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A case-control study into risk factors for acute hepatitis E in the Netherlands, 2015–2017

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SUMMARY

Objectives: A case-control study was performed (2015–2017) to identify risk factors for acute hepatitis E in the Netherlands.

Methods: A questionnaire on potential sources of hepatitis E virus (HEV) exposure, health and socio-demographics was completed by 376 patients with acute hepatitis E, and 1534 controls matched for age, gender and region of residence.

Results: Traditional Dutch dry raw sausages of pork muscle meat, called “cervelaat”, “snijworst”, and “boerenmetworst” were reported by 72% of the patients, and 46% of controls (aOR 3.0; 95%CI 2.2–4.1), with a population attributable fraction (PAF) of 48%. Direct contact with pigs and working with a septic tank were strong risk factors (aOR 3.1; 95%CI 1.3–7.3 and aOR 6.9; 95%CI 1.2–40.8, respectively), with a low PAF (2% and 1%, respectively). Host risk factors were pre-existing liver disease (aOR 3.8; 95%CI 2.0–7.1), diabetes (aOR 2.1; 95%CI 1.4–3.2), immunosuppressive medication (aOR 2.5; 95%CI 1.5–4.1), and gastric acid inhibitors (aOR 2.3; 95%CI 1.7–3.1).

Conclusions: Dry raw pork sausages were the major source of HEV infection among our study population. The prevalence and cause of HEV contamination in these pork muscle meat products require further investigation. Infrequently reported, yet strong risk factors were contact with pigs, or a septic tank.

Introduction

Locally acquired hepatitis E has long been considered uncommon in western countries and mainly associated with travel to developing countries.[1] However, the number of autochthonous cases with hepatitis E virus (HEV) genotype 3 (gt3) reported in many European countries has increased by tenfold between 2005 and 2015.[1–4] In the Netherlands, a five-fold increase in the number of laboratory confirmed HEV infections was reported in the national sentinel laboratory surveillance system as from 2014. The majority of HEV infections in the general population resolve with no symptoms or pass as self-limiting hepatitis. Most clinical HEV infections and severe disease are observed in middle-aged and elderly males, who often have other co-existing illnesses.[2] Immunocompromised patients, such as transplant patients, have a risk of developing a chronic HEV infection, often with no or non-specific symptoms, which can progress to liver cirrhosis.[1] An increase in HEV seropositive blood donors was also observed in the Netherlands, with the strongest increase among young blood donors, suggesting a recent increase in infection pressure.[5] It is currently unclear what causes the increase of hepatitis E in the Netherlands and other European countries. [6]

The detection of HEV gt3 strains in humans that are similar to strains found in pigs and wild boar, suggests zoonotic transmission,[7–9] either via contaminated food (such as raw or undercooked pork) or the environment.[10,11] Although pigs are recognized as the main reservoir of HEV gt3, little is known about the actual sources of infection, transmission routes and host risk factors (such as underlying illness and medication) of acute hepatitis E in the Netherlands. To support design of appropriate control and preventive strategies, we conducted a case-control study to identify the most likely specific food and/or environmental sources and potential (host) risk factors for symptomatic hepatitis E in the Netherlands.

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Materials and methods

Study design & data collection

A nationwide case-control study was conducted between June 2015 and October 2017 (28 months). Cases were patients of all ages with a laboratory confirmed acute HEV infection (detection of HEV RNA and/or HEV IgM). In total 23 regional Public Health Laboratories across the Netherlands, that perform HEV diagnostics using routine detection methods (polymerase chain reaction (PCR) or serology using the Wantai or Mikrogen assay (IgM and IgG)) recruited cases into the study. In addition, patients were recruited through municipal health services who were notified about hepatitis E patients by non-participating laboratories. The laboratories are listed in the Acknowledgements ranked by the number of recruited participants. For each patient with hepatitis E, the laboratory sent a study envelope to the general practitioner or hospital specialist that had requested HEV diagnostics. The study envelope contained an envelope for the patient, with an information letter, informed consent form and structured questionnaire. For development of our questionnaire, we used previous hepatitis E questionnaires from studies on risk factors for acute Hepatitis E in the Netherlands, United Kingdom and France. We consulted the Netherlands Food and Consumer Product Safety Authority (NVWA) about specific food items, and we added other potential transmission routes which we identified from literature.[10–14] Study participants were asked about demographics, symptoms of illness, medical history, food consumption, foreign travel, contact with farm animals, with pets and pet food, and contact with water. Food consumption assessment focussed on a wide range of specified pork meat products, but also beef products, game meat, organ meats of any animal, ready to eat products with meat, shellfish, unpasteurized milk, raw vegetables, soft fruit and berries, ready to eat salads, eating outside the home (e.g. restaurants). A recall-period of 2 months before onset of symptoms was used, based on the maximum incubation period of hepatitis E. Cases were asked to complete and submit the self-administered paper questionnaires, together with the informed consent form. The participating laboratory submitted the serum sample to our laboratory for detection and genotyping by real time PCR. For virus identification, a fragment of 493 bp of the gene encoding the major capsid protein (ORF2) was sequenced.[15] A unique identifier was used to link the returned completed case questionnaires with available serum samples.

