Le Infezioni in Medicina, n. 1, 70-78, 2023
doi: 10.53854/liim-3101-10

Prevalence of HEV infection in acute non-ABC hepatitis and prognostic role of extrahepatic manifestations

Giovanna Picchi[1], Alessandra Ricciardi[2], Lara Marola[3], Monica Di Norcia[1], Margherita D'alessandro[4], Rinalda Mariani[4], Pierluigi Cacciatore[5], Federica Sozio[5], Stefano Necozione[3], Vincenza Cofini[3], Giustino Parruti[5], Umbertina Villano[6], Elisabetta Madonna[6], Cinzia Marcantonio[6], Roberto Bruni[6], Elida Mataj[7], Alessandro Grimaldi[1], Anna Rita Ciccaglione[6]

[1]Infectious Diseases Department, Ospedale Regionale "San Salvatore", L'Aquila, Italy;
[2]Infectious Diseases Department, IRCCS Fondazione Policlinico "San Matteo", Pavia, Italy;
[3]Department of Clinical Medicine, Life, Health & Environmental Sciences-MESVA, University of L'Aquila, Italy;
[4]Infectious Disease Department, Ospedale Civile "SS. Nicola e Filippo", Avezzano, Italy;
[5] Infectious Disease Department, Ospedale "S. Spirito", Pescara, Italy;
[6]Viral Hepatitis and Oncovirus and Retrovirus Diseases Unit, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy;
[7]Instituti i Shendetit Publik (ISHP), Tirana, Albania

Article received 8 December 2022, accepted 8 February 2023

SUMMARY

Background: HEV-3 and HEV-4 are emerging cause of zoonotic acute hepatitis in high-income countries. In Europe the disease is underdiagnosed but hyperendemic areas have been identified. We describe a population with acute non-ABC (n-ABC) hepatitis in Abruzzo, the Italian region with the highest seroprevalence reported. The study was included in the surveillance of acute hepatitis E by the Italian Institute of Public Health started in 2004 and implemented in 2015.
Methods: Patients with n-ABC hepatitis during 2004-2018 in all Abruzzo Infectious Disease Departments were tested for HEV-IgM (Wantai®) and HEV-RNA (ORF3). Positive samples were sequenced (Beckman Coulter®) and phylogenetic tree (MEGA 6.06 software) obtained. Clinical data were retrospectively collected and an alimentary risk factors-questionnaire was administered. Categorical and quantitative variables were compared (Chi square test or Fisher test and Wilcoxon test).
Results: 97 hospitalized patients were tested, most cases (91.7%) after 2015. Overall, HEV-IgM resulted positive in 36% and HEV-RNA detectable in 33.3%. All 24 sequences obtained were HEV-3, with two small groups of closely related strands. L'Aquila was the Province with higher positivity rate (44%). Retrospective clinical data were acquired in 86.5% of patients, no one having liver failure. Higher ALT-levels (1282.34 vs 893.25, p=0.0139) and extrahepatic symptoms (OR 16.69, p=0.0018) were strongly associated with HEV-IgM presence. Two small outbreaks are described.
Conclusions: More than one third of n-ABC hepatitis in all Abruzzo are HEV-related. Extrahepatic symptoms correlate with HEV aetiology. Implementing surveillance is mandatory to really understand the extent of the disease.

Keywords: Acute hepatitis, extrahepatic manifestations, hepatitis E virus, viral zoonosis, HEV hyperendemic area.

INTRODUCTION

Hepatitis E virus (HEV) is a virus of Hepeviridae family and is one of the most common cause of acute viral hepatitis worldwide. So far, 8 different genotypes of HEV have been discovered and, of these, HEV-1, -2, -3 and -4 play a major role

