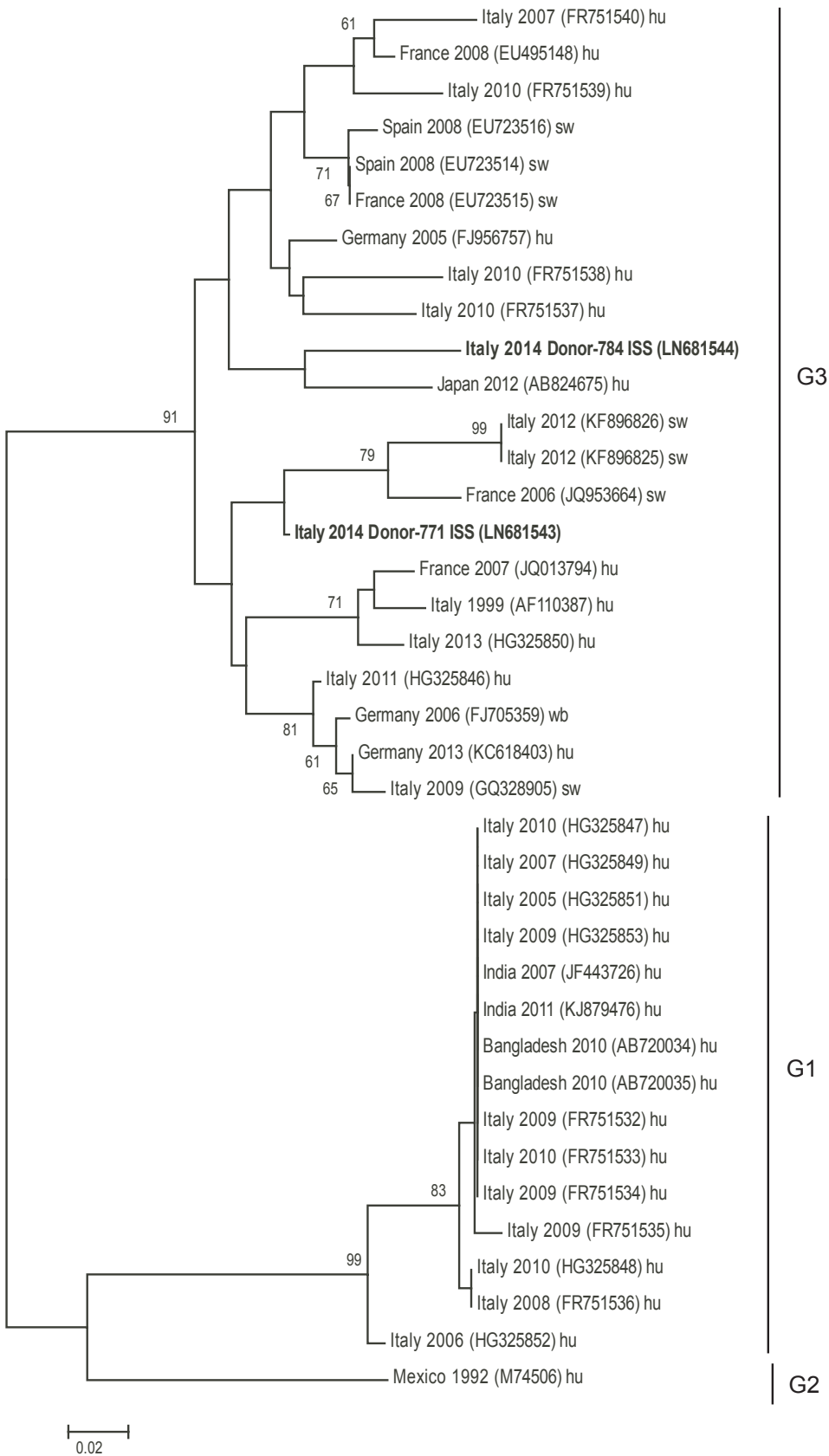
HEV RNA was extracted and amplified by nested RT-PCR using two sets of primers targeting ORF1 (172 bp) and ORF2 (348 bp) as described elsewhere [28,29]. Purified PCR amplicons were subjected to bidirectional automated sequencing. The raw forward and reverse ABI files were assembled into a single consensus sequence using MEGA 6.06 software. The phylogenetic trees were constructed based on the best fit model of nucleotide substitution. The reliability of the phylogenetic trees were determined by bootstrap re-sampling of 1,000 replicates.

