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# Hepatitis E in Italy: A silent presence

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ABSTRACT

Hepatitis E virus (HEV) was discovered in the 1980s and has been considered as being confined to developing countries. The purpose of this critical review was to determine the reported HEV seroprevalence rates in Italy, to identify predisposing factors and individuals at risk and to assess possible importation of HEV by immigrants. A critical review of 159 articles published in PubMed from 1994 to date was done. Only 27 original reports of 50 or more subjects, written in the English or Italian language, were included. Over three decades, the HEV seroprevalence varied from 0.12% to 49%, with the highest rates being reported from the central region of Italy. Risk factors included ingestion of raw pork or potentially contaminated food. The seroprevalence among immigrants ranged from 15.3% to 19.7% in Apulia. Italy has a population of 60 656 000; the total number of individuals surveyed was only 21.882 (0.036%). A national epidemiological survey program is needed to capture more comprehensive seroprevalence data.



**Contents**



## Introduction

Hepatitis E virus (HEV) is the ubiquitous etiological agent of enteric non-A viral hepatitis and it represents an ongoing internationally challenging issue for public health. Annually, HEV is responsible for 3.3 million new symptomatic infections with fatal outcomes in 56 600 individuals worldwide [1,2]. Three decades after its discovery during an outbreak of unexplained hepatitis in Afghanistan [3], not only is its origin unknown but the modes of transmission remain far from being clearly understood in the industrialized world. HEV is a small hepatotropic single-stranded RNA virus, the sole member of the Hepeviridae family, belonging to the

*Abbreviation:* Assay-1, Dia.Pro; Assay-2, Abbott; Assay-3, Wantai; Assay-4, Adaltis; HEV, Hepatitis E virus.

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Hepevirus genus [4]. The genetic variability of HEV, its host range, as well as its classification, has been in constant evolution [5]. Previous genomic sequence analysis had revealed the existence of four well-defined mammalian genotypes and at least 24 sub-genotypes, with a specific geographic distribution [6]. Indeed, the epidemiology and pathogenicity of HEV observes a bimodal pattern that differs in emerging nations and occidental countries. Genotypes 1 and 2 cause large outbreaks and epidemics mainly among young adults in Africa, Central and Southern Asia, Central America and the Middle East [7–12]. The infection is acquired predominately through a fecal–oral route and it is associated with an unusually high mortality rate during pregnancy [13]. Poor sanitation and contaminated water sources are precipitating factors. An increasing number of sporadic and small locally acquired outbreaks have been reported in Northern America, Australia, Europe, China and Japan [14–19]. Genotypes 3 and 4 are less virulent strains identified as the causative agents of subclinical and clinical infection in the elderly population. HEV is mostly transmitted zoonotically with the ingestion of raw and undercooked food. To data, eight genotypes have been detected and 4 of which are confined to animal species: genotype 5 and 6 in Japanese wild boar (*Scrofa scrofa leucomystax*) [20] and genotype 7 and 8 respectively in dromedary camels (*Camelus dromedaries*) and Bactrian camels (*Camelus bactrianus*) [21].

In Europe, HEV seroprevalence estimates ranged in the general population from 7.5% to 31.9% with the average rate being 19.16%; rates increase with age [22]. However, the real prevalence could be underestimated due to the difference in test sensitivity and the frequently asymptomatic course of the disease. Overall, it is likely that current geopolitical instability and the consequent massive immigration would lead towards the local introduction of new pathogenic variants and modify the known epidemiology in Western countries.

## Methods

The critical review is based on a literature search on PubMed, using the keywords “hepatitis E in Italy” and “hepatitis E seroprevalence in Italy”. Studies published from January 1994 and May 2017 were included according to the following criteria: studies provided clear information regarding the seroprevalence rate at the regional or national level and included at least 50 samples in the cohort (Fig. 1). No age restriction was observed and all studies were written in the English or Italian language. The statistical analyses of the reported regional seroprevalences have been done. We used the screening methods to adjust the prevalence value according to the sensitivity and specificity of the assay. Only estimated seroprevalence rates with a lower positive value for C.I. at 95% have been considerate statistically significant.