Corresponding author
Giovanna Picchi
E-mail: giovanna.picchi.inf@gmail.com

in human pathology. HEV was previously considered only as a waterborne agent, with oro-faecal transmission and large diffusion in low-income settings with poor hygienic standards. However, in the last decade HEV-3 and HEV-4 have been increasingly recognized as cause of zoonotic hepatitis in high-income countries including Europe, where at least two million of locally acquired HEV infections every year have been estimated [1-3].
Serologic studies showed the existence of specific European "hot-spots", where HEV is hyperendemic, and Abruzzo-Central Italy is one of these, with a reported seroprevalence of more than 30% in healthy blood-donors [4, 5]. The reason of this peculiar distribution is still unclear but it is probably linked to consumption of kind of raw sausages, considering that HEV-3 and HEV-4 are largely hosted by pigs and wild boars, and that there are molecular, epidemiological and serological evidence supporting its porcine origin [6-8].
Despite the emerging role of HEV in Europe as etiologic factor in acute hepatitis, physicians' perception is still low and the infection is largely underdiagnosed. Some retrospective studies suggest a frequent misinterpretation with drug-related injury (DILI) [9-11].
Therefore, there is a scarce characterization of the clinical picture, which could be indistinguishable from other acute hepatitis. Apart from HEV-1 pregnant women infection, HEV hepatitis is considered a self-limiting disease in immunocompetent patients, despite increased severity was observed in older male, diabetics, alcoholics and in chronic liver disease carriers, in which an acute liver injury (ALI) or an acute-on chronic liver failure (ACLF) can occur [12]. Chronicization and rapid evolution in decompensated cirrhosis have been described in immunocompromised patients.
HEV infection has a high rate of extrahepatic symptoms that may divert clinicians from the hepatic origin of the disease. Extrahepatic manifestations in HEV are frequent and well characterized, especially those regarding the peripheral nervous system like Guillain-Barrè Syndrome and neuralgic amyotrophy, and according to some authors can be more frequent than the hepatitis itself [13].
In this study, we describe the clinical and virological features of a population with acute hepatitis of unknown origin in a HEV hyperendemic area during the period 2008-2018. The study was included in the surveillance of acute hepatitis E by the Italian Institute of Public Health, Department of Infectious Diseases, Unit of Viral Hepatitis.

PATIENTS AND METHODS

Starting from 2004, the Unit of Viral Hepatitis of Italian Institute of Public Health, initiated an HEV Surveillance Project, consisting in performing diagnostic tests on collected blood samples of patients with suspected HEV-acute hepatitis. In 2015 this program was implemented and promoted in the region of Abruzzo, Central Italy, considering the evidence of a very high seroprevalence in this Region [14]. This is a retrospective, multi-centric, clinical study collecting cases of hospitalized acute non-ABC hepatitis occurred in Abruzzo during the period 2008-2018. The 6 participating Centres represent all the Infectious Diseases Departments of the 4 Abruzzo's Provinces (L'Aquila, Pescara, Teramo, Chieti). All serologic and virologic tests were performed at Laboratory of Italian Institute of Public Health, Unit of Viral Hepatitis in Rome.
Acute non-ABC hepatitis was defined as increased serum transaminase (Aspartate Transaminase - AST or Alanine Transaminase - ALT) in patients without evidence of HAV, HBV, HCV. Minor viral hepatitis related to CMV or HSV infections were also excluded.
All patients underwent a specific questionnaire provided by the Italian Society of Infectious and Tropical Diseases (SIMIT), in which the following data were collected: age, sex, nationality, work, animal contact, travel, consumption of raw and liver sausages in the last two months, residence in a rural area, hunting, blood transfusion, drug abuse. For each patient a blood sample was obtained, centrifuged with serum recovery, stored at minus 80°C and later sent refrigerated to ISS. Here a serologic test (Wantai®, Biologic Pharmacy Enterprise, Beijing, People's Republic of China) was performed. All IgM positive samples were tested for HEV-RNA using QiAamp MinElute Virus Spin kit (QIAGEN®Hilden, Germany) for extraction and RealStar HEV RT-PCR kit 1.0 (Altona Diagnostics GmbH®, Hamburg, Germany) for amplification. All samples with detectable HEV-RNA were later tested for genotype (Beckman Coulter®, Inc., Fullerton, CA).
Clinical data regarding onset and type of symptoms, comorbidities and sequelae were retrospec-

tively collected and reviewed. Symptoms were classified in "hepatic", defined as gastroenteric symptoms, jaundice or sub-jaundice, and "extra-hepatic", defined as neurologic, not abdominal pain, others.
Acute HEV hepatitis was diagnosed in all patients with HEV IgM positive serology, HEV IgG positive or negative and HEV-RNA detectable or undetectable. Basing on serology results, patients were allocated in two groups (acute HEV patients and other n-ABC hepatitis), and their available features (age, sex, job, comorbidities, plasmatic liver enzymes and cholestasis index, clinical presentation, risk factors) were analysed. Categorical variables were compared by Chi square test or Fisher test, while quantitative data were compared using Wilcoxon test, for two independent samples. All analyses were performed with SAS software setting p-value <0.05.