### Statistical analysis

Prevalence of anti-HEV antibodies (IgG and IgM) and HEV RNA was calculated and the exact binomial distribution was used to calculate 95% confidence intervals (CI). The association between the study variables and HEV infection was estimated by chi-squared test. Factors independently associated with HEV infection were evaluated by a multivariate binomial regression model. All variables with a *p* value < 0.20 in univariate

**FIGURE 1**

Maximum likelihood phylogenetic tree based on the Tamura-Nei model for ORF1 nucleotide sequences of selected hepatitis E virus strains

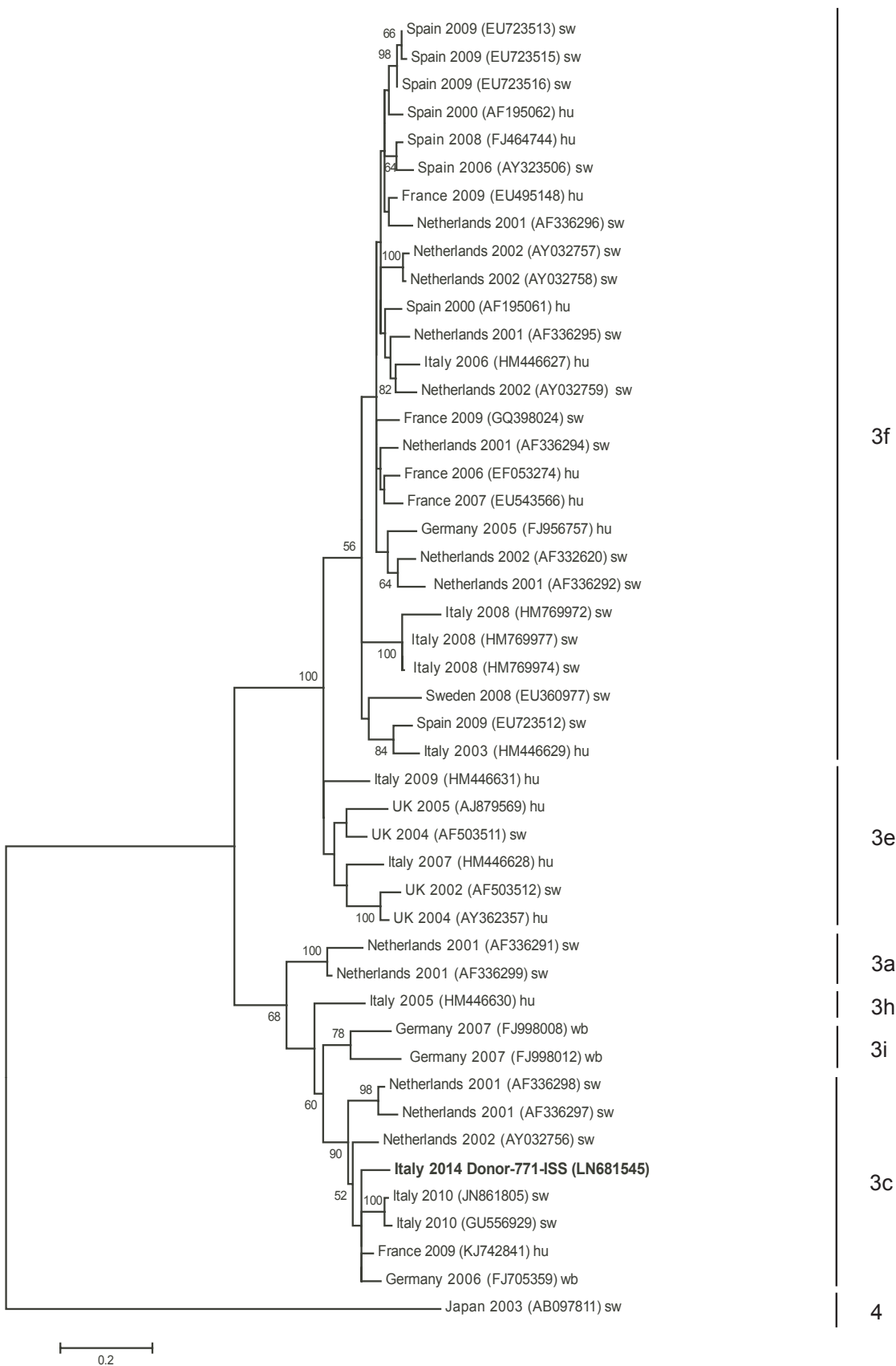


Hu: human; sw: swine; wb: wild boar.

