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Chronic inflammatory demyelinating polyneuropathy and HEV antibody status: A case-control study from Lazio, Italy

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ABSTRACT

Introduction: Few studies have pointed to the possible role of infectious diseases in triggering Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP). Given the association of Hepatitis E Virus (HEV) with Guillain Barrè syndrome, we conducted a case-control study to determine the possible association of HEV infection with CIDP, analyzing possible risk factors for acquiring HEV infection in both CIDP patients and controls.

Materials and methods: 82 CIDP and 260 from the general population have provided some personal information (demographics, anamnestic data and recognized risk factors for HEV infection) and underwent venipuncture blood sampling for virological assays testing for anti-HEV IgG and IgM with ELISA and RNA-HEV performing RT-PCR.

Results: Anti-HEV IgG seropositivity resulted in 32 CIDP patients (39.0%) and in 45 controls (17.3%), indicating a significant association between anti-HEV IgG positivity and CIDP (OR 3.04; 95% CI 1.70–5.43, *p*-value <0.001), but in multivariate logistic regression the only significant associations with anti-HEV positivity were eating pork liver sausages (OR 10.443, 95% CI 2.268–60.12, *p*-value 0.004) and IVIg/SCIg administration (OR 31.32, 95% CI 7.914–171.7, p-value <0.001).

Discussion: The higher prevalence of anti-HEV IgG in CIDP patients than in controls could be justified by chronically administering IVIg/SCIg with a passive acquisition of anti-HEV antibodies. Furthermore, all the 20 CIDP patients who underwent IVIg/SCIg administration reported HEV risk factors, so that they could have acquired the infection.

Conclusions: Further studies in a larger CIDP patient sample in treatment with therapy other than IVIg/SCIg are necessary to rule out the possible confounding effect of IVIg/SCIg.

Abbreviations: CIDP, Chronic Inflammatory Demyelinating Polyradiculoneuropathy; A-CIDP, acute-onset Chronic Inflammatory Demyelinating Polyradiculoneuropathy; AIDP, Acute Inflammatory Demyelinating Polyneuropathy; IVIg, endovenous immunoglobulins; SCIg, subcutaneous immunoglobulins; GBS, GuillainBarrè syndrome; HEV, Hepatitis E virus..

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1. Introduction

Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) is an immune-mediated neuropathy with progressive, stepwise, or recurrent course developing over at least 8 weeks, characterized by paresthesia, sensory loss, symmetric proximal and distal weakness and reduced or abolished deep tendon reflexes in all extremities [1,2]. About 50% of CIDP patients may present a different phenotype (variant CIDP) and, over time, could become a typical form [2,3]. Up to 16% of cases can present with a rapidly progressive onset within 8 weeks (acute-onset CIDP, A-CIDP), which makes it challenging to differentiate from acute inflammatory demyelinating polyneuropathy (AIDP) [4]. The prevalence is 0.8–8.9 cases per 100.000 individuals, with a male predominance and an average age at onset of 48 years [5]. The diagnosis is based on clinical and electrophysiological criteria, eventually with supportive criteria (response to treatment, cerebrospinal fluid analysis, imaging, and nerve biopsy). Treatment is based on immune modulation, including daily/pulsed corticosteroid therapy, endovenous (IVIg)/subcutaneous (SCIg) immunoglobulins and plasma exchange. Cyclophosphamide, mycophenolatemofetil, ciclosporin, azathioprine, and rituximab could be used for steroid/immunoglobulin-sparing or refractory cases [2]. New drugs with different mechanisms of action are under investigation [6].