For comparison, a paper questionnaire similar to the case questionnaire was sent by post to population controls from the general population. For the controls, the questions about possible exposures referred to the 2 months before completion of the questionnaire. We aimed to include 4 controls per each case, inviting new controls during each study month, through random selection from the municipality registry and frequency matched to the cases based on age, gender and area of residence. No blood samples were collected from these population controls.

Additionally, we aimed to enrol controls with better resemblance to the clinical status of the case patients, and without recent HEV exposure, as HEV infection frequently goes unnoticed and is common (27% HEV IgG-antibodies[16]) among the general population. Therefore, eleven laboratories also recruited patients who had been tested for clinical reasons, with a negative outcome for HEV (RNA and IgM negative or RNA and IgM/IgG negative) as HEV-negative controls into the study, using the same logistic procedures as for the case enrolment.

The study protocol titled “HEVIG” (acronym for Hepatitis E Virus Infections in the Community) was approved by the Medical Ethics Committee of the University of Utrecht before enrolment of study participants (protocol number 15-131/C). Written informed consent was obtained from each participant. All personal information was analyzed anonymized and handled carefully with strict confidentiality.

Data analysis

Participants (4 cases, 1 control) with missing data on age and/or sex were excluded from analyses. One case reported to have been tested positive for HEV infection in the preceding year, suggesting possible chronic HEV infection, and was therefore excluded from the analysis.

Statistical analyses were performed using STATA software package version 14. ORs were calculated with 95% confidence intervals and two-tailed p-values, considered significant at $P < 0.05$. To assess the possible association between various exposures and autochthonous acute hepatitis E in the Netherlands, univariate logistic regression analyses and Chi-square tests were performed. Variables that were positively or negatively associated with acute hepatitis E ($P \leq 0.10$) in univariate logistic regression, were entered into a multivariate logistic regression model, together with gender and categories of age. Starting with a full multivariable model, variables were removed by backward selection with a cut-off of $P < 0.05$ to yield a final model with the most relevant independent associations. For all variables of the final model, the population attributable fraction (PAF) with 95% confidence interval was calculated. PAF can be interpreted as the proportion of cases that would be prevented following elimination of the risk factor, assuming the risk factor is causal.[17]

Results

Characteristics of the study population

The questionnaire response rate was 64% among 597 patients with laboratory confirmed acute hepatitis E, who had been invited to the study between June 2015 and October 2017, via regional Public Health laboratories across the Netherlands. The questionnaire response rate among general population control persons was 26% out of 5511 invited individuals. In addition, we received completed questionnaires from 80 laboratory confirmed HEV-negative control persons who had been tested for HEV for clinical reasons by the participating laboratories. Table 1 shows the demographic characteristics of all study participants. After exclusion of participants for reasons described in the methods section, the study population included 376 cases with laboratory confirmed acute hepatitis E (60% male; median age 59, interquartile range 49–69), and 1534 controls, including 79 HEV-negative controls (60% male; median age 60, interquartile range 48–69). Serum samples were available of 351 cases (93%), and HEV RNA was detected in 236 samples (67%). Only HEV gt3 was identified (182/236), and the remaining 54 HEV RNA-positive samples were not typeable. Serum samples of 60 HEV-negative controls (76%) were available (10 HEV RNA and IgM negative and 50 HEV RNA and IgM/IgG negative). A quarter of our participants with hepatitis E (23%; $n = 85$) reported hospital admission for hepatitis E. Most occurring symptoms reported by hepatitis E patients were fatigue, dark urine, nausea, stomach ache and headache (see Table 1).

Host risk factors for acute symptomatic hepatitis E

Table 2 shows the statistically significant risk factors for symptomatic acute hepatitis E in the Netherlands, assessed by logistic regression, and quantified as adjusted odds ratios (aOR), population attributable fractions (PAF) and 95% confidence intervals (95%CI). Multivariate logistic regression analyses, adjusted for age and gender, identified diabetes and pre-existing liver disease as possible host risk factors for symptomatic acute hepatitis E (aOR

Table 1
Demographic and health related characteristics of study participants.

