Title Page

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Title: Hepatitis E virus seroprevalence is associated with neurodegenerative disorders in older people with dementia: a case-control study

Running head: Hepatitis E and neurodegenerative disorders

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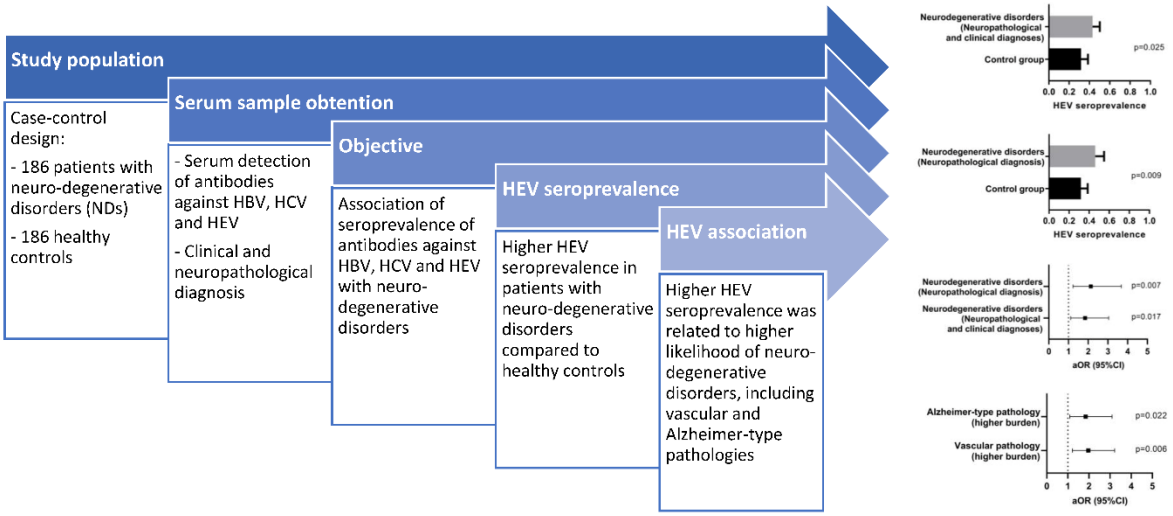
Abstract

In this case-control study, we evaluated the association between serum antibodies against hepatitis E virus (HEV) and central nervous system (CNS) neurodegenerative disorders (NDs) in older people with dementia. The presence of anti-HEV antibodies was related to a higher adjusted odds ratio (aOR) of having CNS-NDs by neuropathological diagnosis (aOR=2.13; p=0.007) and clinical/neuropathological diagnosis (aOR=1.84; p=0.017). Besides, serum anti-HEV antibodies were directly related to neuropathological injury (higher vascular pathology, aOR=1.97; p=0.006) and higher probability of having Alzheimer-type pathology (aOR=1.84; p=0.022). In conclusion, the presence of anti-HEV antibodies was related to higher odds of CNS-NDs and neuropathological injury in older people.

Key Words

Hepatitis E virus; seroprevalence; Alzheimer; vascular pathology; neurodegenerative disorders; older people

Graphical Abstract



Introduction

Neurological disorders are a global public health challenge due to the high burden of deaths and disabilities involving these diseases [1]. Infections have been associated with neurological disorders (such as Parkinson's disease, motor neuron diseases, Alzheimer's disease, and other dementias) and cerebrovascular injury (stroke), supporting the role of infection and inflammation in the etiopathogenesis of these neurological diseases [1, 2]. Microbes can invade the central nervous system (CNS), causing neuroinflammation that leads to critical damage when uncontrolled.

Viral hepatitis is another global public health concern that affects millions of people and causes thousands of deaths due to acute and chronic infections, cirrhosis, and liver cancer. Moreover, increasing evidence suggests a relationship between viral hepatitis infections and neurodegenerative disorders (NDs), particularly hepatitis E virus (HEV) [3].