According to the data available, we focused on fourteen study cohorts: general population, blood donors, pregnant women, the pediatric population, acute hepatitis patients, chronic liver disease patients, hemodialysis patients, immigrants, prisoners, intravenous drug users, HIV co-infected individuals, HIV-exposed and/or infected individuals and workers with contact with potential zoonotic reservoirs (abattoir workers, laboratory workers exposed to biological swine material, animal breeders, veterinarians and farmers) and recipients of renal transplants. Studies that did not meet the above-mentioned criteria, provided duplicate data, personal opinion or international reviews, were excluded from the critical review.

## Results

159 publications were identified by title and abstract through a PubMed search and 27 articles were included in the final data analysis. Based on data published in these articles, we calculated that a total of 21.882 individuals have been tested for anti-HEV IgG and/or anti-HEV IgM, representing only 0,036% of the current Italian population [23]. The seroprevalence rates ranged from 0,12% to 49% among the study cohort [24,25]. The Abruzzi region was found to be a hyper-endemic region with a seroprevalence rate of 49% among blood donors [25]. A seroprevalence study of 132 blood donor residents in Tuscany has reported rates of 9.1% [26], which is similar to the 9% rate of the same cohort of individuals in the Latium region in 2009 (further data not shown). [25]. A seroprevalence of 9% was also reported in the general population of Abbiategrasso, in Lombardy; the highest assessed among the northern regions of Italy [27]. Conversely, the lowest seroprevalence of 1.3% and 2.7% was reported in Piedmont and Apulia among the open population, respectively in the north and south of Italy [28,29]. The highest rate among the southern regions was reported in Calabria (Casanova) with a seroprevalence of 17.8% [27]. Based on the data on age reported by 17 of the 27 studies, we calculated a mean age of 42.28 years for the HEV positive subjects. Moreover, 59,26% were males, according to the information provided by 21 studies. Overall, the seroprevalence increased in association with age and no relevant variation related to gender has emerged from any study. However, only one pediatric study with a prevalence of 0.4% was found in Molise [30].

The study included: 10.527 individuals from the general population cohort, 2776 blood donors, 352 pregnant woman, 264 individuals at pediatric age, 2.609 patients affected by acute hepatitis, 800 individuals in hemodialysis, 118 renal transplant recipients, 430 chronic liver disease patients, 371 at zoonotic risk workers, 510 immigrants and 3.125 at high-risk individuals (100 HIV infected and 1116 with sexual or occupational exposure, 936 intravenous drug users and 973 prisoners).

The selected articles were from 13 different regional areas: Abruzzi (n = 1), Apulia (n = 2), Calabria (n = 2), Latium (n = 2), Lombardy (n = 3), Marche (n = 1), Molise (n = 1), Molise (n = 1), Piedmont (1), Sardinia (n = 1), Sicily (n = 3), Republic of San Marino (n = 1), Tuscany (n = 1), and Veneto (n = 2) (Fig. 2).

The remaining 7 studies provided information at a national level only. Over three decades, 169 cases of hepatitis E were linked to travel in high endemic countries. Nearly 90% of them occurred in travelers returning from Bangladesh, India and Pakistan. The remaining cases were diagnosed in patients who traveled in Angola, Somalia, Morocco and Green Cape. Secondary and intra-familiar infection has been described by two studies with a rate of 2.6% and 4.5% respectively [31,32]. Generally, a higher probability to be positive for anti-HEV antibodies has been associated with immigrants by the different studies focusing on socio-economic and demographic variables (healthy population, prisoners and at-risk categories). Genotype 1 (G1), subtype 1a and 1c, has been isolated in all imported cases of HEV. Genotype 3 (G3), subtype 3e, 3f and 3 h, has been associated with a local source of infection. No significant differences in the clinical course of the disease caused by the G1 and G3 subtypes have been observed in immunocompetent individuals. Potential risk factors for HEV transmission included poor sanitation, person to person contact, family with more than 4 members, parenteral blood contact, male to male contact, professional long exposure with zoonotic reservoirs and raw and undercooked pork meat and shellfish.