	Patients with acute hepatitis E ($n = 376$)		Control persons ($n = 1534$)	
	N	*%*	*N*	*%*
Gender				
Men	225	*59.8*	926	*60.4*
Women	151	*40.2*	608	*39.6*
Median age in years (IQR)	58.5	*(49–69)*	59.5	*(48–69)*
Age groups				
<40	41	*10.9*	236	*15.4*
40–50	62	*16.5*	203	*13.2*
50–60	101	*26.8*	341	*22.2*
60–70	93	*24.7*	426	*27.8*
>70	79	*21.0*	328	*21.4*
Country of birth				
The Netherlands	353	*93.8*	1440	*93.9*
Any other country	21	*5.6*	77	*5.0*
NA	2	*0.5*	17	*1.1*
Level of education				
Low (Elementary school, no education)	26	*6.9*	83	*5.4*
Intermediate (LBO, MAO, MBO, VAO)	272	*72.3*	931	*60.7*
High (HBO, WO)	69	*18.4*	492	*32.1*
NA	9	*2.4*	28	*1.8*
HEV PCR result				
Positive, Cp <30	131	*34.8*	0	*0.0*
Positive, Cp 30–40	102	*27.1*	0	*0.0*
Dubious, Cp >40	3	*0.8*	0	*0.0*
Negative	115	*30.6*	60	*3.9*
Sample not available	25	*6.7*	1474	*96.1*
Hospital admission for hepatitis E	85	*23.0*	NA	*NA*
Health complaints past 2 months				
Jaundice	104	*27.7*	11	*0.7*
Fever	104	*27.7*	100	*6.5*
Nausea	172	*45.7*	205	*13.4*
Vomiting	76	*20.2*	75	*4.9*
Diarrhoea	107	*28.8*	291	*19.4*
Stomach ache	162	*43.3*	265	*17.3*
Light (decoloured) stools	117	*31.1*	84	*5.5*
Dark urine	223	*59.3*	132	*8.6*
Itching	155	*41.2*	266	*17.3*
Fatigue	297	*79.0*	574	*37.4*
Headache	160	*42.6*	460	*30.0*
Neurological symptoms	114	*30.3*	143	*9.3*
NA	4	*1.1*	30	*2.0*
Underlying diseases past 6 months				
No disease or disorders	74	*19.7*	850	*55.4*
Liver disease	37	*9.8*	31	*2.0*
Kidney disease	20	*5.3*	17	*1.1*
Chronic bowel disease	13	*3.5*	24	*1.6*
Chronic lung disease	44	*11.7*	79	*5.2*
Neurological disorder	20	*5.3*	30	*2.0*
Thyroid gland disorder	22	*5.9*	65	*4.2*
Heart disease	33	*8.8*	84	*5.5*
High blood pressure	81	*21.5*	266	*17.3*
Thrombosis	14	*3.7*	28	*1.8*
Malignancy	27	*7.2*	51	*3.3*
Disorder of the immune system	21	*5.6*	19	*1.2*
Diabetes	81	*21.5*	101	*6.6*
Absence of the spleen	3	*0.8*	8	*0.5*
Increased cholesterol	78	*20.7*	216	*14.1*
Stomach ulcer	7	*1.9*	16	*1.0*
Rheumatoid arthritis	50	*13.3*	86	*5.6*
Other chronic rheumatism >3 months	30	*8.0*	43	*2.8*
Epilepsy	3	*0.8*	11	*0.7*
Received (organ) transplant				
No	360	*95.7*	1506	*98.2*
Yes	10	*2.7*	11	*0.7*
NA	6	*1.6*	17	*1.1*
Meat consumption				
No, never	0	*0.0*	4	*0.3*
No, only in the past	1	*0.3*	29	*1.9*
Yes	373	*99.2*	1476	*96.2*
NA	2	*0.5*	25	*1.6*

(*continued on next page*)

Table 1 (*continued*)

	Patients with acute hepatitis E ($n = 376$)		Control persons ($n = 1534$)	
	N	*%*	*N*	*%*
Alcohol consumption				
No, never	61	*16.2*	172	*11.2*
No, only in the past	70	*18.6*	194	*12.7*
Yes	240	*63.8*	1156	*75.4*
NA	5	*1.3*	12	*0.8*
Smoking				
No, never	108	*28.7*	405	*26.4*
Past smoker	200	*53.2*	903	*58.9*
Current smoker	67	*17.8*	220	*14.3*
NA	1	*0.3*	6	*0.4*
Traveling history past 2 months				
No	256	*68.1*	1046	*68.2*
Yes	112	*29.8*	469	*30.6*
NA	8	*2.1*	19	*1.2*

NA; not available.
IQR: interquartile range. PCR: polymerase chain reaction. Cp: crossing point.

2.1; 95%CI 1.4–3.2 and aOR 3.8; 95%CI 2.0–7.1, respectively), as well as the use of immunosuppressive medication and gastric acid inhibitors (aOR 2.5; 95%CI 1.5–4.1 and aOR 2.3; 95%CI 1.7–3.1, respectively). In addition, having "no pre-existing diseases and disorders" was reported less often by cases than controls (aOR 0.2; 95%CI 0.2–0.3). High blood pressure was associated with a lower risk of acute symptomatic hepatitis E (aOR 0.5; 95%CI 0.3–0.7).

Exposure related risk factors for acute symptomatic hepatitis E

The strongest individual risk factor was contact with contaminated water via working with a septic tank (aOR 6.9; 95%CI 1.2–40.8). However, this exposure was rarely reported among the participants (4/376 cases; 4/1534 controls), resulting in a PAF of 1%. The second strongest risk factor was contact with pigs (aOR 3.1; 95%CI 1.3–7.3), which was also reported by few participants (13/376 cases; 29/1534 controls) (PAF 2%).