Concerning risk factors, in a few studies there is a possible association with antecedent events [7–12], such as vaccination, surgery and trauma, flu-like syndrome, and respiratory and gastrointestinal infections [12]. In Guillain Barrè syndrome (GBS), there is a stronger association with infectious events, with some evidence of the association with Hepatitis E Virus (HEV) infection [13,14]. HEV, belonging to the Herpesviridae family, is a single-stranded RNA with four main genotypes responsible for most human infections. HEV1 and HEV2 are endemic in low-income countries and have been found only in humans, while HEV3 and HEV4 are prevalent in high-income countries and are zoonotic infections. Humans are infected by HEV1 and HEV2 through fecal contaminated water (rarely through person-to-person contact); in contrast, infection by HEV3 and HEV4 occurs mainly through ingestion of raw or undercooked liver and meat from infected animals and direct and, possibly, indirect contact with infected animals. HEV3 and HEV4 are found in domestic swine, wild boar, and other wild species such as deer. Infected pork liver and offal pork meat are possible sources of HEV infections, so forestry workers, farmers, veterinarians, hunters, and slaughterhouse workers are at risk of infection. Although transmission occurs mainly through eating food, also iatrogenic transmission is possible through infected blood products and organs [15].

In Italy, based on the most recent studies, the crude prevalence of HEV infection has been estimated to be about 8–9% in blood donors, with a large variability among regions and very high prevalence levels in a couple of them [16–19].

Among the extrahepatic manifestations of HEV infection, some neurological disorders have been reported, such as neuralgic amyotrophy, encephalitis, myelitis, and Guillain Barrè syndrome [13,15]. The pathogenesis of these disorders is unclear; however, the two main hypotheses are an immune reaction against self-antigens for molecular mimicry mechanisms and direct viral damage due to HEV replication in the neurological system. To support the latter hypothesis, HEV-RNA can be detected in the serum and cerebrospinal fluid (with quasi-species compartmentalization in some cases), and in some cases with viral clearance, it can be a resolution of neurological symptoms, anti-HEV IgM can be detected in cerebrospinal fluid, HEV can grow in some neurological cell lines, and it can cross the blood–brain barrier in vivo studies [15]. Concerning the molecular mimicry hypothesis, Lucchese and Kanduc described the correlation between Zika virus infection, Guillain Barrè syndrome, and microcephaly and, searching in protein and nucleic acids databases, noticed that all the aminoacidic sequences (>5 aminoacids) were in common between Zika Virus and the most implicated proteins in demyelinating and axonal neuropathies described in the literature [20].

Given all these findings and the few studies in the literature about the possible association between HEV infection and other demyelinating neuropathies, we conducted a case-control study to determine the possible association of HEV infection with CIDP also analyzing possible risk factors for acquiring HEV infection both in CIDP patients and in controls.

2. Materials and methods

2.1. Study population

For this case-control study, CIDP patients were enrolled in three main Centers for Rare Neurological Disorders in Rome, by prior informed consent, over 30 months between 2019 and 2021 with the following criteria:

- Inclusion Criteria: age > 18 years; diagnosis of definite, probable and possible CIDP using European Federation of Neurological Societies/Peripheral Nerve Society [21] for patients enrolled from 2019 to 2021, confirming their diagnosis and enrolling other patients with a diagnosis of CIDP and possible CIDP using European Academy of Neurology/Peripheral Nerve Society diagnostic criteria [2];
- Exclusion Criteria: pregnancy; alternative diagnosis other than CIDP [2].

The control population consisted of 260 individuals from the general population with similar demographics to CIDP patients enrolled in November 2019. They were the participants in a study on the prevalence of hepatitis viruses (B, C, and E) in the general population, who had been randomly extracted from the list of residents in Rome.

At baseline, every subject has provided some personal information by filling a questionnaire, including some demographics (birthplace and residence, actual or previous living in rural setting, citizenship, schooling), anamnestic data (smoking, sporting and working activities, previous traumas) and recognized risk factors for HEV infection (previous contact with animals, hunting practice, vegetable cultivation, possible fertilizer with manure, eating habits such as consumption of food at risk of contamination with HEV, information about history of therapy with blood products and foreign trips). Every patient underwent venipuncture blood sampling to obtain serum for virological assays.