RESULTS

From January 2004 to September 2018, 97 patients with acute hepatitis n-ABC were tested for HEV in all the Infectious Disease Departments of Abruzzo. The highest number of patients evaluated were from L'Aquila Province (52%, including L'Aquila and Avezzano) followed by Pescara (41%), while Chieti Province (including Chieti and Vasto) and Teramo accounted for 7% together. The large majority of samples (91,7%) were collected in the period 2015-2018, after Surveillance Program implementation. The year with the highest number of specimens sent (49) was 2017.
A diagnosis of acute HEV hepatitis was established in 35 out of 97 patients (36%): 23 out of 35 were HEV-IgM-IgG positive, 12 out of 35 were HEV IgM positive and IgG negative. In 32 out of 35 IgM-positive patients (33.3% overall) HEV-RNA were detectable with a HEV-RNA median value of 10.000 cp/mL (range <100 and >=100.000 cp/mL). Of 32 HEV-RNA positive patients, 70% had a viral load greater than 3 Log and 53.3% greater than 5 Log. In two HEV-IgM-positive with HEV-RNA-undetectable patients, a suboptimal quality of sample storage associated with a long interval from the collection was supposed; in another case the test was not executable because of plasmatic factors interference. In all HEV-RNA positive samples the genotypic sequencing was performed and obtained in 75% of these (24 patients); all were HEV-3, and subtypes found were 3c, 3f, 3e, 3e/3f. For 8 samples in which sequencing was not successful, low HEV-RNA levels were probably implicated. Two small groups of closely related sequences (98.2%-100%) were identified: 5 cases of 3c subtype in patients from 4 nearby towns and 4 cases of 3f subtype in patients from L'Aquila city.
Twelve patients (12%) were HEV IgG+ and IgM negative, serology compatible with previous HEV infection. In 45 patients (46% overall) serology was fully negative. In 5 patients IgG were not executable. Figure 1 summarizes the results of serologic tests.
The HEV-IgM positivity proportions differ widely among Provinces: 44% in L'Aquila, 34.3% in Pescara, 20% in Chieti; no acute HEV-infections were found in Teramo. The positivity rate varies also widely in the different years. Figure 2 shows

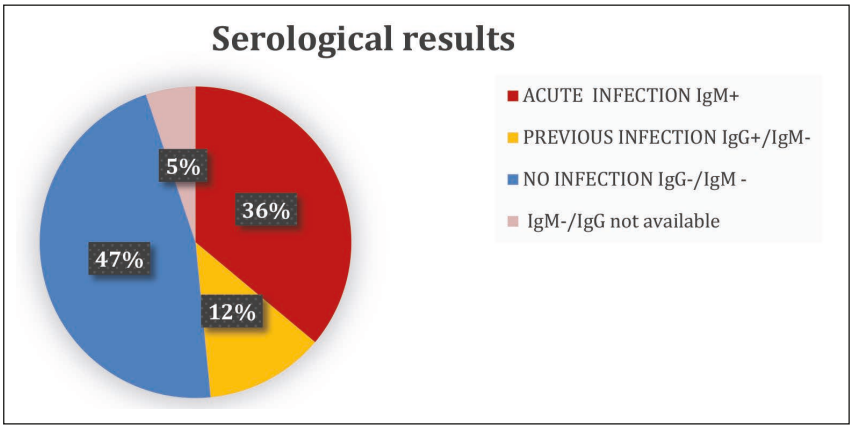

Figure 1 - Patients classification according to serologic tests.

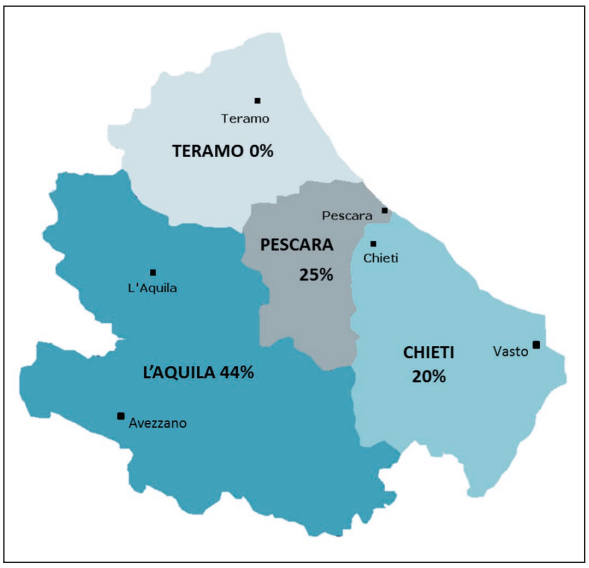

Figure 2 - Map of Abruzzo region with HEV positivity rate for Provinces. Squares represent the Infectious Disease Centers.