HEV infection is a zoonosis transmitted by the fecal-oral route and causes recurrent outbreaks of self-limiting acute hepatitis [4]. HEV acute hepatitis is usually mild and resolves spontaneously, but fulminant liver failure and chronic infections can sometimes occur [3, 4]. HEV causes about 3.3 million symptomatic cases and 44 000 deaths every year worldwide [3]. Anti-HEV IgM antibodies are detected 3–4 days from the onset of jaundice and remain detectable for 3–12 months, while anti-HEV IgG antibodies appear after anti-HEV IgM and persist for years [4]. HEV-related infections represent an emerging public health concern in high-income countries due to zoonotic transmission through animal reservoirs, especially swine [5].

Many extrahepatic manifestations of HEV infection have been described in recent years, including neurological disorders [3]. HEV infects neuronal cells and replicates in the nervous system [6], inducing immune-mediated pathogenesis of the nervous system [3]. Besides, HEV infection also induces lesions in the brain microvascular endothelial cells [7], which contribute to NDs. The most common neurologic manifestations are nerve root and plexus disorder (neuralgic amyotrophy and Guillain-Barré syndrome) [3]. However, to the best of our knowledge, a relationship between anti-HEV antibodies seroprevalence and CNS-NDs, such as Alzheimer's disease, has not been previously reported.

This study aimed to determine the association between the presence of antibodies against different hepatitis viruses (HBV, HCV, and HEV) and CNS-NDs in older people.

Methods

Study design and patients

We conducted a case-control study with participants enrolled between 2007 and 2018 in two previously described cohorts:

1) Patients with CNS-NDs (NDs group) were collected from the Vallecas Alzheimer's Study (VAS) cohort, which included institutionalized patients with dementia followed at the Alzheimer's Centre Queen Sofía Foundation (Madrid) since 2007. The VAS cohort included patients with neuropsychological, neurological, functional, and quality of life data and clinical data evaluations every six months. Around 60% of patients donated their brains to the Centro de Investigación en Enfermedades Neurológicas (CIEN) Tissue Bank, so a full postmortem neuropathological study was available for these patients [9]. At first, 125 patients with a neuropathological diagnosis and serum sample were selected. Later, NDs group was completed with 61 patients randomly selected from among those who had an exclusively clinical diagnosis and a serum sample.

2) Participants without CNS-NDs (Control group) were collected from the Vallecas Project (VP) cohort, a single-center, observational study on cognitive aging with yearly neuropsychological and neurological assessments. The VP cohort includes community-dwelling individuals, 70 to 85 years of age, capable of leading an independent life without any mental disorder (cognitive or psychiatric) and absence of any severe illness at the time of recruitment [8]. Here, we randomly selected 186 participants of the same age as the cases and an available serum sample.

The Ethics Committee of Fundación Centro de Investigación en Enfermedades Neurológicas, Instituto de Salud Carlos III (FCIEN-ISCIII), approved this study. Each patient signed a written informed consent before inclusion in the study, and when a patient was unable to sign, an authorized family member signed the consent.

Data source

Demographic and clinical data were extracted from medical records and stored in the databases of the two cohorts analyzed in this study (VP and VAS). ApoE polymorphisms (E2, E3, and E4 alleles) were determined by real-time PCR. Neuropathological diagnosis, including common neurodegenerative and vascular entities associated with dementia and recently described pathologies as aging-related tau astrogliopathy (ARTAG), and limbic-predominant age-related TDP-43 encephalopathy (LATE), was performed by a pathologist (AR) as previously described [10]. The clinical diagnosis of dementia was carried out using a previously described protocol [8].

Immunoassays

The serum samples analyzed were collected from each subject at approximately halfway through their stay in the study cohorts (VP and VAS). Serum samples were tested following the manufacturer's instructions for antibodies against hepatitis C virus (HCV; Murex anti-HCV kit v. 4.0, DiaSorin, Saluggia, Italy), hepatitis B virus (HBc; Murex anti-HBc total v. 3.0, DiaSorin, Saluggia, Italy), and hepatitis E virus (Abia HEV IgG, AB Diagnostic Systems GmbH, Berlin, Germany). The analytical sensitivity of Abia HEV IgG was 0.25 IU/ml and diagnostic performance was very high (sensitivity of 100% and specificity of 99% according to manufacturer information).