In order to assess the seroprevalence of hepatitis E, different enzyme immunoassays (EIA) have been used to detect class G and M immunoglobulins against HEV. The commercially available assays were based on two methodologies: ELISA and Western Blot. The majority of studies (22 over 27) used one or more Elisa assays [25–28,30–47], while in 5 articles were used both serological assay types [24,29,48–50]. HEV RNA was detected if samples were positive for IgM and/or IgG in 12 of the selected studies [25,28,29,32,36,37,39,42,44,46,47,50].

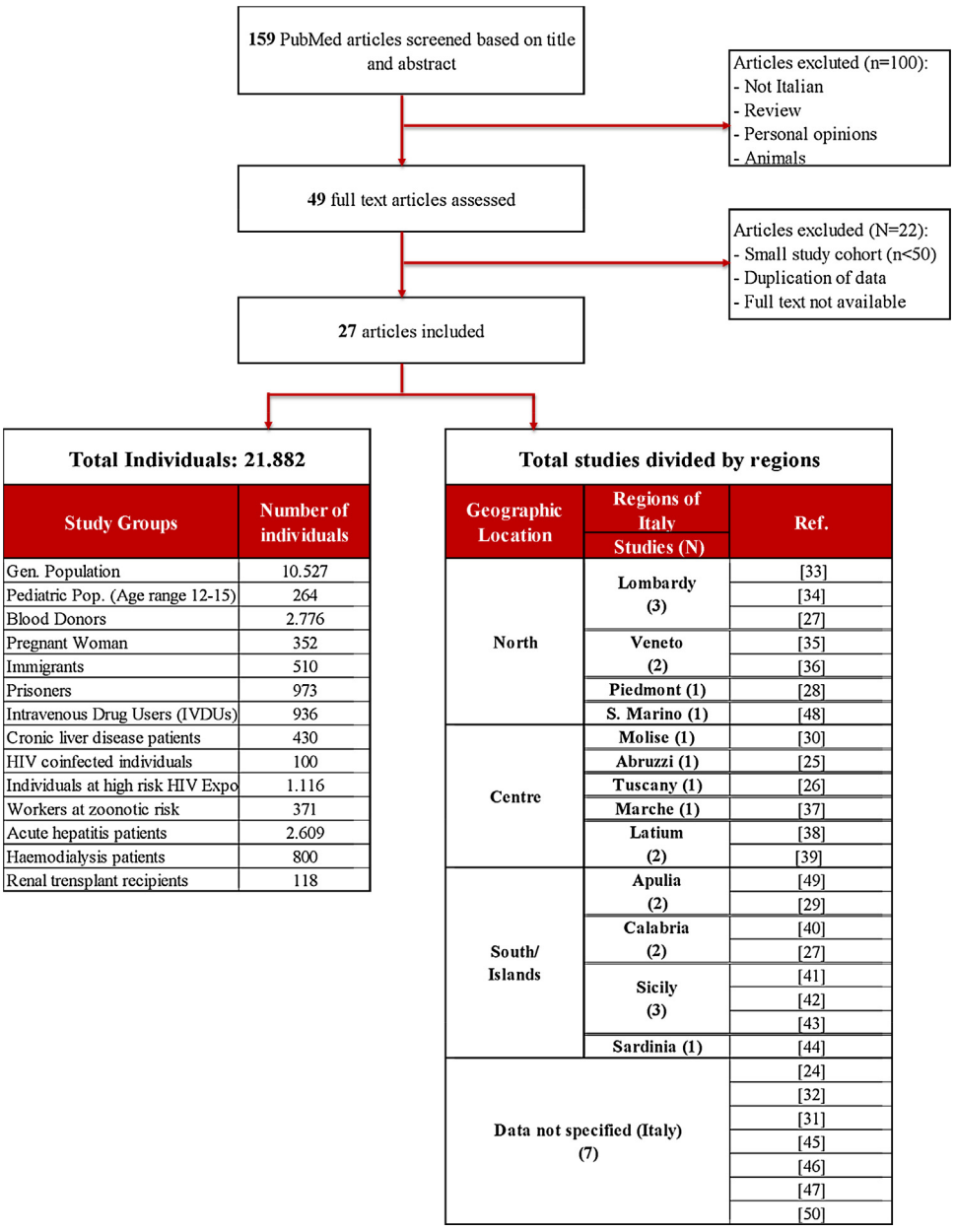


**Fig. 1.** Protocol utilized to select articles for the review.

**Table 1**
Comparative sensitivity of each commercial assay by regions and study population.

| Assay | Regions | Study population (N) | Anti-HEV | | HEV-RNA (%) |
|---|---|---|---|---|---|
| | | | IgG (%) | IgM (%) | |
| Assay-1 | [a]Calabria | GP (876) | [b]17.8% | – | – |
| | [a]Lombardy | GP (2.489) | [b]9.0% | | – |
| | Sardinia | BD (402) | 5.0% | – | – |
| | Sicilia | GP (44) | 4.7% | – | – |
| | Apulia | GP (450) | 2.7% | 0.22% | 0.22% |
| | | BD (151) | 1.3% | – | – |
| Assay-2 | Veneto | GP (1.889) | 2.6% | | |
| | Republic of S. Marino | GP (2.233) | 1.5% | – | – |
| | Molise | Ped (264) | 0.4% | – | – |
| Assay-3 | Abruzzi | BD (313) | [b]49.0% | 0.6% | 0.6% |
| | Piedmont | GP (73) | 1.3% | – | – |
| Assay-4 | Tuscany | BD (132) | 9.1% | – | – |

GP: general population; BD: blood donors; Ped: pediatric study.
[a] Data from the same study [27].
[b] Seroprevalence rate with lower positive value at C.I. 95%.