2.2. Virological assays

Every serum sample was tested for anti-HEV IgG antibodies using the Wantai ELISA (Enzyme-Linked Immunoadsorbent Assay) test according to the manufacturer's recommendation (Biologic Pharmacy Enterprise, Beijing, People's Republic of China). The positive samples were then tested for the presence of IgM anti-HEV antibodies (by the d by the Wantai HEV-IgM ELISA) as well as for the presence of HEV RNA by RealStar HEV RT-PCR version 1.0 kit manufactured by Altona Diagnostics, Hamburg, Germany, which is based on probes and primers specific for the ORF3 region of HEV. According to the manufacturer's instructions, the viral RNA extracted from 200 μL plasma was reverse transcribed and subsequently amplified on the Rotor-Gene Q 5/6 plex Platform. The reported sensitivity is 95%, and the lower detection limit is 50 IU/mL of HEV-RNA. All the commercial assays used in the present study have CE marking and can detect infection by all the four HEV genotypes infecting humans (genotypes 1–4).

2.3. Statistical analysis

The statistical analysis was performed by RStudio IDE for R software (version 4.2.3.2). The Rstudio package used was Epitools. To estimate the existence and the strength of the association between CIDP and anti-HEV IgG positivity crude and adjusted (by sex, age, and area of residence), Odds Ratios and their Confidence Intervals (CIs) were computed by normal approximation (Wald). The *p*-value of the chi-squared test

estimated the association between anti-HEV IgG positivity and other categorical variables. The relationship between HEV infection and the continuous variables was evaluated by the Mann–Whitney *U* test, a non-parametric test for two independent populations.

Univariate and multivariate logistic models analyzed risk factors for anti-HEV IgG positivity.

The variables with a *p*-value <0.10 in univariate analysis were evaluated by a multivariate Logistic model; sex and age were included in the model independently from the p-value. A backward selection (based on the Akaike Information Criterion (AIC)) to perform the multivariate model was used. The coefficient of the Logistic model was used to estimate the adjusted Odds Ratio for each of the model's variables. The variables sex and age were included in the model independently from the *p*-value.

3. Results

Table 1 shows the main demographic, clinical, and anamnestic characteristics of CIDP patients and controls. Apart from sex and age, no other variable showed a significant difference between patients and controls.

Thirty-two CIDP patients (39.0%) tested positive for anti-HEV IgG; none were anti-HEV IgM and/or HEV RNA positive. Among controls, 45 (17.3%) were anti-HEV IgG positive. These data indicated a strong significant association between anti-HEV IgG positivity and CIDP [Odds Ratio (OR), 3.04; 95% Confidence Interval (CI), 1.70–5.43, *p*-value <0.001] (Table 2).

Univariate and multivariate logistic regression were used to identify the potential at-risk exposures associated with anti-HEV IgG positivity both in CIDP and controls (Tables 3 and 4).

As regards CIDP patients, evidence of a positive association with anti-HEV positivity was found in univariate analysis with several exposures (i.e. eating pork liver sausages, eating berries, blood transfusion and IVIg/SCIg administration), but only eating pork liver sausages (OR 10.443, 95% CI 2.268–60.12, *p*-value 0.004) and IVIg/SCIg administration (OR 31.32, 95% CI 7.914–171.7, p-value <0.001) resulted significantly and independently associated with anti-HEV IgG positivity, as resulting from multivariate analysis (Table 3). Indeed, the strong and independent association between IVIg/SCIg administration and anti-HEV IgG positivity might challenge the above-reported association between CIDP and anti-HEV IgG positivity (Table 2), thus suggesting a spurious association.

Excluding CIDP patients in IVIg/SCIg treatment, we compared anti-HEV IgG seropositivity in remaining CIDP patients and controls. Among patients in other therapies than IVIg/SCIg or without therapy, 14 out of 60 were anti-HEV IgG seropositive (23,3%), with a slight difference compared to control subjects (17,3%). However, analyzing the difference between these two groups, we did not find any significant difference ($p = 0{,}278$).