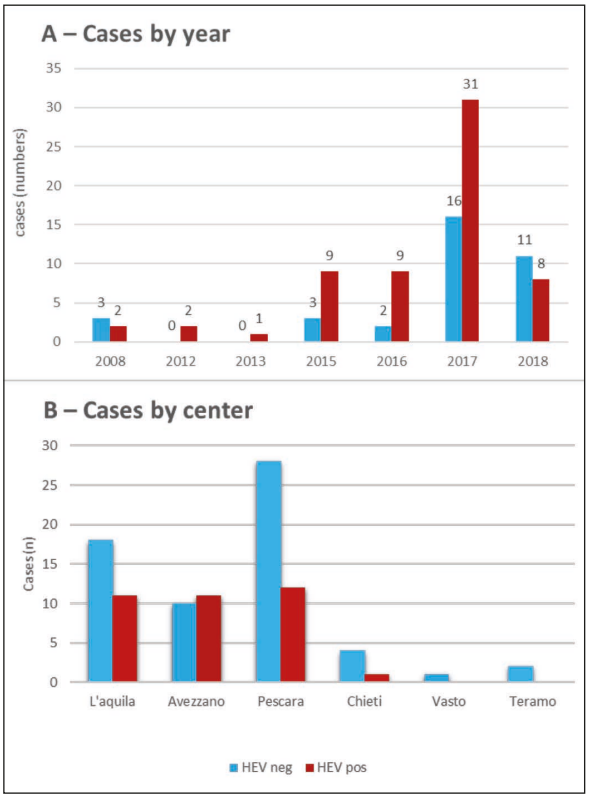

Figure 3 - HEV positive and negative cases by Year (A) and Infectious Diseases Center (B).

positivity rates for Province. Figure 3 describes positive and negative cases by Infectious Diseases Center and year.

The retrospective collection of clinical data was obtained for 86.5% of the patients.

Overall, symptoms observed were: asthenia, fever, sweating, abdominal pain, nausea, vomit, anorexia, diarrhoea, cervical, chest or shoulder pain, diffuse or localized arthralgia and myalgia, hyposthenia, paraesthesia, vertigo, skin lesions.

Time of hepatic symptoms resolution for acute HEV hepatitis varied between 2 and 14 days (data not shown), with slower liver enzymes normalization of approximatively few weeks. No patient had hepatic decompensation or needing for antiviral therapy. Interestingly, almost all patients with extrahepatic symptoms of acute HEV hepatitis exhibited thoracic and/or arms pain or generic arthralgia, sometimes with paraesthesia. For 3 patients a diagnosis of neuralgic amyotrophia was confirmed, while for other 3 electromyography showed signs of sensitive neuropathy, and one patient presenting with vertigo had a clinical diagnosis of vestibular neuritis. All these patients resolved symptoms in medium-long term, except one who had stable chronic arthralgia. Two of these patients had also concomitant diagnosis: one of autoimmune thyroiditis, in which is not possible to exclude a trigger role of HEV, and one of tachy-brady-syndrome, for which a connection is unlikely.

Table 1 summarizes the characteristics of all patients with acute hepatitis n-ABC, divided into patients with acute HEV hepatitis and patients without evidence of HEV (acute hepatitis n-ABCE), and statistical analysis. A specific regression analysis for clinical features was not applicable for every parameter but showed elevated odds ratios for extrahepatic symptoms (OR 16.69 [1.96-141] IC 95%, p=0.0018) and neurologic symptoms (OR 14 [1.62-120.4 IC 95%] p=0.0047, data not shown).

Figure 4 describes the virological, clinical and geographical features of the two small outbreaks of closely related 3f and 3c subtypes observed.

DISCUSSION

Despite in recently published metanalysis Italy and all Mediterranean countries experienced several hepatitis HEV outbreaks, the number of illnesses reported were surely affected by lack of

Table 1 - General and clinical features of all patients, of Acute HEV patients and of Non ABCE patients.