Sequences of hepatitis E virus isolated from blood donors 771 and 784 in Italy in 2014 are shown in bold. The numbers at the nodes indicate bootstrap values  $\geq 60\%$ . Sequences are denoted by country, year of isolation (or publication if not available), GenBank accession number in parenthesis, and source.

**FIGURE 2**

Maximum likelihood phylogenetic tree based on the Tamura-Nei model for ORF2 nucleotide sequences of selected hepatitis E virus strains



Hu: human; sw: swine; wb: wild boar.

Sequence of hepatitis E virus isolated from blood donor 771 in Italy in 2014 is shown in bold. The numbers at the nodes indicate bootstrap values  $\geq 60\%$ . Sequences are denoted by country, year of isolation (or publication if not available), GenBank accession number in parenthesis, and source.

**TABLE 1**

Prevalence of anti-hepatitis E virus IgG antibodies and univariate and multivariate analysis of factors potentially associated with infection in blood donors, central Italy, 2014 (n = 313)

Characteristics	Tested	HEV IgG+	Univariate analysis			Multivariate analysis		
	N	n (%)	PR	95% CI	p value	APRR	95% CI	p value
<b>Sex</b>								
Female	61	24 (39)	1.00	Ref	Ref	Ref	Ref	Ref
Male <sup>a</sup>	252	129 (51)	1.30	0.84–2.01	0.24	0.87	0.56–1.35	0.52
<b>Age group (years)</b>								
18–34	54	19 (35)	1.00	Ref	Ref	Ref	Ref	Ref
35–44	82	37 (45)	1.28	0.74–2.23	0.19	1.35	0.77–2.35	0.29
45–54	102	51 (50)	1.42	0.84–2.41				
≥55 <sup>a</sup>	75	46 (61)	1.74	1.02–2.97				
<b>Environment</b>								
Birth in rural area <sup>a</sup>	107	61 (57)	1.28	0.92–1.76	0.14	1.17	0.84–1.64	0.39
Living in rural area	164	84 (51)	1.10	0.80–1.51	0.56	NI	NI	NI
<b>Educational level</b>								
Secondary school	93	48 (52)	1.00	Ref	Ref	NI	NI	NI
High school	166	77 (46)	0.90	0.63–1.29	0.80	NI	NI	NI
Master's degree	54	28 (52)	1.00	0.63–1.60				
<b>Professional exposure</b>								
Work with animals	28	17 (61)	1.27	0.77–2.11	0.35	NI	NI	NI
<b>Contacts with</b>								
Pigs	170	87 (51)	1.10	0.81–1.53	0.53	NI	NI	NI
Wild boar	51	29 (57)	1.20	0.80–1.80	0.37	NI	NI	NI
Other wild animals	37	21 (57)	1.19	0.75–1.88	0.47	NI	NI	NI
Horses	114	53 (47)	0.92	0.66–1.29	0.65	NI	NI	NI
Poultry	194	98 (51)	1.10	0.79–1.52	0.60	NI	NI	NI
Sheep	124	58 (47)	0.93	0.67–1.29	0.67	NI	NI	NI
Cattle	113	62 (55)	1.20	0.87–1.67	0.26	NI	NI	NI
Other animals	80	38 (48)	0.96	0.67–1.39	0.84	NI	NI	NI
Dogs <sup>a</sup>	253	116 (46)	0.74	0.51–1.08	0.12	0.73	0.50–1.07	0.11
Cats	205	100 (49)	1.00	0.71–1.39	0.97	NI	NI	NI
<b>Hobbies</b>								
Hunting	32	20 (63)	1.32	0.83–2.11	0.25	NI	NI	NI
Gardening	207	105 (51)	1.12	0.80–1.58	0.51	NI	NI	NI
Vegetable gardening <sup>a</sup>	200	106 (53)	1.27	0.90–1.80	0.17	1.23	0.87–1.78	0.24
<b>Eating habits</b>								
Pork	312	152 (49)	0.49	0.07–3.48	0.47	NI	NI	NI
Game	249	125 (50)	1.15	0.76–1.73	0.51	NI	NI	NI
Salami	311	151 (49)	0.49	0.12–1.96	0.31	NI	NI	NI
Raw dried pork sausage	311	152 (49)	0.98	0.14–6.98	0.98	NI	NI	NI
Raw dried wild boar sausage	225	115 (51)	1.18	0.82–1.71	0.37	NI	NI	NI
Raw dried pork liver sausage <sup>a</sup>	253	139 (55)	2.35	1.36–4.08	0.002	2.14	1.23–3.74	0.007
Undercooked/raw meat	80	37 (46)	0.93	0.64–1.34	0.70	NI	NI	NI
Consumption of vegetables from own kitchen garden	244	124 (51)	1.21	0.81–1.81	0.36	NI	NI	NI
Frequent consumption of vegetables from own garden	186	98 (53)	1.22	0.87–1.69	0.24	NI	NI	NI
Occasional consumption of vegetables from own garden	59	27 (46)	0.92	0.61–1.40	0.70	NI	NI	NI
<b>Travel</b>								
Travel to non-endemic areas	259	127 (49)	1.02	0.66–1.55	0.93	NI	NI	NI
Travel to endemic areas	118	65 (55)	1.22	0.88–1.68	0.22	NI	NI	NI