Among controls, only two at-risk exposures such as eating pork liver sausages (OR 2.377, 95% CI 1.075–5.080, *p*-value 0.028) and eating raw seafood (OR 2.093, 95% CI 1.003–4.276, p-value 0.045) are resulted significantly associated with anti-HEV positivity in univariate analysis. Both were confirmed to be independent predictors of anti-HEV positivity by multivariate analysis (Table 4).

Table 1
Demographic and anamnestic features of CIDP patients and control subjects.

	CIDP[a] patients (N° 82)		Controls[b] (N° 260)		
	N°	%	N°	%	P-value
Females	29	35.4	100	38.5	
Males	53	64.6	160	61.4	0.695
Age (mean ± sd) (yrs)	65.68 (±15.2)		65.13 (±9.9)		0.544
Resident in Lazio region	75	92.6	234	99.6	0.001
Resident in non-rural area	64	79.0	231	95.9	<0.001
Smoking history[c]	22	39.3	39	24.5	0.040
	25	30.9	97	37.9	0.289
Practising sport [c]	38	47.5	123	48.2	1.000
Traumatism history[c]	24	31.2	77	30.4	0.889
Education > 8 y	74	91.4	239	94.8	0.283
IVIg/SCIg therapy	20	25.0	–	–	
Other or no therapy	60	75.0	–	–	–

N°, Number of participants.
[a] CIDP, Chronic Inflammatory Demyelinating Polyradiculoneuropathy.
[b] Controls were general population adults participating in a voluntary HEV screening program, adjusted by age, sex, and area of residence.
[c] Assessed over a lifetime.

4. Discussion

This case-control study is the first to analyze the possible association between HEV and CIDP. Even if we enrolled 82 CIDP patients, this study sample must be considered important given the rarity of the disease. Possible risk factors for past HEV infection (anti-HEV IgG positivity) were also determined in the CIDP patients and controls.

Only one other recent study, testing for anti-HEV IgG and IgM seropositivity 99 serum patient samples with acute neurological manifestations, found an anti-HEV IgG seroprevalence of 25% in AIDP/CIDP group (28 patients), not significant compared to controls [22].

Concerning environmental risk factors for CIDP, some eating habits, such as eating rice three times a week and eating fish at least once a week, decreased the risk of acquiring CIDP [12]; at the same time, some antecedent events could precede the symptoms' onset [7–12]. Data from the Italian CIDP database have shown that in 15.5% of patients an antecedent event such as vaccination (1.5%), surgery (1%), trauma (0.5%), infectious diseases (12%) such as flu-like syndrome, respiratory and gastrointestinal infections, with an average time from the antecedent event and symptoms' onset of 16.5 days, ranging from 1 to 42 days [12]. Similar data have emerged from a collaborative European study that analyzed 268 CIDP patients, showing in the 6 weeks before disease onset an antecedent event in 10.4% of cases, in particular infectious disease in 9.3% (gastrointestinal or respiratory infections) and 1.2% after vaccination [9]. Interestingly, both studies have shown a stronger association between previous infections and A-CIDP [9,12], suggesting different pathogenetic mechanisms in A-CIDP similar to GBS, compared to those in typical CIDP.

It is well known that in the immunopathogenesis of CIDP, both cellular and humoral immunity against Schwann cells/myelin antigens are involved, a view also supported by the efficacy of immunomodulatory treatments [3]. Although it is not established, given the reported association with connective tissue diseases, infections and malignancies, molecular mimicry mechanism due to cross-reactivity against neural components may be involved in the immunopathogenesis of CIDP [23].