Outcome variables

The primary outcome was the presence of a CNS neurodegenerative disorder (by clinical or neuropathological diagnosis; n=186). The secondary endpoint was a neuropathological injury in the brain postmortem study (n=125) (see **Supplementary Table 1**): i) presence of vascular pathology (high vs. low burden); ii) presence of Alzheimer-type pathology (high vs. low burden) according to international guidelines criteria [11].

Statistical analysis

Stata IC 15.1 (StataCorp, Texas, USA) was used for statistical analysis and GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA) for making figures. The level of significance was established at 0.05 (2-tailed).

The differences between groups were evaluated using the Mann-Whitney U test for continuous variables and Chi-square test or Fisher's exact test for categorical variables. The association with outcome variables was assessed using multivariate logistic and ordinal regression analyses adjusted by covariables (age, sex, smoker, hypertension, dyslipidemia, diabetes mellitus, and APOEε4 allele). First, we performed univariate regression analyses. Then, we performed multivariate regression analyses adjusted by the most significant covariables, which were selected using a stepwise forward selection method (pin <0.05 and pout <0.10) and providing the odds ratio (OR) and 95% confidence intervals (95%CIs). Collinearity between variables was assessed using the Variance Inflation Factor (VIF), and interaction between independent variables in multivariate logistic regression models was studied using the likelihood-ratio test (chunk test).

Results

Participant characteristics

Characteristics of the participants are summarized in **Table 1**. Briefly, patients with CNS-NDs (NDs group) showed a lower percentage of males (p=0.016) and smokers (p<0.001) than people without neurological disorders (Control group). At the same time, the NDs group had a higher frequency of diabetes mellitus (p<0.001) and APOEε4 allele (p<0.001). A full description of neurological disorders can be found in **Supplementary Tables 1 & 2**.

Table 1. Characteristics of the elderly population, stratified by the presence of neurological disorders (dementia).

Characteristic	Controls, No. (%)a (n=186)	Patients With CNS NDs, No. (%)a (n=186)	*p-value*
Male sex	56 (30.1%)	36 (19.4%)	**0.016**
Age at time of study, y	83 (80 – 86)	83 (79 – 87)	0.467
Age at disease onset, y	-	76 (71 – 80)	-
Duration of disease, y	-	7 (5 – 9)	-
Smoker	56 (30.1%)	22 (12%)	**<0.001**
Comorbid conditions			
Hypertension	113 (60.8%)	116 (63.7%)	0.555
Dyslipidemia	99 (53.2%)	90 (49.2%)	0.437
Diabetes mellitus	25 (13.4%)	41 (22.4%)	**0.025**
***APOE* genotype**			
E2/E2	2 (1.1%)	0 (0.0%)	**<0.001**
E2/E3	13 (7.0%)	7 (3.8%)	
E2/E4	2 (1.1%)	1 (0.5%)	
E3/E3	144 (77.8%)	80 (43.0%)	
E3/E4	24 (13.0%)	83 (44.6%)	
E4/E4	0 (0.0%)	15 (8.1%)	
E4 allele carriers	26 (14.1%)	99 (53.2%)	**<0.001**

Statistics: Values are expressed as the median and interquartile range (IQR) for continuous variables and absolute count (percentage) for categorical variables.
Abbreviations: y, years; APOE, Apolipoprotein E gene; E2, E3 and E4, APOE alleles.

HEV seroprevalence and CNS neurodegenerative disorders

There were no significant differences in seroprevalences of anti-HCV (4.3% vs. 8.4%; p=0.810) and anti-HBV (24.7% vs. 23.6%; p=0.805) antibodies between NDs and Control groups. However, NDs group showed higher anti-HEV seroprevalence than control group, both with the clinical/neuropathological diagnosis (43% vs. 31.7%; p=0.025; **Figure 1A**) and the neuropathological diagnosis only (46% vs. 31.7%; p=0.009; **Figure 1B**). In adjusted logistic regression analyses, the presence of anti-HEV antibodies was related to higher adjusted odds ratio (aOR) of having CNS-NDs (**Figure 1C**, full description in **Supplementary Table 3**) with the clinical/neuropathological diagnosis (aOR=1.84 (95%CI=1.11 – 3.04); p=0.017) and neuropathological diagnosis only (aOR=2.13 (95%CI=1.23 – 3.68); p=0.007).