APRR: adjusted prevalence rate ratio; CI: confidence Interval; HEV IgG+: donors testing positive for IgG anti-hepatitis E virus antibodies; NI: not included; PR: prevalence ratio.

<sup>a</sup> Variables initially included in the multivariate binomial regression model because of a p value < 0.20 in the univariate analysis.

analysis were initially included in the model, while age and sex were included independently from the p value. A backward selection was then performed and only variables with a p value < 0.10 (by the log-likelihood ratio test) were retained [30]. Statistical analyses were performed using STATA, version 12.

## Results

### Serological testing and anti-hepatitis E virus prevalence

From February to March 2014, 327 blood donors attended the blood transfusion unit, of whom 313 (81% male; age: 18–68 years, median: 48 years) were suitable for donation and agreed to participate to the study and to complete the questionnaire. Almost all were Italian citizens (99%) and resided in Abruzzo (98%), where 84% of donors were also born.

The overall anti-HEV IgG prevalence was 49% (153/313; 95% CI: 0.43–0.54). As shown in Table 1, prevalence was higher, although not significantly, in men ( $p=0.24$ ). Likewise, no statistically significant differences were found for educational level ( $p=0.80$ ), professional exposure (work with animals) ( $p=0.35$ ) and urban or rural area of birth ( $p=0.14$ ) or living ( $p=0.56$ ). A high anti-HEV IgG prevalence (>35%) was observed in all age groups, without significant differences among them ( $p=0.19$ ) (Table 1). However, the prevalence increased with age, and people older than 55 years showed the highest rate.

Two of 313 donors (0.6%; 95% CI: 0.08–2.3) were positive for anti-HEV IgM, and both were also positive for anti-HEV IgG (Table 2).

### Factors associated with anti-hepatitis E virus IgG positivity

Several potential risk exposures showed in the univariate analysis an association with anti-HEV IgG positivity with a p value < 0.2 (Table 1): birth in a rural area ( $p=0.14$ ), contact with dogs ( $p=0.12$ ), home vegetable gardening ( $p=0.17$ ) and eating raw dried pig liver sausage ( $p=0.002$ ). In the multivariate analysis, also adjusting for age and sex, only eating raw dried pig liver sausage was independently associated with anti-HEV IgG positivity (adjusted prevalence rate ratio=2.14; 95% CI: 1.23–3.74;  $p=0.007$ ).

### Detection, quantification and genotyping of hepatitis E virus RNA

Three donors (1; 95% CI: 0.20–2.80) showed evidence of acute or recent HEV infection. Two donors (0.6%; 95% CI: 0.08–2.3) were positive for HEV RNA (Table 2). One of them (donor 784) had low-level viraemia (100 IU/mL) and was anti-HEV IgG- and IgM-positive, while the other (donor 771) had 10,000 IU/mL of HEV RNA and was anti-HEV IgG- and IgM-negative. The third one (donor 207), who tested positive for both IgG and IgM, was HEV RNA-negative. These three donors were completely asymptomatic and their blood donation

showed normal alanine aminotransferase (ALT) levels. All three were tested again for HEV RNA, anti-HEV IgG and IgM four to eight months later (Table 2). Donor 207 was examined five months later and remained HEV RNA-negative, while donors 784 and 771 had become HEV RNA-negative four and eight months after the first blood sampling, respectively. Anti-HEV IgG and IgM were positive in all samples obtained during the follow-up. Therefore, seroconversion occurred in the donor who was negative at the time of donation while IgG and IgM persisted in the two others.