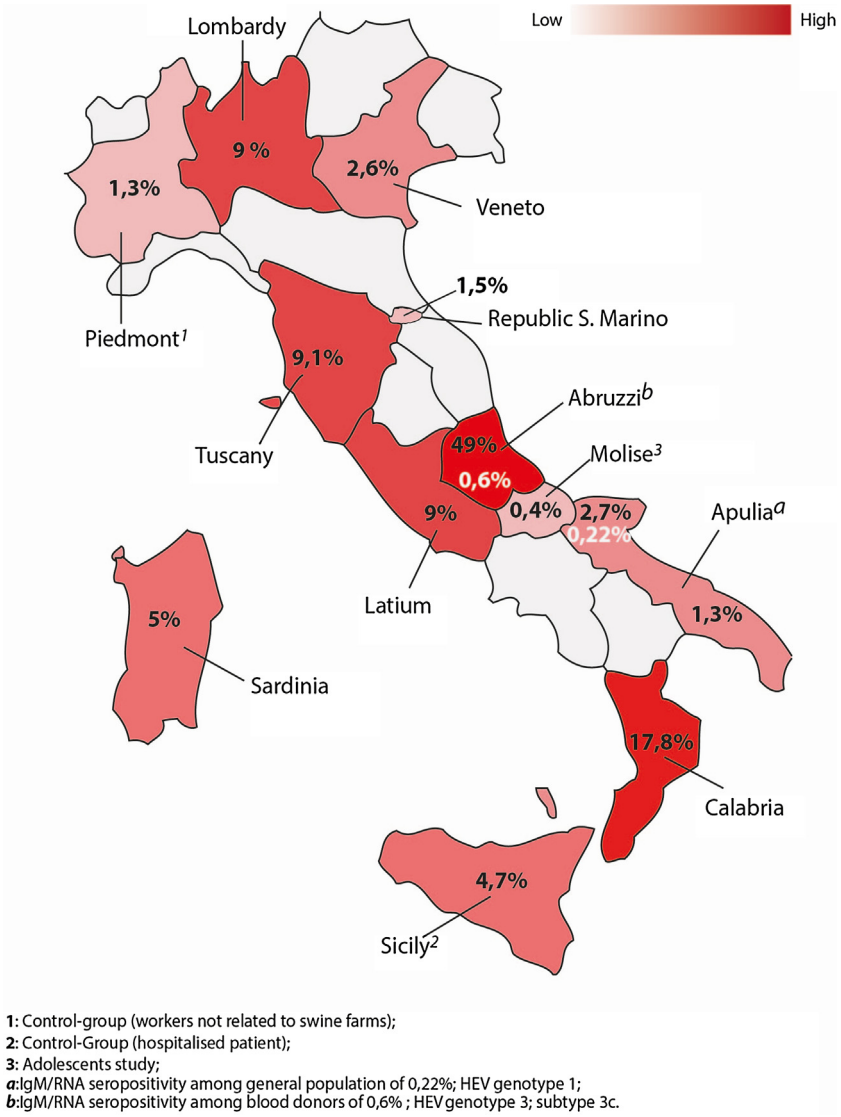


**Fig. 2.** Map of Italy showing different HEV seropositivity rates among blood donors and general population. .

Table 1 shows the reported anti-HEV IgG seroprevalence according to the assay used in the Italian regions. Assay 1 (Dia.pro) was used in the majority of studies and the reported seroprevalence varied from 17.8% in Calabria to 2.7% and 1.3% in Apulia, respectively found in general population and blood donors [27,29]. Assay-2 (Abbott) was used in three regions to detect the anti-HEV IgG in two studies among the general population (seroprevalence ranging from 2.6% and 1.5%) and in a pediatric study (seroprevalence 0.4%) in three regions [35,48,30]. The highest and the lowest seroprevalence in general population were both detected by assay-3 (Wantai), respectively in Abruzzi (49%) and in Piedmont (1.3%) [25,28]. Both the Abruzzi and Apulia studies have provided data on anti-HEV IgM and HEV RNA with an overall prevalence ranging from 0.6% to 0.22%, among blood donors and general population, respectively. In the former study the genotype found was 3 while in the latter study was 1 [25,29]. A recent study on Italian blood donors has compared the diagnostic performances of assay-1 and assay-3, demonstrating an overall concordance of 96%. Even thought assay-3 detected a slightly lower positivity rate than assay-1, no significant difference in sensitivity was observed [47]. However, our Bayesian analysis of the reported seroprevalence rates, among Italian regions, has shown that the screening methodology was adequate in term of specificity only in two studies. Thus, the estimated HEV prevalence in Abruzzi, Calabria and Lombardy reflects the real spreading of infection [25,27].