Various pork meat products were identified as risk factor for acute hepatitis E. The highest odds ratios were found for three different dry sausages of minced raw pork meat, that are consumed sliced on bread, called "cervelaat" (aOR 2.0; 95%CI 1.4–2.7), "snijworst" (aOR 1.9; 95%CI 1.3–2.6), and "boerenmetworst" (aOR 1.8; 95%CI 1.3–2.4). Consumption of any of these dry raw sausages was reported by 72% of the cases, compared to 46% of the controls (combined aOR 3.0; 95%CI 2.2–4.1), resulting in a total PAF of 48%. Other ready-to-eat (but not all raw) pork products that were identified as risk factors included pre-packed liver sausage/pate (aOR 1.5; 95%CI 1.1–2.1; PAF 18%), pork shoulder ham (aOR 1.5; 95%CI 1.1–2.0; PAF 21%) and smoked bacon (aOR 1.6; 95%CI 1.2–2.2; PAF 15%). Among pork products that require cooking, "fresh pork sausage" of minced pork meat was identified as risk factor (aOR 1.7; 95%CI 1.2–2.2; PAF 26%). Cases were more likely to usually buy meat at the supermarket (aOR 1.6; 95%CI 1.0–2.6; PAF 34%). Only nine cases (2.4%) reported "I don't eat pork", compared to 20% of controls (aOR 0.2; 95% CI 0.1–0.4). Due to this low number of cases who reported "I don't eat pork" plus one vegetarian case, a subanalysis within this group that doesn't consume pork was not feasible. Pre-packed lettuce (aOR 1.5; 95%CI 1.1–2.0; PAF 18%) was the only risk factor among all investigated fruits and vegetables.

Traveling abroad during the past 2 months and consumption of shellfish were not identified as risk factor for acute hepatitis E. Negative associations were observed for a few pork food products. Pork butt ("procureurlapje"), cordon bleu, and chipolata sausage were associated with lower risk of acute hepatitis E (aOR 0.6; 95%CI 0.4–0.8, aOR 0.2; 95%CI 0.1–0.4 and aOR 0.4; 95%CI 0.2–0.6, respectively). Compared to controls, cases also reported less consumption of steak (beef) and wild duck (aOR 0.7; 95%CI 0.5–1.0 and aOR 0.3; 95%CI 0.1–0.6, respectively), and less consumption of forest fruits such as raspberries, blackberries, blueberries, red berries and black currant (aOR 0.4; 95%CI 0.3–0.6). Recreation in the woods was also associated with lower risk of acute hepatitis E (aOR 0.5; 95%CI 0.4–0.6).

Sub-analyses performed among 236 RNA-positive patients versus 1534 control persons, as well as sub-analyses among all 376 cases versus 79 HEV-negative controls, yielded similar but less risk factors with lower statistical significance (data not shown) due to low numbers of participants in those sub-analyses.

Discussion

With 376 hepatitis E cases and 1534 controls, this study is to our knowledge the largest case-control study on risk factors for acute hepatitis E in Europe. We identified host, food, and environmental risk factors for acute hepatitis E between 2015 and 2017 in the Netherlands, which provides relevant information for control and preventive strategies and concrete advice to vulnerable high-risk groups. From the viewpoint of the general population, the main risk factors for acute symptomatic hepatitis E in the Netherlands were pork meat products; dry raw pork sausages, pre-packed liver sausage or pate, smoked bacon, pork shoulder ham, and fresh pork sausage were identified as independent risk factors within the study period. Correspondingly, the statement "I don't eat pork" was hardly reported by cases, as compared to controls. Transmission of HEV via raw and processed pork products has been strongly suggested in other studies before.[10,11,18] A substantial role of meat products in the exposure to HEV was recently also suggested by a study among blood donors in our country, showing a significantly lower prevalence of HEV exposure among vegetarian blood donors compared to meat-eating donors.[19]

Assuming a causal relation, half (48%) of all hepatitis E cases would be prevented if the risk factors dry raw pork sausages such as "cervelaat", "boerenmetworst" and "snijworst" would be eliminated. The same type of dry raw pork sausages were recently also identified as risk factors for exposure to HEV, in a recent study on risk factors for anti-HEV IgG antibodies (indicative of long-term exposure) among blood donors in the Netherlands.[20] In a recent similar case-control study by Faber et al. in neighbouring country Germany, dry raw sausages were not identified as risk factors for hepatitis E.[21] Their main risk factors were liver sausage or pate and boiled sausage such as frankfurter/wiener type sausages. However, production methods of sausages can vary between different regions and countries. Comparison of risk factors and

Table 2
Univariate and multivariate logistic regression analyses of risk factors associated with acute hepatitis E in the Netherlands 2015-2017.
The model was based on backward selection of variables with $P < 0.10$ in univariate analyses, and adjusted for age and gender. Only variables with $P < 0.05$ in uni- and/or multivariate analyses are shown.