The phylogenetic analysis performed using partial nucleotide sequences of ORF1 (172 bp) and ORF2 (348 bp) is shown in Figures 1 and 2, respectively. ORF1 sequence was amplified from both HEV RNA-positive donors, while ORF2 amplification was successful only in donor 771, owing to very low-level viraemia in donor 784. The phylogenetic tree based on ORF1 sequences showed that both isolates grouped with HEV3 sequences (Figure 1). The ORF2 based tree indicated that the isolate from donor 771 belonged to subtype 3c and clustered with very high bootstrap values with human and swine 3c sequences from Italy (Figure 2).

## Discussion

The observed anti-HEV IgG prevalence is among the highest prevalence rates reported in blood donors from developed countries [11,12,24]. The demographic data and the age distribution of anti-HEV IgG-positive donors suggest that HEV infection is hyperendemic in the Abruzzo region and virus exposure occurs relatively early in life. However, we observed an increasing prevalence with age which is likely to reflect a cumulative exposure to HEV over time.

Such a high anti-HEV IgG prevalence was unexpected, since all studies performed so far among Italian blood donors reported much lower figures (0.7–9.1%) [19,21–23]. Indeed, differences in anti-HEV IgG prevalence seem to be largely due to the different sensitivity and specificity of the assays employed [2,11,13,15] and most previous studies, by now outdated, used assays less sensitive than the assays currently in use. In the present study we used the Wantai IgG ELISA, a validated assay which in various comparative studies proved to be the most sensitive test available for anti-HEV IgG detection, also showing high specificity [2,31,32].

Recent studies using this assay for blood donors in Europe showed varying IgG seroprevalence: 4.6% in Scotland [12], 10% and 16%, respectively, in the north-west and south-west of the United Kingdom (UK) [15,33], 13.6% in Upper Austria [17], 20.0% in Catalonia, Spain [16], 19.8% in Denmark [18], 26.7% in the Netherlands [34], 39.1% in southern France [24] and 52.6% in the Midi-Pyrenees region of south-western France [11].

As these latter studies used the same Wantai IgG ELISA assay, sensitivity and specificity could not be a variable affecting the results. Therefore such differences

TABLE 2

Serological and virological features in blood donors with acute/recent hepatitis E virus infection, central Italy, 2014 (n = 3)

Donor	Age (years)	Baseline sample						Follow-up sample			
		HEV RNA	HEV RNA load (IU/mL)	HEV IgM (OD) <sup>a</sup>	HEV IgG (OD) <sup>b</sup>	ALT (U/L)	GT	TI (months)	HEV RNA	HEV IgM (OD)	HEV IgG (OD)
771	50	Pos	10,000	Neg (0.000)	Neg (0.001)	13	3c	8	Neg	Pos (0.370)	Pos (2.954)
784	50	Pos	100	Pos (2.466)	Pos (0.423)	10	3	4	Neg	Pos (0.766)	Pos (1.844)
207	46	Neg	NA	Pos (0.602)	Pos (2.785)	16	NA	5	Neg	Pos (0.432)	Pos (2.764)

ALT: alanine aminotransferase; GT: genotype; HEV: hepatitis E virus; HEV IgG: anti-HEV IgG antibodies; HEV IgM: anti-HEV IgM antibodies; NA: not applicable; neg: negative; OD: optical density; pos: positive; TI: time interval.

<sup>a</sup> Cut-off OD value for IgM anti-HEV: 0.260.