## Discussion

To the best of our knowledge, the current article represents the first critical review of HEV IgG seroprevalence in Italy. The sera samples of 21.882 individuals were collected from 1978 to 2015 as reported in the 27 publications included in the final analysis. The seroprevalence ranged from 0.12% to 49% with the highest rates being in the central region. However, a great geographical variability was observed among the Italian regions with a average prevalence of 10,25% among blood donors. These findings lay the groundwork for the hypothesis that diverse predisposing factors such as dietary habits, environmental characteristics, different distributions of zoonotic reservoirs, contaminated surface waters, the socio-economic and hygienic level, would sustain the spreading of HEV infection along the Italian regions interdependently.

The majority of autochthonous cases of HEV in Italy seem to be due to the zoonotic transmission of the infection from domestic and wild animals. To date, HEV infections have been demonstrated in domestic pig, wild boars, rabbits, wild dears and goats in Italy [51–55]. Not surprisingly, nearly half of the wild boars in the Latium

**Table 2**
Laboratory diagnosis of HEV infection: Anti-HEV IgG Assays.

| IgG assay | Assay type | Antigen for coating | Strain | Sensitivity | Specificity |
|---|---|---|---|---|---|
| Abbott [91] | – | Recombinant ORF-2 and ORF-3 proteins | Burmese | 91% | 96% |
| Adaltis (EIAgen) [92] | Qualitative indirect | Synthetic antigens from ORF-2 and ORF-3 | – | 80.% | 62.9% |
| Dia.Pro [93] | Qualitative indirect | 4 synthetic peptides with conservative epitopes of ORF-2 and ORF-3 Genotypes 1, 2, 3, and 4 | Burmese and Mexican | 98% | 96% |
| DSI [93] | – | Recombinant ORF-2 and ORF-3 peptides Genotype 1,2 and 3 | – | 72% | 99% |
| MP Diagnostics [95] | – | 3 recombinant ORF-2 proteins and 33-amino acid sequence from ORF-3. Genotype 2,3 | – | 98% | 97% |
| Wantai [94] | Qualitative indirect | Recombinant ORF-2 protein (PE2) Genotype 1 | Chinese | 99,80% | 99.9% |
| W. Blot (Mikrogen) [93] | Quantitative indirect | 4 recombinant proteins (O2 N, O2 M and O2) from ORF-2 and ORF-3 Genotype 1,3 | – | 62% | 99% |