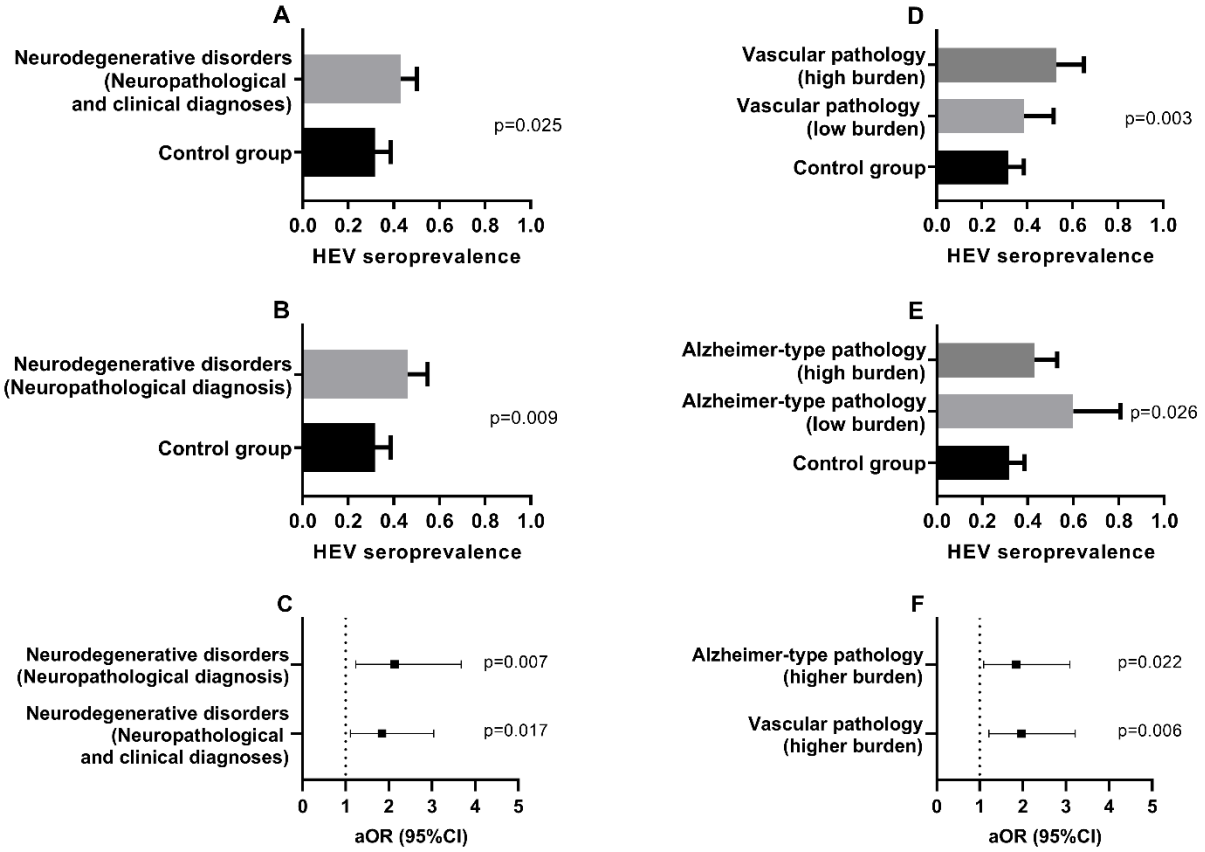


Figure 1. Relationship of hepatitis E virus (HEV) seroprevalence with neurodegenerative disorders (A–C) and neuropathological injury (D–F). P values and adjusted odds ratios (aORs) (with 95% confidence intervals [CIs]) were calculated using multivariate regression analysis.