Based on the hypothesis of molecular mimicry and the previously cited study [20], we searched in protein and nucleic acid databases such as NCBI (through the Blast tool [24]), Human Protein Atlas [25] and Immune Epitope Database [26] to investigate the possible similarity in antigenic terms between aminoacidic sequences of HEV proteins and the most important myelin and paranodal proteins more likely involved in the pathogenesis of CIDP and acute/chronic paranodopathy [11].The most relevant similarity was observed between the viral capsid protein encoded by ORF2 and the myelin protein zero (identity ranging from 100% (9/9) in stretches of 9 aminoacids and 90% (9/10) in stretches of 10 aminoacids), with a less significant similarity between myelin basic protein and viral RNA-polymerase and between myelin protein 2, connexin 32 and peripheral myelin protein 22 and viral capsid protein. Although in shorter aminoacidic stretches, some similarity was also observed between the most important proteins of the paranode (such as contactin-1 and contactin-associated protein-1) and non-structural viral

Table 2
Crude and Adjusted Odds Ratios (patients vs controls) for anti-HEV IgG positivity in CIDP patients.

	N°	N° anti-HEV IgG+	Prevalence %	95% CI	Crude OR	95% CI	P-Value	Adjusted OR	95% CI	P-Value
Controls[a]	260	45	17.3	12.7-21.9	1	–	–	1	–	–
CIDP[b]	82	32	39.0	29.5-49.6	3.06	1.77–5.29	<0.001	3.04	1.70–5.43	<0.001

N°, Number of participants; CI, Confidence Interval; OR, Odds Ratios.
[a] Controls were general population adults participating in a voluntary HEV screening program, adjusted by age, sex, and area of residence.
[b] CIDP, Chronic Inflammatory Demyelinating Polyradiculoneuropathy.

Table 3
Univariate and multivariate analysis of sociodemographic and risk factors[a] associated with anti-HEV IgG positivity in CIDP patients (N° 82).

					Univariate analysis			Multivariate analysis		
		N° Tested	HEV+ (N)	HEV+ (%)	OR	95% CI	P	OR	95% CI	P
Sex	*Male*	53	19	35.8	1	–	–			
	Female	29	13	44.8	1.454	0.578–3.657	0.426	1.904	0.540–6.989	0.317
Age (yrs)	*21–54*	17	6	35.3	1	–	–			
	55–64	20	6	30.0	0.786	0.198–3.123	0.732	1.015	0.975–1.057	0.452
	>65	45	20	44.4	1.467	0.462–4.658	0.515			
Place of residence	*Rural area*	64	27	39.1	1	–	–			
	Urban area	17	7	41.2	1.092	0.368–3.243	0.874			
Years of schooling	*0–8 yrs*	7	3	42.9	1	–	–			
	≥9 yrs	74	29	39.2	0.859	0.179–4.122	0.85			
Work with animals	*No*	74	27	36.5	1	–	–			
	Yes	7	5	71.4	4.352	0.79–23.98	0.071			
Domestic contact with animals[b]	*No*	23	6	26.1	1	–	–			
	Yes	58	26	44.8	2.302	0.794–6.677	0.120			
Wild animals contact[c]	*Yes*	64	23	35.9	1	–	–			
	No	18	9	50.0	1.783	0.620–5.123	0.280			
Other animals' contact[d]	*No*	12	3	25.0	1	–	–			
	Yes	70	29	41.4	2.122	0.528–8.524	0.281			
Hunting	*No*	73	28	38.4	1	–	–			
	Yes	7	4	57.1	2.143	0.446–10.30	0.332			
Vegetable gardening	*No*	55	18	32.7	1	–	–			
	Yes	26	14	53.8	2.398	0.923–6.231	0.070			
Eating vegetables from own or friends' garden	*No*	46	16	34.8	1	–	–			
	Yes	35	16	45.7	1.579	0.642–3.884	0.319			
Using manure to fertilize the garden	*No*	48	20	58.3	1	–	–			
	Yes	18	9	50.0	1.400	0.472–4.154	0.544			
Eating pork sausages	*No*	31	12	38.7	1	–	–			
	Yes	49	19	38.8	1.003	0.398–2.525	0.995			
Eating pork liver sausages	*No*	69	24	34.8	1	–	–			
	Yes	12	8	66.7	**3.75**	**1.024–13.74**	**0.037**	**10.443**	**2.268–60.12**	**0.004**
Eating wild boar sausages	*No*	68	27	39.7	1	-	–			
	Yes	13	5	38.5	0.949	0.281–3.209	0.933			
Eating salami e pork meat[e]	*No*	10	2	20.0	1	–	–			
	Yes	72	30	41.7	2.857	0.566–14.42	0.188			
Eating berries	*No*	56	18	32.1	1	–	–			
	Yes	25	14	56.0	**2.687**	**1.020–7.078**	**0.042**			
Eating game meat[e]	*No*	70	29	41.4	1	–	–			
	Yes	11	3	27.3	0.530	0.129–2.171	0.372			
Eating raw seafood	*No*	63	24	38.1	1	–	–			
	Yes	17	7	41.2	1.138	0.382–3.388	0.817			
IVIg/SCIg	*No*	60	14	23.3	1	–	–			
	Yes	20	17	85.0	**18.62**	**4.753–72.94**	**<0.001**	**31.32**	**7.914–171.7**	**<0.001**
Blood or blood products transfusion	*No*	53	15	28.3	1	–	–			
	Yes	28	17	60.7	**3.915**	**1.491–10.28**	**0.005**			
Travelling abroad	*No*	38	14	36.8	1	–	–			
	Yes	43	18	41.9	1.234	0.504–3.022	0.645			