region and in Tuscany (central Apennines area), had serological markers of HEV infection [56,57]. A similar high seroprevalence was demonstrated among swine in Southern (Calabria) and Northern Italy but was remarkably low Piedmont region [58–61]. Moreover, in Northwestern Italy (Lombardy and Emilia Romagna), HEV contamination was demonstrated in the majority of slurry samples from pig production facilities [62]. In addition, the majority of indigenous cases of acute HEV were clearly related to the ingestion of raw or undercooked local pork meat [37]. HEV contamination has been assessed in pork meat-derived products and in the Italian production chain. Interestingly, a high nucleotide homology has been proven in human, swine and contaminated food samples from the same geographical regions and, as expected, intermixed swine and human genomic sequences were found [63–66]. In fact, the inadequate cooking of commercially available pork products does not inactivate effectively HEV infectivity [67]. In order to prevent food-borne HEV infection, consuming these products cooked at a temperature of 71 °C for a minimum of 20 min, has been experimentally proven to be necessary [68]. Moreover, HEV has been detected in bagged ready-to-eat (RTE) vegetables, posing a further concern regarding food safety and new potential consumers' risks [69]. Genotype 3 appears to occur in the majority of the locally acquired acute HEV cases, both in human and zoonotic reservoirs. However, genotype 4 has been recently reported as an emerging indigenous pathogen in Italy as well in France and Germany, and it seems no longer confined to Japan, China and Southeastern Asia [70,71]. In fact, a small outbreak was reported in the Latium region, in 2011. The isolated strain differed genetically from the identified European 4d and 4f strains and it resembled the subtype 4d strain isolated in China among the swine population [18]. Furthermore, a genotype 4 strain, phylogenically related to the human strain isolated during the outbreak in central Italy, was identified in swine farms in Northern Italy and provided further evidence of a plausible cross-species infection and introduction of a new HEV variant in a different geographic region [72].

Although the ingestion of shellfish has been reported since 1980 as a risk factor in our cohort of patients with HEV, only lately has its role been assessed as an indicator of marine pollution [41]. The bivalve molluscan shellfish samples were analyzed in independent studies in Italy, France, Spain and Denmark for their ability to concentrate the viral particles filtrated during the feeding process [73–76]. However, all the aforementioned studies did not support the contamination of the marine environment. No positive samples collected in the potentially contaminated sites were found. This result could have been caused by either an undetectable quantity of viral particles or a short-lived environmental persistence of HEV. Nevertheless, a recent study in Shandong Province in China assessed the seroprevalence among 1028 seafood-processing workers of whom 22.20% were anti-HEV IgG antibody positive. The increase in seroprevalence was associated with working-time, thus to a higher likelihood to being exposed to contaminated raw seafood and semi-finished products [77]. Interestingly, time of exposure was the only independent variable linked with a higher anti-HEV prevalence found in our cohort of workers at zoonotic risk [28].

The waterborne route of infection has been largely recognized globally. Still, its epidemiological impact in the industrialized countries is unknown. However, HEV particles have been traced in the Latium region and in the Tiber River that runs from the central Apennines region to the Tyrrhenian Sea, in Italy [78]. Overall, these findings suggest that different factors could determine the endemicity we observed in Italy and thus the need for further investigation.

A recent seroepidemiological study compared a group of residents in two different regions: Lombardy (Abbiategrasso, Milan) and Calabria (Cittanova); reporting a twofold increased HEV prevalence in in the southern region [27]. The authors have explained the difference observed, as likely consequence of the lowest socioeconomic and hygienic/sanitary conditions in Calabria. Since only the Dia.pro essay was used to determine HEV IgG positive, the result might to reflect the real spreading of HEV, with a north-to-south gradient. According to this finding, the highest prevalence observed among blood donors in Abruzzi region might be a consequence of the inadequate sanitation and poor hygienic conditions that followed the devastating earthquake that struck L'Aquila in 2009, causing over 80 000 evacuated from their homes, As matter of the fact, an increase of enteric transmitted diseases was reported subsequently to the catastrophic environmental and geological changes [79]. Moreover, the high prevalence rate in the Abruzzi region is likely due in part to the highly sensitive Wantai assay used [80]. Both the reported IgM and RNA seroprevalence among blood donors in Abruzzi region was 0.6% [25].