	n (%) of 376 cases	*n* (%) of 1534 controls	Univariate analyses		Multivariate analyses		PAF (95%CI)
			OR (95% CI)	P-value	aOR (95% CI)	*P*-value	
A. Demographics and medical condition							
Gender				0.852		0.067	
Men	225 *(59.8)*	926 *(60.4)*	1.0		1.0		
Women	151 *(40.2)*	608 *(39.6)*	1.0 (0.8–1.3)		1.3 (1.0–1.8)		NA
Age groups in years				0.035		<0.001	
<40	41 *(10.9)*	236 *(15.4)*	1.0		1.0		
40–50	62 *(16.5)*	203 *(13.2)*	1.8 (1.1–2.7)		1.7 (1.0–3.0)		NA
50–60	101 *(26.9)*	341 *(22.2)*	1.7 (1.1–2.5)		1.1 (0.7–1.8)		NA
60–70	93 *(24.7)*	426 *(27.8)*	1.3 (0.8–1.9)		0.7 (0.4–1.1)		NA
>70	79 *(21.0)*	328 *(21.4)*	1.4 (0.9-2.1)		0.6 (0.3–1.0)		NA
Level of education				<0.001			
Low (elementary school, no education)	26 *(6.9)*	83 *(5.4)*	1.0				
Intermediate (secondary/vocational education)	272 *(72.3)*	931 *(60.7)*	0.9 (0.6–1.5)				
High (college or university)	69 *(18.4)*	492 *(32.1)*	0.4 (0.3–0.7)				
NA	9 *(2.4)*	28 *(1.8)*	1.0 (0.4–2.5)				
Underlying diseases							
Liver disease	37 *(9.8)*	31 *(2.0)*	5.3 (3.2–8.7)	<0.001	3.8 (2.0–7.1)	<0.001	0.07 (0.05–0.08)
Kidney disease	20 *(5.3)*	17 *(1.1)*	5.0 (2.6–9.7)	<0.001			
Chronic bowel disease	13 *(3.5)*	24 *(1.6)*	2.2 (1.1–4.5)	0.020			
Chronic lung disease	44 *(11.7)*	79 *(5.2)*	2.4 (1.7–3.6)	<0.001			
Neurological disorder	20 *(5.3)*	30 *(2.0)*	2.8 (1.6–5.0)	<0.001			
Heart disease	33 *(8.8)*	84 *(5.5)*	1.7 (1.1–2.5)	0.018			
High blood pressure	81 *(21.5)*	266 *(17.3)*	1.3 (1.0–1.7)	0.059	0.5 (0.3–0.7)	<0.001	NA
Thrombosis	14 *(3.7)*	28 *(1.8)*	2.1 (1.1–4.0)	0.028			
Malignancy	27 *(7.2)*	51 *(3.3)*	2.2 (1.4–3.6)	0.001			
Disorder of the immune system	21 *(5.6)*	19 *(1.2)*	4.7 (2.5-8.9)	<0.001			
Diabetes	81 *(21.5)*	101 *(6.6)*	3.9 (2.8–5.4)	<0.001	2.1 (1.4–3.2)	0.001	0.11 (0.06–0.15)
Increased cholesterol	78 *(20.7)*	216 *(14.1)*	1.6 (1.2–2.1)	0.001			
Rheumatoid arthritis	50 *(13.3)*	86 *(5.6)*	2.6 (1.8–3.7)	<0.001			
Other chronic rheumatism >3 months	30 *(8.0)*	43 *(2.8)*	3.0 (1.9–4.9)	<0.001			
No diseases or disorders	74 *(19.7)*	850 *(55.4)*	0.2 (0.2–0.3)	<0.001	0.2 (0.2–0.3)	<0.001	NA
Medication							
Antibiotics/antivirals	71 *(18.9)*	165 *(10.8)*	1.9 (1.4–2.6)	<0.001			
Anti-inflammatory /immunosuppressive medication	66 *(17.6)*	66 *(4.3)*	4.7 (3.3–6.8)	<0.001	2.5 (1.5–4.1)	<0.001	0.11 (0.06–0.13)
Gastric acid inhibitors	182 *(48.4)*	334 *(21.8)*	3.4 (2.7–4.3)	<0.001	2.3 (1.7–3.1)	<0.001	0.27 (0.20–0.33)
Cholesterol reducers	117 *(31.1)*	313 *(20.4)*	1.8 (1.4–2.3)	<0.001			
Antihypertensives	117 *(31.1)*	367 *(23.9)*	1.4 (1.1–1.8)	0.004			
Chemotherapy	8 *(2.1)*	12 *(0.8)*	2.8 (1.1–6.8)	0.028			
Insulin	34 *(9.0)*	34 *(2.2)*	4.4 (2.7–7.2)	<0.001			
Received (organ) transplant				0.004			
No	360 *(95.7)*	1506 *(98.2)*	1.0				
Yes	10 *(2.7)*	11 *(0.7)*	3.8 (1.6–9.0)				
NA	6 *(1.6)*	17 *(1.1)*	1.5 (0.6–3.8)				
Alcohol consumption				<0.001			
No, never	61 *(16.2)*	172 *(11.2)*	1.0				
No, only in the past	70 *(18.6)*	194 *(12.7)*	1.0 (0.7–1.5)				
Yes	240 *(63.8)*	1156 *(75.4)*	0.6 (0.4–0.8)				
NA	5 *(1.3)*	12 *(0.8)*	1.2 (0.4–3.5)				
B. Food consumption							
Usually buy meat at the supermarket	341 *(90.7)*	1296 *(84.5)*	1.8 (1.2–2.6)	0.002	1.6 (1.0–2.6)	0.046	0.34 (0.00–0.55)
No pork food products	9 *(2.4)*	305 *(19.9)*	0.1 (0.1–0.2)	<0.001	0.2 (0.1-0.4)	<0.001	NA
Prepared/Cooked pork products							
Fresh sausage/ Roast sausage	239 *(63.6)*	796 *(51.9)*	1.6 (1.3–2.0)	<0.001	1.7 (1.2–2.2)	0.001	0.26 (0.11–0.35)
Dutch "Slavink"	188 *(50.0)*	594 *(38.7)*	1.6 (1.3–2.0)	<0.001			
Cordon bleu	111 *(29.5)*	579 *(37.7)*	0.7 (0.5–0.9)	0.003	0.6 (0.4–0.8)	0.002	NA
Ground meat "half beef, half pork"	230 *(61.2)*	817 *(53.3)*	1.4 (1.1–1.7)	0.006			
Pork chop	164 *(43.6)*	510 *(33.3)*	1.6 (1.2–2.0)	<0.001			