N°, Number of participants; HEV+, Anti-HEV IgG positive; OR, Odds Ratios; CI, Confident Interval; P, P-value.
[a] Exposure to risk factors was assessed over a lifetime.
[b] Dogs, cats, other domestic animals.
[c] Wild boars, deers, porks.
[d] Rabbits, rodents, horses, chickens, sheep, cattle, dogs, cats, other (turtles, birds, fishes, hamsters, squirrels, donkeys, elephants, camels).
[e] Raw or undercooked meat.

proteins of the virus (such as ORF3 and RNA polymerase), with identity up to 83% (5/6).

In our study, we observed a higher prevalence of IgG anti-HEV in CIDP patients than in controls (OR = 3.04; 95% CI, 1.70–5.43), but the resulting association could be spurious, i.e. due to a third factor acting as a confounder. Indeed, by performing multivariate logistic regression analysis, the chronic administration of IVIg/SCIg resulted in the most strongly independent risk factors associated with anti-HEV IgG

Table 4
Univariate and multivariate analysis of sociodemographic and risk factors[a] associated with anti-HEV IgG positivity in controls (N° 260).

					Univariate analysis			Multivariate analysis		
		N° Tested	HEV+ (N)	HEV+ (%)	OR	95% CI	P	AdjOR	95% CI	P
Sex	*Male*	160	27	16.9	1		–			
	Female	100	18	18.0	1.081	0.561–2.085	0.816	1.181	0.591–2.324	0.633
Age (yrs)	*21–54*	42	6	14.3	1		–			
	55–64	71	15	21.1	1.607	0.571–4.525	0.366	1.029	0.995–1.067	0.101
	>65	147	24	16.3	1.171	0.444–3.084	0.750			
Place of residence	*Rural area*	231	42	18.2	1		–			
	Urban area	10	1	10.0	0.5	0.062–4.054	0.508			
Years of schooling	*0–8 yrs*	13	2	15.4	1		–			
	≥9 yrs	239	42	21.1	1.173	0.251–5.486	0.840			
Work with animals	*No*	210	32	15.2	1		–			
	Yes	26	7	26.9	2.049	0.797–5.271	0.130			
Domestic contact with animals[b]	*No*	105	19	18.1	1		–			
	Yes	128	19	14.8	0.789	0.393–1.582	0.504			
Wild animals contact[c]	*yes*	189	30	15.9	1		–			
	No	71	15	21.1	1.420	0.712–2.832	0.318			
Other animals' contact[d]	*No*	52	9	17.3	1		–			
	Yes	208	36	17.3	1.000	0.448–2.233	1.000			
Hunting	*No*	232	40	17.2	1		–			
	Yes	22	4	18.2	1.067	0.343–3.321	0.911			
Vegetable gardening	*No*	179	29	16.2	1		–			
	Yes	76	15	19.7	1.272	0.637–2.538	0.494			
Eating vegetables from own or friends' garden	*No*	162	24	14.8	1		–			
	Yes	87	18	20.7	1.500	0.763–2.949	0.238			
Using manure to fertilize the garden	*No*	117	14	12.2	1		–			
	Yes	46	7	15.2	1.295	0.486–3.449	0.604			
Eating pork sausages	*No*	122	15	12.3	1		–			
	Yes	137	29	21.2	1.915	0.972–3.774	0.058			
Eating pork liver sausages	*No*	215	31	14.4	1		–			
	Yes	43	13	30.2	**2.572**	**1.210–5.466**	**0.012**	**2.377**	**1.075–5.080**	**0.028**
Eating wild boar sausages	*No*	204	31	15.2	1		–			
	Yes	54	13	24.1	1.769	0.851–3.678	0.123			
Eating salami e pork meat[e]	*No*	33	6	18.2	1		–			
	Yes	227	39	17.2	0.934	0.361–2.412	0.887			