	N	*All patients*	*Acute HEV patients*	*N ABCE patients*	*P value**
Male n (%)	95	71 (73.20%)	30 (85.71%)	41 (66.1)	P=0.0861
Age (years) *Mean (SD)* *Median (range)*	88	50 (16.01) 52 (20-80)	53 (13.66) 52 (31-80)	49 (17.08) 50 (20-80)	P=0.1845
Farming job n (%)	96	3 (3.13 %)	1 (2.86%)	2 (3.28%)	P=0.4483
Italian nationality n (%)	97	89 (91.75%)	33 (94.29%)	56 (90.32%)	P=7069
Comorbidities n (%)	82	29 (35.37%)	14 (45.16%)	15 (29.41%)	p=0.1618
Immunitary deficit n (%)	81	2 (2.47%)	1 (3.23%)	1 (2%)	P=0.4784
AST (UI/l) *Mean (SD)* *Median (range)*	82	701.23 (730.25) 374.5 (18-2936)	772.16 (789.03 419 (54-2936)	658.12 (696.67) 341 (18-2827)	P=0.547
ALT (UI/l) *mean (SD)-* *median (range)*	84	1041.48 (1004.50) 813.5 (57-4721)	1282.34 (959.58) 1168.50 (95-4312)	893.25 (1011.75) 452 (57-4721)	*P=0.0139*
Total Bilirubin (mg/dl) *mean (SD)* *median (range)*	78	5.12 (6.76) 2.26 (0.4-30.4)	5.08 (6.75) 2.70 (0.44-29.90)	5.15 (6.83) 2 (0.40-30.40)	P=0.6905
GGT (UI/l) *mean (SD)* *median (range)*	76	383.64 (337.30) 340 (16-1852)	322.52 (233.04) 346 (16-922)	417.33 (380.91) (16-852)	P= 0.5219
ALP (UI/l) *mean (SD)* *median (range)*	67	265.82 (199.05) 200 (70-1125)	293.56 (249.69) 218 (70-1125)	249.31 (170.65) 190 (70-826)	p= 0.7311
Risk factors					
Animal contact	82	45 (54.88%)	17 (58.62%)	28 (52.83%)	p=0.6494
Gardening	80	22 (27.50%)	9 (31.03%)	13 (25.49%)	P=0.6115
Beetroot	77	16 (20.78%)	7 (25.00%)	9 (18.37%)	P=0.5641
Pork sausages	81	57 (70.37%)	23 (76.67%)	34 (66.67%)	P=0.4515
Liver pork sausages	77	31 (40.26%)	15 (53.57%)	16 (32.65%)	P=0.0927
Liver boar sausages	72	9 (12.50%)	4 (14.81%)	5 (11.11%)	p=0.7201
Vegetables	77	27 (35.06%)	10 (35.71%)	17 (34.69%)	P=1.0000
Fruits	77	23 (29.87%)	8 (29.63%)	15 (30.00%)	P=1.0000
Travel	87	8 (9.20%)	3 (9.68%)	5 (8.93%)	P=1.0000
CLINICAL PRESENTATION					
Gastroenteric/Hepatic symptoms	79	31 (39.24%)	10 (33.3%)	21 (42.86%)	p=0.4798
Jaundice/sub-jaundice	80	34 (42.5%)	14 (46.67%)	20 (40.0%)	p=0.6427
Extrahepatic symptoms	80	9 (11.25%)	8 (25.81%)	1 (2.04%)	*p=0.0018*
Neurologic symptoms	80	8 (10.00%)	7 (22.58%)	1 (2.04%)	*p=0.0047*
Not abdominal pain	78	7 (8.97%)	7 (23.33%)	0 (0.00%)	NA