HEV seroprevalence and CNS neuropathological injury

Taking as reference the control group, we found a significant direct correlation between anti-HEV seroprevalence and a higher burden of vascular pathology (p=0.003; **Figure 1D**) and Alzheimer-type pathology (p=0.026; **Figure 1E**). This relationship was confirmed by adjusted ordinal regression (**Figure 1F**, full description in **Supplementary Table 4**) for high burden of vascular pathology (aOR=1.97 (95%CI=1.21 – 3.21); p=0.006) and Alzheimer-type pathology (aOR=1.84 (95%CI=1.09 – 3.09); p=0.022). Besides, anti-HEV antibodies were directly related with vascular score (aOR=1.81 (95%CI=1.13 – 2.91); p=0.014) and Alzheimer score (aOR=1.80 (95%CI=1.07 – 3.05); p=0.027).

Collinearity between variables was discarded, as all significant variables that remained in logistic regression models presented a VIF value below 5. Regarding interactions, we did not find any significant interaction between HEV seroprevalence and other covariates for any of the multivariate regression analyses (see **Supplementary Table 5**).

Discussion

Our study shows that anti-HEV antibody seroprevalence was associated with CNS-NDs (mainly Alzheimer-type) and vascular pathology in the brain of older people with dementia. These results indicate a high HEV seroprevalence in older adults who developed CNS-NDs, supporting the evidence of the relationship between HEV infection and the development of neurological disorders.

In our study, HEV seroprevalence in patients with CNS-NDs was higher than in previous reports in the general Spanish population [12]. In line with our data, Fritz-Weltin *et al.* found that anti-HEV IgG seroprevalence was significantly higher in patients with central nervous system infections of unknown cause (30.7% in the whole group and more than 50% in older than 60 years) than in healthy controls (17%) [13]. This result suggests that the contribution of HEV infection to the nervous system pathology may be underestimated, and HEV screening should be done in patients with CNS neurodegenerative disease when other infectious causes have been excluded.

The cause of nervous system damage in patients with HEV infection has not been fully elucidated. Direct HEV replication in the nervous system and/or immune-mediated pathology may account for HEV extrahepatic manifestations. On the one hand, HEV can infect neuronal cells *in vitro* [6], suggesting that neurological disorders may be caused by virus replication in the nervous system. HEV can enter the brain by neuronal transport or crossing the blood-brain barrier [14]. In line with this theory, HEV RNA strands were detected in the brain and spinal cord of Mongolian gerbils and rabbits after HEV intraperitoneal inoculation [7, 14]. Pathological changes were also seen, including degeneration and necrosis of neurons, inflammatory cell infiltration, microglial nodules, and Purkinje cell necrosis [7]. HEV RNA was also detected in brain tissues of mice and rhesus macaques that were injected intravenously with HEV [6], as well as in the cerebrospinal fluid (CSF) of patients with neurological manifestations [6, 15]. Furthermore, ribavirin therapy cleared the virus in the plasma of a patient with chronic HEV, but viral RNA persisted in the CSF [3].

On the other hand, immune-mediated pathogenesis may also be behind HEV-related neurological disorders, as indicated by its higher frequency in immunocompetent than in immunocompromised patients [3]. In line with this, anti-ganglioside GM1 and GM2 antibodies have been found in HEV-infected patients. Besides, HEV infection in the brain induces mitochondrial apoptosis and pro-inflammatory response, leading to microvascular endothelial cell injuries [3, 7, 14]. Perivascular cells are also targets of HEV in the CNS. Shi et al. [7] reported damage in the brain's blood vessel wall in HEV infected gerbils, such as swelling endothelium and loss of endothelial junctional complexes.

Our study may be considered preliminary and has some limitations. Firstly, our study has a case-control design that can introduce biases and limit the interpretation of the data. For example, factors associated with degenerative disorders, such as obesity and activity, were not included in the statistical analysis due to the lack of these data. In addition, smokers are more likely to develop neurodegenerative disorders, but it is a protective factor in our study. It is probably a bias associated with the predominance of older women in the cases group, who tend to have a lower smoking habit. Secondly, the small sample size could reduce the possibility of finding significant differences in some of the comparisons. Thirdly, we did not have data regarding HEV infection in the brain of patients, and we did not evaluate the presence of HEV viremia in these patients. HEV generally causes acute hepatitis, and HEV RNA disappears from the body after a few weeks, which makes it very difficult to detect [4].