Eating berries	*No*	171	29	17.0	1		–			
	Yes	85	15	17.6	1.049	0.528–2.083	0.891			
Eating game meat[e]	*No*	208	32	15.4	1		–			
	Yes	49	11	22.4	1.592	0.738–3.437	0.233			
Eating raw seafood	*No*	198	28	14.1	1		–			
	Yes	61	16	26.2	**2.159**	**1.076–4.332**	**0.028**	**2.093**	**1.003–4.276**	**0.045**
Blood or blood products transfusion	*No*	224	39	17.4	1		–			
	Yes	32	5	15.6	0.878	0.318–2.423	0.802			
Travelling abroad	*No*	79	12	15.2	1		–			
	Yes	180	32	17.8	1.207	0.586–2.489	0.610			

N°, Number of participants; HEV+, Anti-HEV IgG positive; OR, Odds Ratios; CI, Confident Interval; P, P-value; AdjOR, Adjusted Odds Ratios.
[a] Exposure to risk factors was assessed over a lifetime.
[b] Dogs, cats, other domestic animals.
[c] Wild boars, deers, porks.
[d] Rabbits, rodents, horses, chickens, sheep, cattle, dogs, cats, other (turtles, birds, fishes, hamsters, squirrels, donkeys, elephants, camels).
[e] Raw or undercooked meat.

positivity in multivariate analysis (OR = 31.32; 95% CI, 7.91–171.7). Among CIDP patients, also eating pork liver sausages was strongly and independently associated with anti-HEV IgG positivity (OR = 10.44; 95% CI, 2.26–60.12). However, eating pork liver sausages is a well-recognized risk factor for HEV infection, constantly cited in every analysis of risk factors for this infection, while the association with IVIg/SCIg administration was reported to be a HEV risk factor for the first time just right in the present study. However, excluding CIDP patients in IVIg/SCIg treatment and comparing seropositivity in remaining CIDP patients and controls, we did not find a significant difference between CIDP patients in other or without therapies and control subjects. These data suggested that the association between CIDP and anti-HEV IgG seropositivity could depend on the therapy itself, namely patients' passive acquisition of heterologous anti-HEV IgG antibodies present in the IVIg/SCIg administered to CIDP patients.

This is supported by Ankcorn et al., who analyzed 245 serums of subjects with primary or secondary antibody deficiency in replacement treatment with immunoglobulins. No one had detectable HEV-RNA while IgG anti-HEV seroprevalence was 38,8% and, interestingly, all IVIg/SCIg products tested contained variable levels of anti-HEV IgG [27]. Another study in subjects with primary immunodeficiency demonstrated a significant difference in anti-HEV IgG seroprevalence in patients treated with IVIg/SCIg (68%) compared to those without therapy (16%). All 23 tested IVig/SCIg batches contained detectable IgG anti-HEV at different concentrations without detectable levels of IgM and/or RNA-HEV. They also analyzed seronegative patients before and after IVIg/SCIg infusion, and 67% of them became seropositive for anti-HEV IgG [28].