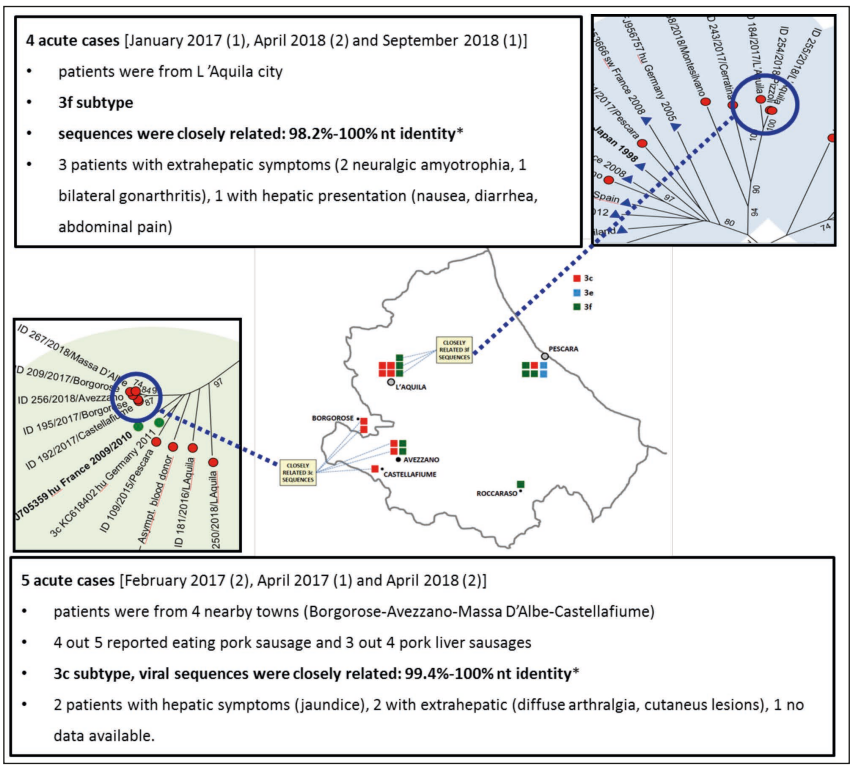

Figure 4 - Description of 2 small outbreaks occurred in 2017-2018.

aggressive investigation and rigorous case finding [15]. As a large collection of acute hepatitis of possible HEV origin, our study showed how HEV hepatitis may be significant as a health problem in susceptible adult population of high-income countries. Considering Abruzzo region, more than one third of acute hepatitis n-ABC are caused by HEV and all cases are autochthonous as proven by the HEV-3 genotype. Cases were collected from all Infectious Diseases Centers, describing completely the regional situation. Epidemiological differences between areas are evident and confirm that, as evidenced by repeated outbreak and seroprevalence study (15, 16), L'Aquila Province is a hyper endemic area that needs to be constantly monitored, with the highest positivity rates in a contest of high number of specimens sent.

In this contest, the surveillance program and the high grade of suspicion by clinicians in order to correctly diagnose this disease are essential. Considering only the 2015-2018 period, there is however a growing trend in positive cases over the first three years, in the absence of documented clusters and despite of a stable number of specimens sent. This trend is confirmed in Italy by the Epidemiological System of Acute Hepatitis bulletin [17]. A possible role of small outbreaks must be considered.

The HEV 3 genotype and subtypes found are in line with those of De Sabato L. et al in Central Italy (Lazio), who showed that the high circulation of these subtypes in food may act as active carrier and may be vehicles of transmission in humans among countries; further evidence comes from the paper published by Garbuglia in 2019 in the same area, in which the introduction of new strain harboured by pork products or live animals imported from outside, in a population with high consumption of raw meat, may cause large and potentially threatening outbreaks [12, 14, 18].

As in similar case collections, patients with acute HEV patients are more frequently males (85.71%), aged nearly 50 years old and with comorbidities (40%). At presentation, laboratory parameters showed elevated serum transaminase with a median of AST 701.23 (18-2936) UI/l and ALT 1041.48 (57-4721) (UI/l), with total bilirubin 5.12 (0.4-30.4) (mg/dl). A statistically significant higher level of

ALT was found in HEV positive patients; this is already known in other acute viral hepatitis, and discriminates it from non-infective ones, which probably accounts for the majority of alternative diagnosis. High levels of HEV-RNA were generally observed, consistent with serologic data of a very recent infection in almost half of the acute-HEV patients, being IgG negative in 12 out of 35. It is unclear if high-levels of viremia, like those found here, can be related with the clinical severity, but a relation with high levels of ALT has been reported [19].

In this study all patients were hospitalized but there were no cases of acute liver injury (ALI), acute-on chronic liver failure (ACLF) or death. This is quite different from published data in England that, despite incomplete, have shown a mortality rate of 1-4%, particularly in elderly, with pre-existing liver disease or immunocompromised [20].

Similarly, in a retrospective collection of HEV cases in the Marche Region, mortality rate was as high as 8%, death and hospitalization being associated with older age and severe comorbidities but related also with delay in diagnosis [21].

In our study, the observed favourable outcome may be due to the relatively younger age of our cohort and the lack of severe comorbidities. Despite this, we believe that early diagnosis can potentially improve the prognosis in patients with comorbidities or immunosuppression, mainly implementing supportive treatment and offering them rapid antiviral therapy.