In conclusion, anti-HEV antibody seroprevalence was related to higher odds of CNS-NDs and neuropathological injury in older people. Although preliminary, our results associate HEV with vascular and Alzheimer-type pathology in CNS postmortem study, which may have important

implications for preventing this devastating disease. Additional studies should be made to confirm our findings and corroborate the impact of HEV on CNS-NDs.

List of abbreviations

Hepatitis B virus (HBV)
Hepatitis C virus (HCV)
Hepatitis E virus (HEV)
Central nervous system (CNS)
Neurodegenerative disorder (ND)
Enzyme-linked immunoassay (ELISA)
Polymerase chain reaction (PCR)
Apolipoprotein E (ApoE)
Antibodies against Hepatitis B core protein (anti-HBc)
Aging-related tau astrogliopathy (ARTAG)
Limbic-predominant age-related TDP-43 encephalopathy (LATE)
Odds Ratio (OR)
Adjusted Odds Ratio (aOR)
95% Confidence Intervals (CIs)
Cerebrospinal fluid (CSF)

Declarations

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding authors upon reasoned request.

Author contributions

Funding acquisition: SR and AR.
Study concept and design: SR, AR and MC.
Patients' selection and clinical data acquisition: AR, MC, IBG, MAZS
Sample preparation and immunoassays: SVM, MJMG, and MC.
Statistical analysis and interpretation of data: FPG, SR, and IM.
Supervision and visualization: SR and AR.
Writing – original draft preparation: FPG, SR, and IM.
Writing – Review & Editing: AR and MC.
Supervision and visualization: SR.

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Authors' information

Not applicable.

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Supplementary data

Supplementary Table 1. Summary of neuropathological diagnosis in older patients (n=126) with dementia according to international guideline criteria [1].

	Vascular pathology		Alzheimer pathology	
	Low burden	**High burden**	**Low burden**	**High burden**
Braak stage (1-6)	5.5 (5; 6)	5 (5; 6)	3 (2; 4)	5 (5; 6)
Alzheimer probability (1-4)	4 (4; 4)	4 (4; 4)	3 (2; 3)	4 (4; 4)
Vascular score (1-20)	5 (4; 6)	10 (8; 12)	10 (7; 11)	8 (5; 10)
Global Deterioration Scale (GDS)	5.5 (5; 6)	5 (5; 5)	6 (5; 6)	6 (6; 7)
Functional Assessment Staging of Alzheimer's Disease (FAST)	10 (7.25; 11)	9 (7; 11)	9.5 (7; 10)	10 (7; 11)
Clinical Dementia Rating (CDR)	3 (2.25; 3)	3 (2; 3)	3 (2; 3)	3 (2; 3)

Statistics: Values are expressed as median (interquartile range).

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Supplementary Table 2. Summary of neurological disorders in elderly patients.

	Neuropathological/clinical diagnosis	Neuropathological diagnosis	Clinical diagnosis
Patients diagnosed	186	125	61
Alzheimer disease	126 (67.7%)	98 (52.7%)	29 (15.6%)
Vascular dementia	22 (11.8%)	12 (6.5%)	10 (5.4%)
Dementia NOS	15 (8.1%)	-	15 (8.1%)
Lewy body dementia	10 (5.4%)	10 (5.4%)	-
Parkinson disease	5 (2.7%)	-	5 (2.7%)
Others	8 (4.3%)	5 (2.7%)	2(1%)
N/A	-	61 (32.8%)	125 (67.2%)

Statistics: Values are expressed as absolute count (percentage).
Abbreviations: NOS, not otherwise specified; N/A, not available.

Supplementary Table 3. Logistic regression analysis for the relationship between HEV seroprevalence and CNS neurodegenerative disorders.