Nevertheless, there is no way to distinguish anti-HEV IgG antibodies that are passively acquired following IVIg/SCIg from those acquired naturally after exposure to the virus. In addition, all the 20 CIDP patients who underwent IVIg/SCIg administration reported recognized HEV risk factors and, therefore, could have acquired the infection. A prevalence as high as that found in our sample of CIDP patients (if true) has been documented among blood donors only in some Italian hyperendemic areas (in Abruzzo e Sardinia regions) [16,17,19]. Instead, the prevalence

found in controls (17.3%) could be in line with data reported for blood donors residing in Lazio, considering that in our study controls have a median age of about 65 years [18,19]. Further studies are necessary to completely exclude the relation with anti-HEV IgG seropositivity in a larger sample of CIDP patients in treatment with immunomodulatory therapy other than IVIg/SCIg to rule out the possible confounding effect of IVIg/SCIg. It is also important for future studies to consider the possible influence of IVIg/SCIg therapy in antibody seropositivity for pathogens.

Additionally, the observed similarities in antigenic terms between viral and myelin/paranodal proteins could lead to a future better understanding of the pathogenesis of CIDP, underlying the importance of studying infectious diseases as triggers of this autoimmune disease, particularly in A-CIDP subtype, given previous evidence about its stronger association with infections [9,12].

5. Conclusions

The results of our study do not support the existence of a causal correlation between HEV and CIDP. However, our investigation might be important and useful given the paucity of studies about environmental risk factors for CIDP especially considering the size of our CIDP patients' sample and the rarity of this neuromuscular pathology. Besides, this study is important to contribute to a future multidisciplinary approach to reveal the complex interactions between immunological responses and genetic and environmental factors in CIDP, whose pathogenesis is only partially known.

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Ethical standards

The study involving human participants was reviewed and approved by the local Ethics Committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The patients/participants provided their written informed consent before being included in the study.

CRediT authorship contribution statement

Federica Moret: Writing – original draft, Data curation, Writing – review & editing, Investigation. **Enea Spada:** Writing – review & editing, Formal analysis, Resources. **Marco Ceccanti:** Investigation, Data curation. **Edoardo D'Andrea:** Investigation, Data curation. **Umbertina Villano:** Formal analysis, Resources. **Elisabetta Madonna:** Formal analysis, Resources. **Paola Chionne:** Formal analysis, Resources. **Alberto Carocci:** Formal analysis, Resources. **Giulio Pisani:** Formal analysis, Resources. **Laura Fionda:** Investigation, Data curation. **Giovanni Antonini:** Investigation. **Antonio Petrucci:** Investigation. **Roberto Bruni:** Formal analysis, Writing – review & editing, Resources. **Anna Rita Ciccaglione:** Formal analysis, Writing – review & editing, Resources. **Gloria Taliani:** Conceptualization, Methodology, Supervision. **Marco Rivano Capparuccia:** Data curation, Investigation. **Eduardo Nobile-Orazio:** Writing – review & editing. **Maurizio Inghilleri:** Conceptualization, Supervision, Data curation, Investigation. **Chiara Cambieri:** Supervision, Data curation, Investigation, Validation.

Declaration of competing interest

ENO reports personal fees for Advisory or Scientific Board from ArgenX – Belgium, Danthus - USA, Takeda - Italy and USA, CSL-Behring - Italy and USA, Janssen – USA, Kedrion – Italy, LFB – France, Roche – Switzerland, Sanofi - USA. GA reports conference honoraria, advisory board and travel grants from Kedrion, Alnylam, Alexion, Takeda, Argenx. The other authors report no potential conflicts of interest.

Data availability

Anonymized data supporting the conclusions of this article will be made available by the authors without undue reservation.

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