Extrahepatic presentation, mainly represented by not abdominal pain and neurologic symptoms, was observed in 25.8% of patients and strongly associated with HEV-IgM positivity. Presence of these symptoms is widely recognized in autochthonous HEV acute hepatitis and may have a predictive role in diagnosing it. To our knowledge, in this paper extrahepatic presentation is systematically recorded and described in Italy for the first time, probably because of the major awareness of physicians. As seen in 8 patients with a defined non-hepatic disease, extrahepatic symptoms can overstep hepatic ones in importance. For this reason, testing for HEV is recommended also in neurologic disorders especially in highly endemic areas, regardless of liver disease. Of notice, in the HEV-3f small outbreak observed in L'Aquila city, 2 patients had very similar neurologic presentation, and another one had only non-hepatic symptoms (arthralgia). A possible role of HEV-3 subtypes is not in the scope of this study, but there are many speculations about differences that subtypes may have in defining both type and severity of clinical features including liver damage and ALT levels [22-24]. Moreover, in this same outbreak the only one patient with metabolic comorbidities had a typical acute hepatitis presentation, suggesting a possible concurrence of viral subtype and pre-existing comorbidities in determining the clinical picture.

No differences were observed between populations regarding work, comorbidities, and or risk factors such as food or animal contact in the last two months. A farming job was very rare (3 patients overall), while animal contact (nearly 50%) and gardening (25%) were very common habits in all patients and consumption of pork sausages (58.76%) and liver pork sausages (31.98%) were wide spread with no intra-regional differences. This large diffusion may explain the lack of significance of these risk factors but may determine a difference with other regions, as previously reported between Abruzzo and Lazio [14].

At the same time, an IgG seroprevalence of close to 20% was observed in active patients without HEV, statistically unrelated to recent dietary risk factors (data not presented). It is however mandatory that all patients with a hepatic pre-existing disease or immunocompromised should avoid consumption of pork and liver-pork sausages, as though an accurate veterinary surveillance with one-health approach in this area.

Although there are no complete data about the alternative diagnosis of HEV-negative patients in this study, most of them were diagnosed as toxic hepatitis or drug-induced liver injury (DILI); this is often a diagnosis made by exclusion, and these data emphasize the needing of a systematic HEV evaluation in all acute hepatitis, not only in hyperendemic area, as recommended by EASL guidelines. HEV should be also ruled out in patients diagnosed for other viral hepatitis because many data from developing countries suggest a clinical relevance of HEV superinfection in HBV chronic hepatitis, and this cannot be excluded in autochthonous HEV cases [4, 25-27].

These data were systematically collected and analysed until 2018, and the delay in submission is mainly due to the COVID-19 pandemic; this rep-

resents a limitation of the study. Surveillance was not interrupted and a huge HEV cluster in Abruzzo was described and published in 2019 [16]. This cluster was removed from the analysis so as not to falsify it. Since 2020, being surveillance still active, a very shorter number of samples has been sent to National Institute of Health, mainly for difficulties in health system access and for the presence of mainly SARS-CoV-2 infected patients in all Infectious Diseases Departments of Abruzzo.

Despite the limitations of heterogeneity and incompleteness of data retrospectively collected, this study recognizes HEV as an important cause of non-ABC viral hepatitis, often misdiagnosed because of atypical presentation and lack of clinical suspicion. Overall, 36% of acute hepatitis n-ABC in Abruzzi were related to HEV, reaching 44% in L'Aquila Provinces. Surveillance implementation allowed clinicians to recognize a great majority of cases, probably undiagnosed during the previous period.

Extrahepatic presentation is quite common and may be a predictor of HEV aetiology, especially in middle-aged men with unexplained high levels of ALT. In a hyperendemic region HEV should be routinely excluded in all patients with other hepatic disease and also with neurological symptoms, regardless of the presence of a clear hepatic damage. Further studies are needed to assess risk factors for disease severity and a possible role of subtypes in clinical presentation.

Conflict of interest

The authors certify that there is no conflict of interest regarding the material discussed in the manuscript.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Acknowledgements

The authors acknowledge Lucia Orneto who helped in collecting data.

REFERENCES

[1] Adlhoch C, Avellon A, Baylis SA, et al. Hepatitis E virus: Assessment of the epidemiological situation in humans in Europe, 2014/15. *Clin Virol.* 2016; 82, 9-16.

[2] Hewitt PE, Ijaz S, Brailsford SR, et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. *Lancet* 2014; 384, 1766-1773.

[3] Bura M, Łagiedo M, Michalak M, Sikora J, Mozer-Lisewska I. Hepatitis E virus IgG seroprevalence in HIV patients and blood donors, west-central Poland. *Int J Infect Dis.* 2017; 61, 20-22.

[4] EASL Clinical Practice Guidelines on hepatitis E virus infection, *J Hepatol.* 2018; 68(6), 1256-1271.

[5] Spada E, Pupella S, Pisani G, et al. A nationwide retrospective study on prevalence of hepatitis E virus infection in Italian blood donors. *Blood Transfus*. 2018; 16 (5), 413-421.