A) By clinical/neuropathological diagnosis	**OR (95%CI)**	**p-value**	**aOR (95%CI)**	**p-value**
Presence of anti-HEV antibodies	1.62 (1.06 – 2.48)	**0.025**	1.84 (1.11 – 3.04)	**0.017**
Sex (male)	0.93 (0.90 – 0.97)	**<0.001**	-	n.s.
Age (years)	0.56 (0.34 – 0.90)	**0.017**	0.93 (0.89 – 0.97)	**0.002**
Smoker	0.32 (0.18 – 0.55)	**<0.001**	0.31 (0.17 – 0.58)	**<0.001**
Hypertension	1.14 (0.74 – 1.73)	0.555	-	n.s.
Dyslipidemia	0.69 (0.49 – 0.96)	**0.027**	0.55 (0.36 – 0.82)	**0.004**
Diabetes mellitus	1.86 (1.08 – 3.21)	**0.026**	2.17 (1.11 – 4.27)	**0.024**
APOEε4 allele	6.96 (4.20 – 11.53)	**<0.001**	7.26 (4.19 – 12.55)	**<0.001**

B) By neuropathological diagnosis	**OR (95%CI)**	**p-value**	**aOR (95%CI)**	**p-value**
Presence of anti-HEV antibodies	1.86 (1.17 – 2.98)	**0.009**	2.13 (1.23 – 3.68)	**0.007**
Sex (male)	0.55 (0.32 – 0.95)	**0.032**	-	n.s.
Age (years)	0.94 (0.90 – 0.98)	**0.005**	0.93 (0.88 – 0.98)	**0.006**
Smoker	0.40 (0.22 – 0.72)	**0.002**	0.37 (0.19 – 0.72)	**0.003**
Hypertension	1.08 (0.68 – 1.73)	0.744	-	n.s.
Dyslipidemia	0.65 (0.44 – 0.94)	**0.023**	0.57 (0.37 – 0.89)	**0.013**
Diabetes mellitus	1.90 (1.05 – 3.44)	**0.035**	-	n.s.
APOEε4 allele	6.63 (3.85 – 11.40)	**<0.001**	7.62 (4.22 – 13.74)	**<0.001**

Statistics: First, we performed univariate logistic regression analyses. Then we performed multivariate logistic regression analyses adjusted by the most significant variables, which were selected by a stepwise forward selection method (pin <0.05 and pout <0.10). Significant differences are shown in bold.
Abbreviations: CNS: central nervous system; ND: neurodegenerative disorder; HEV: hepatitis E virus; OR: odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confidence interval; p-value: level of significance; n.s., not significant.

Supplementary Table 4. Ordinal regression analysis for the relationship between HEV seroprevalence and CNS neuropathological injuries.