(*continued on next page*)

Table 2 (*continued*)

	n (%) of 376 cases	*n* (%) of 1534 controls	Univariate analyses		Multivariate analyses		PAF (95%CI)
			OR (95% CI)	P-value	aOR (95% CI)	*P*-value	
Pork butt/ "Procureurlapje"	*24 (6.4)*	349 *(22.8)*	0.2 (0.1–0.4)	<0.001	0.2 (0.1–0.4)	<0.001	NA
Smoked sausage	218 *(58.0)*	756 *(49.3)*	1.4 (1.1–1.8)	0.003			
Chipolata	29 *(7.7)*	367 *(23.9)*	0.3 (0.2–0.4)	<0.001	0.4 (0.2–0.6)	<0.001	NA
Smoked bacon	147 *(39.1)*	508 *(33.1)*	1.3 (1.0–1.6)	0.029	1.6 (1.2–2.2)	0.004	0.15 (0.07–0.21)
Dry raw pork sausages[a]	270 *(71.8)*	700 *(45.6)*	3.0 (2.4–3.9)	<0.001	3.0 (2.2–4.1)	<0.001	0.48 (0.39–0.54)
Dutch "Boerenmetworst"	141 *(37.5)*	366 *(23.9)*	1.9 (1.5–2.4)	<0.001	1.8 (1.3–2.4)	<0.001	0.17 (0.09–0.22)
Dutch "Cervelaat"	165 *(43.9)*	403 *(26.3)*	2.2 (1.7–2.8)	<0.001	2.0 (1.4–2.7)	<0.001	0.22 (0.13–0.28)
Dutch "Snijworst"	107 *(28.5)*	266 *(17.3)*	1.9 (1.5–2.5)	<0.001	1.9 (1.3–2.6)	0.001	0.14 (0.07–0.18)
Sausages consumed sliced on bread							
Liver cheese/ Leberkäse	78 *(20.7)*	247 *(16.1)*	1.4 (1.0–1.8)	0.032			
Pork shoulder ham	242 *(64.4)*	742 *(48.4)*	1.9 (1.5–2.4)	<0.001	1.5 (1.1–2.0)	0.010	0.21 (0.06–0.32)
Pariser/ Bologna sausage	69 *(18.4)*	176 *(11.5)*	1.7 (1.3–2.4)	<0.001			
Snack sausages	60 *(16.0)*	165 *(10.8)*	1.6 (1.1–2.2)	0.005			
Spreadable pork							
Prepacked liver sausage/ pate[b]	202 *(53.7)*	599 *(39.1)*	1.8 (1.4–2.3)	<0.001	1.5 (1.1–2.1)	0.005	0.18 (0.05–0.28)
Beef							
Steak	176 *(46.8)*	839 *(54.7)*	0.7 (0.6–0.9)	0.006	0.7 (0.5–1.0)	0.030	NA
Filet Americain/ Steak tartare	179 *(47.6)*	598 *(39.0)*	1.4 (1.1–1.8)	0.002			
Ox sausage	50 *(13.3)*	271 *(17.7)*	0.7 (0.5–1.0)	0.043			
Smoked beef	138 *(36.8)*	444 *(29.0)*	1.4 (1.1–1.8)	0.003			
Wild game meat							
Deer	15 *(4.0)*	112 *(7.3)*	0.5 (0.3–0.9)	0.023			
Duck	10 *(2.7)*	130 *(8.5)*	0.3 (0.2–0.6)	<0.001	0.3 (0.1–0.6)	0.002	NA
Ready-made products with meat							
Soup with meat[c]	81 *(58.3)*	453 *(46.8)*	1.6 (1.1–2.3)	0.012			
Shellfish							
Mussels	68 *(18.1)*	357 *(23.3)*	0.7 (0.5–1.0)	0.031			
Oysters	7 *(1.9)*	68 *(4.4)*	0.4 (0.2–0.9)	0.026			
Fruits and vegetables							
Vegetables or fruit from a vegetable garden	67 *(17.8)*	428 *(27.9)*	0.6 (0.4–0.7)	<0.001			
Vegetables or fruit purchased from a farm	38 *(10.1)*	288 *(18.8)*	0.5 (0.3–0.7)	<0.001			
Sun/ semi-dried tomatoes	67 *(17.8)*	376 *(24.5)*	0.7 (0.5–0.9)	0.006			
Prepacked lettuce	230 *(61.2)*	859 *(56.0)*	1.2 (1.0–1.6)	0.070	1.5 (1.1–2.0)	0.015	0.20 (0.06–0.31)
Fresh herbs	95 *(25.3)*	493 *(32.1)*	0.7 (0.6–0.9)	0.010			
Forest fruits[d]	75 *(20.0)*	546 *(35.6)*	0.5 (0.3–0.6)	<0.001	0.4 (0.3–0.6)	<0.001	NA
Dried fruits	40 *(10.6)*	238 *(15.5)*	0.6 (0.5–0.9)	0.017			
Homemade apple juice	8 *(2.1)*	71 *(4.6)*	0.4 (0.2–0.9)	0.033			
C. Outdoor activities & contact with animals							
Restaurant visit			<0.001				
<1 time per month	223 *(59.3)*	768 *(50.1)*	1.0				
>1 time per month	153 *(40.7)*	766 *(49.9)*	0.7 (0.5–0.9)				
Outdoor swimming (lake/ river/ sea)	38 *(10.1)*	214 *(14.0)*	0.7 (0.5–1.0)	0.049			
Recreation in the woods	172 *(45.7)*	1026 *(66.9)*	0.4 (0.3–0.5)	<0.001	0.5 (0.4–0.6)	<0.001	NA
Working with a septic tank	4 *(1.1)*	4 *(0.3)*	4.1 (1.0–16.5)	0.046	6.9 (1.2–40.8)	0.033	0.01 (0.00–0.01)
Contact with pigs	13 *(3.5)*	29 *(1.9)*	1.9 (1.0–3.6)	0.067	3.1 (1.3–7.3)	0.011	0.02 (0.01–0.03)