Furthermore, we found a similar seroprevalence rate among volunteer blood donors and the general population. This finding ought to denote that this specific cohort could represent the prevalence in the Italian population, in general [24,40]. Moreover, we observed that in the majority of cases HEV infection was asymptomatic, anicteric and self-limiting and a normal level of transaminases has been also reported. On the other hand, this implies that the biochemical and serological screening currently performed; in order to select the healthy blood donors may be unable to identity viremic donors. Indeed, viremic blood donors with a normal ALT level have been reported in Germany and Japan [81,82]. HEV RNA has been detected in blood donations and cases of transfusion-transmitted infection have been reported worldwide [83–87]. The contami-

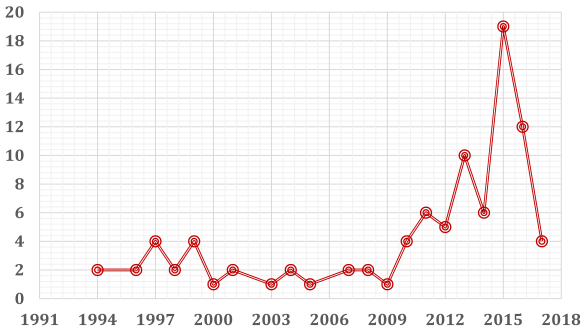


**Fig. 3.** Number of HEV studies in Italy over two and one-half decades.

nated blood could have an under-recognized role as a potential new source of infection and it requires further investigations in Italy.

We were unable to provide a clear understanding of the potential impact of the immigration phenomenon among the Italian population since only two studies, in the same region, were included in the final analysis [29,49]. However, according to a recent retrospective study on a small cohort of symptomatic migrants, hepatitis E appeared to be the main cause of acute viral hepatitis [88]. Nevertheless, 5% of 40 fecal samples, from asymptomatic immigrants were positive for HEV RNA supporting a plausible role of immigrants as " symptom-free HEV carriers" [89].

The current study suggests the existence of a great variability in the seroprevalence of HEV in Italy. This result could be partially explained by the heterogeneity in the sensitivity and specificity of the immunoassays used, by small number of studies included as well as the number of samples tested; and the narrow window of collecting samples period. All samples from the general population and the blood donors have been analyzed with four commercially available ELISA test: Dia.pro, Abbott, Wantai, Adaltis. Performance comparison among different assays was reported by a recent study, which showed a very good concordance between the Dia.pro and Wantai assay [47].

The global burden of hepatitis E is still underestimated due to the sub-optimal commercial assays available [90].

In fact, serological assays based on different genotypes, using recombinant proteins or synthetic peptides, vary greatly in term of sensitivity and specificity (Table 2) [91–95]. Moreover, the assay sensitivity is higher in symptomatic cases than in the asymptomatic ones [91]. Nevertheless, the specificity of the screening methodology, to obtain valid value of HEV prevalence, differ according to the infection endemicity. However, these limitations empathize the necessity of a comparably standard seroprevalence study at a national level, in order to estimate the real prevalence of Hepatitis E and to create an interventional plan directed at a regional level. Clearly, different dietary habits can't alone determine the variability observed in our studies.

## Conclusion

The World Health Organization defines as an emerging zoonosis any disease that is "newly recognized or newly evolved, or has shown an increase in incidence or expansion in geographical, host or vector range" [96]. We do not know whether HEV is truly an emerging infection or whether it is due to an increased awareness and understanding of HEV in the Western countries (Fig. 3).

Although a phylogenetic and evolutionary analysis has stated that HEV might have been present in the Italian territory since the early 90s, nowadays it remains a silent and understudied entity [66]. At the present, HEV is undoubtedly endemic in Italy. However, the lack of commercially approved diagnostic assays [95], the need for correct timing of the detection of HEV and the absence of standard treatment have all made HEV one of the most unrecognized infectious diseases. HEV should always be considered in the differential diagnosis of acute viral hepatitis.

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## Competing interests

None declared.

## Ethical approval

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