<sup>b</sup> Cut-off OD value for IgG anti-HEV: 0.161.

are likely to be genuine, representing true geographical variation in HEV prevalence due to different types and levels of zoonotic exposure. In addition to evident zoonotic exposure, such as professional contact with animals and consumption of contaminated food, other risk factors possibly linked to zoonotic transmission (e.g. ingestion of contaminated water or consumption of shellfish) might play a role in acquiring HEV infection, but their relative importance remains unknown [2]. In the present study, professional exposure to animals was reported by only 9% of blood donors while 54% and 16% reported contact with pigs and contact with wild boar, respectively. This reflects the common habit in the studied area to rear pigs for personal consumption and to hunt boars. However, these three risk factors were not associated with a higher risk of HEV positivity; this seems in contrast with previous studies [2,33], but the high prevalence we observed, mainly related to dietary habits, could have masked the effect of other possible risk factors. In our study, anti-HEV IgG positivity was independently associated with eating raw dried pig liver sausage, a food product widely diffused in the study region. Therefore, it may play a predominant role in HEV transmission, clearly detectable even when analysing a small population. Our data are also in agreement with a study carried out among blood donors from south-western France, where eating uncooked pork liver sausages and being male were independent predictors of anti-HEV prevalence [24]. In addition, cases of an HEV outbreak were linked with eating raw pig liver sausage in south-eastern France [35]. The risk of infection by eating raw pig liver sausages is likely to be higher than other infection modes because liver tissue is the main target of HEV replication and the viral concentration is therefore expected to be very high: an individual is likely to be exposed to a higher viral dose when eating raw liver compared with other raw meat or sausages [35].

We cannot exclude that other risk factors that only played a minor role in our studied population may be more prominent when increasing the sample size. Other studies showed that particular eating habits might

explain the spread of infection in a region [2,11,24,35-37]. According to Legrand-Abrevanel et al., eating game meat was the only independent predictor of HEV infection in French transplant recipients [36]. Offal and wild boar consumption emerged as unique independent predictors of HEV infection in a German study [37]. Moreover, a recent study in south-western France carried out in a large population, identified offal and eating uncooked pork liver sausages as risk factors [24].

Several studies have shown that HEV circulates widely in Italian pigs. An HEV RNA prevalence of 42–64.6% was found in pigs in northern Italy and the viral sequences belonged to HEV<sub>3</sub> (specified in one study as subtypes 3c and 3f) [38,39]. HEV RNA belonging to genotype 3 has been detected in both raw and dried pig liver sausages sold at a grocery store in Rome [40].

Also the two HEV RNA-positive donors in our study harboured HEV<sub>3</sub> genotype; one of them, for whom subtyping was successful, belonged to subtype 3c and clustered together with other human and swine strains from Italy [41]. Preparation and consumption of pig liver sausages are widespread throughout the Abruzzo region. However, consumption of pig liver sausage could not by itself explain the high anti-HEV IgG prevalence we saw among blood donors from Abruzzo, because pig liver sausage represents a traditional and widely consumed food also in other regions such as Latium and Molise. Nevertheless, in a seroprevalence study performed during 2009 among 101 blood donors from Latium (89% male; median age: 42 years, range: 20–62 years) we found an overall anti-HEV IgG prevalence of 9% with the same assay employed in this study (data not shown). This finding suggests that other factors in addition to food-borne transmission may have contributed to the high prevalence. Specific geographical and demographic factors, not reported in other studies, may have had a synergistic effect. Firstly, the geographical characteristics of the territory (mostly mountainous, forested and sparsely populated) favoured the uncontrolled expansion of the wild boar population and may have led to contamination of

soil and watercourses. Secondly, almost all study participants were born and lived in Abruzzo. More than half of them lived in rural areas, had a potentially high risk of exposure to HEV-contaminated environment and had similar dietary habits.

In this study, the proportion of HEV RNA-positive blood donors was 0.6% (2/313), to our knowledge one of the highest rates reported among qualified blood donors worldwide [10,12,13,24,31,42,43]. Interestingly, the three donors showing evidence of acute infection were asymptomatic and had normal serum ALT levels. The different patterns of HEV RNA and anti-HEV IgG and IgM observed in each of them suggests they were in three different phases of acute infection (ramp-up phase of viraemia, early control and full control of infection). This picture is corroborated by the follow-up results: HEV RNA clearance occurred in all examined samples, associated with seroconversion or persistence of IgG and IgM antibodies.