A) High burden of vascular pathology	**OR (95%CI)**	**p-value**	**aOR (95%CI)**	**p-value**
Presence of anti-HEV antibodies	1.97 (1.26 – 3.10)	**0.003**	1.97 (1.21 – 3.21)	**0.006**
Sex (male)	0.55 (0.32 – 0.94)	**0.029**	-	n.s.
Age (years)	0.95 (0.91 – 0.99)	**0.014**	0.95 (0.91 – 0.99)	**0.023**
Smoker	0.38 (0.21 – 0.69)	**0.001**	0.37 (0.20 – 0.69)	**0.002**
Hypertension	1.16 (0.74 – 1.83)	0.514	-	n.s.
Dyslipidemia	0.66 (0.46 – 0.95)	**0.027**	0.66 (0.44 – 0.98)	**0.041**
Diabetes mellitus	1.71 (0.98 – 2.99)	0.057	-	n.s.
APOEε4 allele	4.27 (2.66 – 6.84)	**<0.001**	4.36 (2.66 – 7.13)	**<0.001**
B) High burden of Alzheimer-type pathology	**OR (95%CI)**	**p-value**	**aOR (95%CI)**	**p-value**
Presence of anti-HEV antibodies	1.67 (1.06 – 2.63)	**0.026**	1.84 (1.09 – 3.09)	**0.022**
Sex (male)	0.53 (0.31 – 0.91)	**0.021**	-	n.s.
Age (years)	0.93 (0.89 – 0.97)	**0.001**	0.92 (0.88 – 0.97)	**0.001**
Smoker	0.39 (0.22 – 0.70)	**0.002**	0.35 (0.18 – 0.68)	**0.002**
Hypertension	1.10 (0.70 – 1.75)	0.678	-	n.s.
Dyslipidemia	0.68 (0.47 – 0.99)	**0.042**	0.62 (0.41 – 0.95)	**0.029**
Diabetes mellitus	1.71 (0.97 – 3.02)	0.063	-	n.s.
APOEε4 allele	7.32 (4.34 – 12.35)	**<0.001**	8.33 (4.76 – 14.59)	**<0.001**
C) High vascular score	**OR (95%CI)**	**p-value**	**aOR (95%CI)**	**p-value**
Presence of anti-HEV antibodies	1.92 (1.23 – 3.01)	**0.004**	1.81 (1.13 – 2.91)	**0.014**
Sex (male)	0.55 (0.32 – 0.94)	**0.028**	-	n.s.
Age (years)	0.96 (0.92 – 1.00)	**0.029**	-	n.s.
Smoker	0.39 (0.21 – 0.70)	**0.002**	0.41 (0.22 – 0.77)	**0.005**
Hypertension	1.13 (0.72 – 1.78)	0.593	-	n.s.
Dyslipidemia	0.68 (0.47 – 0.98)	**0.037**	0.66 (0.44 – 0.97)	**0.036**
Diabetes mellitus	1.60 (0.93 – 2.74)	0.090	-	n.s.
APOEε4 allele	3.94 (2.48 – 6.26)	**<0.001**	4.03 (2.50 – 6.49)	**<0.001**
D) High Alzheimer score	**OR (95%CI)**	**p-value**	**aOR (95%CI)**	**p-value**
Presence of anti-HEV antibodies	1.65 (1.05 – 2.60)	**0.031**	1.80 (1.07 – 3.05)	**0.027**
Sex (male)	0.53 (0.31 – 0.90)	**0.019**	-	n.s.
Age (years)	0.93 (0.89 – 0.97)	**0.002**	0.92 (0.8 – 0.97)	**0.002**

Smoker	0.39 (0.22 – 0.70)	**0.002**	0.35 (0.18 – 0.67)	**0.002**
Hypertension	1.10 (0.70 – 1.75)	0.677	-	n.s.
Dyslipidemia	0.69 (0.48 – 0.99)	**0.046**	0.62 (0.40 – 0.95)	**0.027**
Diabetes mellitus	1.75 (0.99 – 3.09)	0.053	-	n.s.
APOEε4 allele	7.99 (4.71 – 13.56)	**<0.001**	8.88 (5.05 – 15.61)	**<0.001**

Statistics: First, we performed univariate ordinal regression analyses. Then we performed multivariate ordinal regression analyses adjusted by the most significant variables, which were selected by a stepwise forward selection method (pin <0.05 and pout <0.10). Significant differences are shown in bold.
Abbreviations: CNS: central nervous system; ND: neurodegenerative disorder; HEV: hepatitis E virus; OR: odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confidence interval; p-value: level of significance; n.s., not significant.

Supplementary Table 5. Study on interactions between HEV seroprevalence and independent covariates in the multivariate logistic regression.

Type of model	Likelihood-ratio	Likelihood-ratio test (p-value)
A) Logistic regression for clinical/neuropathological diagnosis		
With interactions	114.72	0.840
Without interactions	112.65	
B) Logistic regression for neuropathological diagnosis		
With interactions	86.89	0.646
Without interactions	84.39	
C) Ordinal regression for a high burden of vascular pathology		
With interactions	72.31	0.269
Without interactions	67.12	
D) Ordinal regression for a high burden of Alzheimer-type pathology		
With interactions	96.79	0.543
Without interactions	93.70	
E) Ordinal regression for high vascular pathology		
With interactions	60.76	0.180
Without interactions	55.87	
F) Ordinal regression for high Alzheimer's score		
With interactions	65.05	0.206
Without interactions	59.14	

Statistics: First, we performed multivariate regression analyses with interactions between HEV seroprevalence and independent covariates. Then we performed multivariate regression analyses without interactions. We calculated the likelihood ratio for both models and employed the likelihood-ratio test (chunk test) to establish the significance (p-value) of the interactions.