OR: odds ratio. aOR: adjusted odds ratio. PAF: population attributable fraction (only assessed for positive associations). 95%CI: 95% confidence interval. NA: not available.

[a] Combined for the variables "Boerenmetworst", "Cervelaat" and "Snijworst".

[b] Combined variable, created based on "liver sausage" and "canned liver pate".

[c] These products were added later to the questionnaire and were analyzed for $n = 1107$ participants.

[d] Combined variable, created based on "raspberries", "blackberries", "blueberries", "red berries" and "black currant".

production methods between countries, considering international food trade, could strengthen our understanding of transmission routes for hepatitis E virus infection in Europe.

The majority of dry raw pork sausages are produced and consumed in Europe and are generally made from raw pork and beef muscle meat, sometimes spiced, stuffed in a casing.[22] The sausages are sometimes fermented, followed by a long period of continuous air-drying. To completely inactivate HEV, it is recommended to heat meat products to 70 °C for at least 20 min.[23] However, unlike liver sausages and pate, dry raw pork sausages do not pass a heating step.[24] These sausages are generally consumed unheated, as they are meant to be consumed sliced on bread. Salting, fermentation and drying of the sausages largely inactivates pathogenic bacteria, but the persistence of viable HEV after these processes is likely.[18] However there is no evidence to substantiate this, so further research is required to demonstrate that dry raw pork sausages indeed contain infectious HEV. The Netherlands Food and Consumer Product Safety Authority (NVWA) recently detected HEV RNA in meat samples of various types of dry raw pork sausages using an accreditated method [personal communication with

Ingeborg Boxman, NVWA]. The NVWA also investigates the production methods and control of HEV in these products.

A recent risk assessment on classification and control of HEV in meat products, conducted by the Dutch Meat Products Association,[25] provided a plausible explanation for HEV contamination in dry raw pork sausages. Pork liver is not a listed ingredient for the production of dry raw pork sausages. However, pork diaphragm muscle is used as an ingredient, according to that report, and small amounts of pork liver often remain attached to the diaphragm muscle. By no longer using the diaphragm muscle, the risk of HEV in these sausages would be reduced.[25] Following the report, mid-2017, the Dutch Meat Products Association advised its members to stop using the diaphragm muscle of pigs in unheated products. A subtle decrease in the number of HEV infected patients in the national sentinel laboratory surveillance system has been observed since the second half of 2017. The number of HEV infections in 2017 and 2018 were approximately 60%–70% compared to 2015 and 2016, which are the years with the highest numbers of HEV infections observed in the Netherlands.

Another risk factor identified in this study, was consumption of fresh pork sausage. It is plausible that pork diaphragm, sometimes containing small pieces of liver, is also used for the minced meat in this type of sausage. Fresh sausage is baked before consumption, although this is a popular sausage to heat on a barbeque or grill. Possibly, the heating conditions of a barbeque are not sufficient for inactivation of HEV.[26] Additional routes of HEV contamination in processed pork meat products, could be through the use of pig-derived blood products as food additive to enhance colour and texture of the sausages (e.g. red blood cells, haemoglobin and collagen). Boxman et al.[15] recently identified HEV RNA in such pig-derived blood products.[27] The HEV found in the liquid blood products is most likely infectious, as these are not heated during production. In addition, residual blood in the pig muscle tissue could be a modest source of HEV contamination.[28] However, because of the low viral load that ends up in meat products via pig-derived blood products, as well as by residual blood, the risk of symptomatic HEV infection is likely negligible.[25]

Other meat products that we identified as risk factors for acute hepatitis E were pre-packed liver sausage or pate, smoked bacon and pork shoulder ham. During infection in pigs, the virus is abundantly present in the liver, and HEV RNA has been detected in liver sausages and pork pate samples in the Netherlands.[29] This type of sausages is often briefly cooked during processing. However, it is unknown if the remaining load of viable HEV is likely to cause infection. A case-control study in Germany also identified pre-cooked liver sausage as risk factor for acute hepatitis E.[21] Smoked bacon and pork shoulder ham are cooked products that are meant to be consumed sliced on bread, which were identified as risk factors in our study. However, consumption of these products was inter correlated with other risk factor pork meat products: participants who reported consumption of dry raw sausages, also more often reported consumption of smoked bacon (41% versus 27%) and pork shoulder ham (62% versus 40%).