[6] Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. *Lancet Infect Dis.* 2008; 8 (11), 698-709.

[7] Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E. *Lancet* 2012; 379 (9835), 2477-2488.

[8] Kamar N, Dalton HR, Abravanel F, Izopet J. Hepatitis E virus infection. *Clin Microbiol Rev.* 2014; 27 (1), 116-138.

[9] Sanabria-Cabrera J, Sanjuán-Jiménez R, Clavijo E, et al. Incidence and prevalence of acute hepatitis E virus infection in patients with suspected Drug-Induced Liver Injury in the Spanish DILI Registry. *Liver Int.* 2021; 41 (7), 1523-1531.

[10] Davern TJ, Chalasani N, Fontana RJ, et al. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. *Gastroenterol*. 2011; 141(5), 1665-1672.

[11] Dalton HR, Fellows HJ, Stableforth W, et al. The role of hepatitis E virus testing in drug-induced liver injury. *Aliment Pharmacol Ther*. 2007; 26 (10), 1429-1435.

[12] Manka P, Bechmann LP, Coombes JD, et al. Hepatitis E Virus Infection as a Possible Cause of Acute Liver Failure in Europe. *Clin Gastroenterol Hepatol.* 2015; 13 (10), 1836-1842.

[13] Mclean BN, Gulliver J, Dalton HR. Hepatitis E virus and neurological disorders, *Pract Neurol.* 2017; 17 (4), 282-288.

[14] Lucarelli C, Spada E, Taliani G, et al. High prevalence of anti-hepatitis E virus antibodies among blood donors in central Italy, February to March 2014. *Euro Surveill.* 2016; 21 (30).

[15] Horn J, Hoodgarzadeh M, Klett-Tammen CJ, et al. Epidemiologic estimates of hepatitis E virus infection in European countries. *J Infect.* 2018; 77 (6), 544-552.

[16] Garbuglia AR, Bruni R, Villano U, et al. Hepatitis E Outbreak in the Central Part of Italy Sustained by Multiple HEV Genotype 3 Strains, June-December 2019. *Viruses.* 2021; 13 (6), 1159.

[17] Istituto Superiore di Sanità - EpiCentro - SEIEVA (Sistema epidemiologico integrato dell'epatite virale acuta). Epatite E Available at: http://www.epicentro.iss.it/epatite/epatite-e [accessed 03 February 2023]

[18] De Sabato L, Di Bartolo I, Lapa D, Capobianchi MR, Garbuglia AR. Molecular Characterization of HEV Genotype 3 in Italy at Human/Animal Interface. *Front Microbiol.* 2020; 11, 137.
[19] Kamar N, Izopet J, Rostaing L. Hepatitis E virus infection. *Curr Opin Gastroenterol.* 2013; 29 (3), 271-278.
[20] Dalton HR, Stableforth W, Thurairajah P, et al. Autochthonous hepatitis E in Southwest England: natural history, complications and seasonal variation, and hepatitis E virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease. *Eur J Gastroenterol Hepatol.* 2008; 20 (8), 784-790.
[21] Tarantino G, Ortolani A, Marinelli K, et al. Locally acquired hepatitis E virus in Marche Italy: Clinical/laboratory features and outcome. *Dig Liver Dis.* 2020; 52 (4), 434-439.
[22] Minosse C, Biliotti E, Lapa D, et al. Clinical Characteristics of Acute Hepatitis E and Their Correlation with HEV Genotype 3 Subtypes in Italy. *Pathogens.* 2020; 9 (10), 832.
[23] Abravanel F, Dimeglio C, Castanier M, et al. Does HEV-3 subtype play a role in the severity of acute hepatitis E? *Liver Int.* 2020; 40 (2), 333-337.
[24] Subissi, L, Peeters, M, Lamoral S, et al. Subtype-specific differences in the risk of hospitalisation among patients infected with hepatitis E virus genotype 3 in Belgium, 2010-2018. *Epidemiol. Infect.* 2019; 147, e224.
[25] Nasir M, Wu GY. HEV and HBV Dual Infection: A Review. *J Clin Transl Hepatol.* 2020; 8 (3), 313-321.
[26] Tseng TC, Liu CJ, Chang CT, et al. HEV superinfection accelerates disease progression in patients with chronic HBV infection and increases mortality in those with cirrhosis. *J Hepatol.* 2020; 72(6), 1105-1111.
[27] Schulz M, Schott E. An unusual cause for a hepatic flare in a chronic HBV carrier. *Hepat Mon.* 2014; 14 (9).