These findings have the following implications: Firstly, a small but not negligible proportion of the predominantly male blood donors (nearly 1%) in Abruzzo have evidence of acute/recent HEV infection but are completely asymptomatic and have normal ALT. Secondly, anti-HEV IgM is detectable in only a proportion of viraemic donors, as reported by others [13,42,43]. Thus, infective donations may escape from being identified unless viraemia is examined, and such asymptomatic and viraemic donors are potentially able to cause transfusion-transmitted hepatitis E in blood recipients. Finally, the coexistence of both IgM and HEV RNA is of short duration [13]. Overall, neither ALT measurement nor anti-HEV IgM testing seems appropriate to prevent transfusion-transmitted HEV infection.

Although the presence of HEV RNA in blood donors is not a rare event, only a few cases of transfusion-transmitted hepatitis E have been reported in developed countries [25-27] and such cases have never been reported in Abruzzo or elsewhere in Italy. It is possible that the vast majority of transfusion-transmitted infections remain asymptomatic and unrecognised, or that transmission is hampered by low infectious dose and/or high anti-HEV titre in blood donation as well as immunity of the recipient, especially important in high prevalence regions [44].

We were able to trace two recipients who had received red blood cell concentrates from the two viraemic donors identified by us. These recipients remained uninfected, without markers of HEV infection, up to seven months after transfusion and were then lost to follow-up.

Some important issues need to be addressed before considering implementation of HEV nucleic acid testing in blood screening. These include: the duration of HEV viraemia, the minimal infectious dose, the possible protective effect of anti-HEV antibodies in donors

and recipients (e.g. nearly half of the Abruzzo population, including blood recipients, has anti-HEV IgG), the true incidence of symptomatic and asymptomatic acute infections in the general population, and the frequency of chronic HEV infection in blood recipients.

Published data about all these issues are scarce. In Italy, notification of acute hepatitis E to the Italian surveillance system for acute viral hepatitis (SEIEVA) began only in 2007, and from 2007 to 2014, only 145 cases of acute infection were reported [45]. This low annual incidence markedly disagrees with the anti-HEV IgG prevalence found in our study and also in previous Italian studies [19-23,46-48]. However, virtually all acute viral hepatitis cases reported to SEIEVA are symptomatic (and hospitalised), while most cases of HEV<sub>3</sub> and HEV<sub>4</sub> acute infection are subclinical or completely asymptomatic [1,2] and thus pass unrecognised. It is also possible that the discrepancy may, at least in part, be due to under-reporting to the surveillance system because of low awareness of healthcare professionals.

Some limitations of this study need to be discussed. We are aware that the sample of blood donors analysed by us was small. However, this limited size made a complete virological analysis possible at affordable cost (all donors were tested for anti-HEV IgG and IgM and HEV RNA) as well as an evaluation of risk factors for infection. Our findings cannot be representative of the Italian population of blood donors because the study was limited to a restricted geographical area. However, a similar epidemiological scenario could exist in other countries with similar dietary habits and should be the object of further studies.

In addition, our study sample enrolled only 19% women, lower than the annual percentage (30%) of female blood donors in Italy [46]. However, our study covered a period of only two months and this short period has probably reduced the chance to enrol female blood donors who have a longer inter-donation interval than men. Several studies have shown that reasons for exclusion or long inter-donation intervals are more frequent for women, e.g. lower body weight, lower iron levels, lower blood pressure, minor transient health problems, pregnancy and breastfeeding [49,50].

## Conclusion

Anti-HEV IgG prevalence among the predominantly male blood donors from Abruzzo was high and associated with eating raw dried pig liver sausage. HEV RNA was detected in two donors, who harboured HEV<sub>3</sub> and were completely asymptomatic, without ALT abnormalities. More data are required before considering implementing HEV nucleic acid testing in blood donors. Recommendations for blood donors and immunocompromised patients against eating undercooked pork meat would help reduce the risk of HEV infection and chronic liver disease.



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## Conflict of interest

None declared.

## Authors' contributions

Spada E and Ciccaglione AR participated in study's design, data analysis, manuscript drafting and supervision of the study; Lucarelli C and Taliani G contributed to data analysis, interpretation of data and helped manuscript drafting; Pezzotti P performed statistical analysis and assisted in study design, analysis and interpretation of data; Bruni R, contributed to study design and assisted in the analysis and interpretation of the data; Chionne P, Madonna E, La Rosa G and Pisani G performed laboratory analysis and contributed to the interpretation of data. Marcantonio C, Dell'Orso L, Ragone K and Tomei C contributed to samples and data collection. All authors read and critically revised the first as well as subsequent drafts to this manuscript and approved the final version.

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