Pre-packed lettuce was the only non-meat food product that we identified as a risk factor for acute hepatitis E. No association was found with any other raw vegetables or fruits, which are produced and consumed in the same way as prepacked lettuce. However, we cannot exclude the possibility of environmental pig manure contamination of lettuce with HEV. Viral contamination in the lettuce supply chain could occur via food handlers' hands or rinsing water.[30] Possibly HEV could internalize in lettuce through cut edges, which was previously demonstrated for norovirus.[31] In a previous study conducted in three European countries, 5 out of 146 (3.4%) fresh lettuce tested positive for HEV RNA.[32]

Environmental transmission routes 'working with a septic tank' and 'contact with pigs' were also identified as risk factors for acute hepatitis E, but seem to have a very low impact on population level, with population attributable fractions of respectively 1% and 2%. These associations are in line with results from previous case-control studies [20,21] and were not unexpected, as water of septic tanks is contaminated by pig manure, and HEV infection is very common in pigs in the Netherlands.[8] In sewage water, surface water and waste water, HEV RNA sequences have been detected similar to sequences found in autochthonous patients, swine and wildlife from the same geographical area.[13] Higher HEV-IgG-seroprevalence have been found in humans with occupational exposure to pigs in Europe.[33,34] However, a recent study among people living nearby a pig livestock-dense area in the Netherlands, did not show an association between residential proximity to pig farms and anti-HEV seroprevalence.[35]

This study identified risk groups based on host conditions such as health status. Reporting no underlying diseases or disorders was very strongly associated with a lower risk of hepatitis E. Comorbidity such as pre-existing liver disease and diabetes were observed more often among patients with symptomatic acute hepatitis E, than among controls. Pre-existing liver conditions among hepatitis E patients have been described frequently in literature as a predisposing factor to severe disease progression of hepatitis E.[2,36–38] Diabetes has previously been reported as associated with HEV in another case-control study.[21] Patients with diabetes might be more susceptible for a symptomatic course of HEV infection because of impaired immune responses[39] and possibly, diabetes could impair hepatocyte-regenerating capacity.[40] Having a high blood pressure was associated with lower risk in multivariate analyses. Although it may be likely that people with high blood pressure consume less salty meat products, such as dry sausages and liver pate, this was not observed in the present study. We observed an association between the use of anti-inflammatory or immunosuppressive medication and an increased risk of symptomatic acute hepatitis E. Users of this kind of medication are likely more prone to infections because of lower immunity. We also identified the use of gastric acid inhibitors as a risk factor, which was negatively associated with reporting "no underlying diseases". Furthermore, susceptibility to foodborne infections might increase due to lower intragastric acidity.[41]

Several other food products in our study were associated with a lower risk of acute hepatitis E. Reporting consumption of steak and wild duck were associated with lower risk of acute hepatitis E as well, suggesting that people who eat more expensive types of meat, might eat less pork meat. Other food products associated with a lower risk of acute hepatitis E were the pork meat products cordon bleu, pork butt ("procureurlapje"), chipolata sausage, which are all usually heated thoroughly before consumption. Consumption of forest fruits and recreation in the woods were associated with lower risk of acute hepatitis E, which could be indicators for a healthy lifestyle, maybe even less comorbidity, and therefore a smaller chance of a symptomatic course of HEV infection.

In summary, consumption of dry raw pork sausages was the most important risk factor on population level for acute hepatitis E during the study period. Since in this study comorbidity was more often observed in patients, it seems that this group is more prone to a symptomatic course of HEV infection. As HEV can cause serious illness in immunocompromised patients and in patients with pre-existing liver disease the nutritional advice for vulnerable groups has been adjusted by the Netherlands Nutrition Centre, NVWA and RIVM, with specific mention of the high-risk meat products that we identified. Research is needed into the prevalence and infectivity of HEV in these pork food products. Also, the production methods of these sausages should be reviewed and

adjusted. Some meat producers in the Netherlands have already adjusted their production methods by no longer using the diaphragm muscle in dry raw pork sausages [personal communication with Martijn Bouwknegt, author of reference 25]. Last but not least, direct contact with pigs or working with a septic tank should be considered as strong risk factors in working condition regulations, although the impact is low on the population level.

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Declarations of interests

None.

Competing interests

The authors have no competing interests to declare.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Contributions

The study concept and design was developed by WvP and AH. Data collection was performed by AT,AH and HV. AT, AH, HV, EF and WvP analyzed and interpreted the data. Statistical analysis was performed by AT and AH. Drafting of the manuscript was performed by AT and AH. WvP obtained funding. The study was supervised by EF, WvP and AH. All authors were involved in critical revision of the manuscript and all authors read and approved the final manuscript.

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Ethics approval and consent to participate

Before enrolment of participants, the study protocol titled “HEVIG” (acronym for Hepatitis E Virus Infections in the Community) (protocol number 15-131) was approved by the Medical Ethics Committee of the University of Utrecht. Written informed consent was obtained from each participant included in the study.

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