

Pork products associated with human infection caused by an emerging phylotype of hepatitis E virus in England and Wales

B. SAID*, M. USDIN, F. WARBURTON, S. IJAZ, R.S. TEDDER AND
D. MORGAN

National Infection Service, Public Health England, Colindale, London, UK

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SUMMARY

Since 2010, human hepatitis E infections have increased in England and Wales. Most cases are locally acquired and caused by hepatitis E virus genotype 3 (HEV G3). HEV G3 is linked to the consumption of pork products. The increase is associated with the emergence of a new phylotype, HEV G3-group 2 (G3-2, also known as G3abcdhij). Sixty individuals with confirmed hepatitis E infection and no history of travel outside the UK were recruited: 19 were infected with HEV G3-group 1 (G3-1 or G3efg) and 41 with G3-2. Epidemiological data relating to usual shopping habits and consumption of ham and sausages were analysed together with typing data to identify any associations with HEV phylotype. Study participants who purchased ham and/or sausage from a major supermarket were more likely to have HEV G3-2 infection (Relative risks 1.85, $P = 0.06$, CI 0.97–3.53). The HEV G3-2 phylotype has not been detected in indigenous UK pigs and it is suggested that human infections could be the result of consumption of products made from pork originating outside the UK. This does not infer blame on the supermarket but the epidemiology of HEV is dynamic and reflects complex animal husbandry practices which need to be explored further.

Key words: Epidemiology, hepatitis E, zoonoses, zoonotic foodborne diseases.

INTRODUCTION

Between 2010 and 2015, Public Health England's (PHE) enhanced surveillance for human cases of hepatitis E detected increasing numbers of reference laboratory confirmed hepatitis E virus (HEV) infection in England and Wales [1]. Similarly across Europe increasing numbers of human infections have been reported from France, Germany, the Netherlands, Finland, Hungary, Italy and Scotland [2]. The indigenous HEV in Europe is genotype 3 (G3). Whilst infection with HEV G3 usually

causes mild disease, in the immunocompromised, such as people with HIV infection and particularly those who have received solid organ transplants, this infection may result in persistent infection leading to progressive liver disease. The rising incidence of hepatitis E evidences a much larger incidence of subclinical infection and is of public health concern.

Investigation into the molecular epidemiology of the virus has shown a divergence into two distinct groups or phylotypes, these are: HEV G3, group 1 (G3-1, also known as G3efg) and HEV G3, group 2 (G3-2, also known as G3abchij) [3, 4]. Whilst available sequence data are limited many European countries report infections linked to HEV G3-2 [2]. In England and Wales, the dominant subtype in human

* Author for correspondence: B. Said, Emerging Infections and Zoonoses, National Infection Service, Public Health England, 61 Colindale Avenue, London NW9 5EQ, UK.
(Email: bengusaid@phe.gov.uk)

cases is also HEV G3-2 replacing HEV G3-1, which was dominant between 2003 and 2009 [3]. The emergence of the new phylotype HEV G3-2 was observed around the same time as the increase in hepatitis E cases was noted and is responsible for the majority of the increase since 2010 [3].

A case-control study undertaken in 2011–2012 showed that HEV G3 infection was associated with the consumption of processed pork products, in particular the consumption of sausages and ham purchased from a major supermarket chain [5]. Similarly, an analysis of questionnaires completed by 73 HEV-infected donors, who were recruited to a joint PHE and National Health Service Blood and Transplant (NHSBT) study of blood donors in 2012–2013, identified the consumption of pork products from this supermarket as a risk factor for HEV viraemia [6]. This study explores that link in further detail.

It is known that HEV is enzootic in pigs worldwide. In 2013 a UK pig abattoir study showed that nearly 93% of pigs sampled had serological evidence of infection by HEV and that around 6% of pigs were viraemic at the point of slaughter [7]. Viruses characteristic of HEV G3-2 were not found in these animals. In addition, a small survey of retail sausages in the UK found 10% of sausages from a single batch contained detectable HEV RNA at point of sale [8].

Following on from these studies, which show that HEV is prevalent in the UK pig population and that human HEV G3 infection is associated with eating processed pork products, the aim of the present study was to determine whether there are any associations between the phylotype of HEV (i.e. G3-1 or G3-2) and the source or type of sausages or ham consumed by infected individuals in England and Wales.

METHODS

Hepatitis E cases, confirmed and typed by the reference laboratory (as described below) were interviewed to examine their food habits with regard to specific processed pork products (sausages and ham) and to test whether there was any association between exposure and the phylotype (G3-1 or G3-2) of the infecting HEV.

Study population

Individuals infected with HEV were recruited to the study from: (i) the cohort of blood donors who

participated in the joint PHE/ NHSBT prevalence and transmission study in 2012–2013 [9], referred to as the ‘donor cohort’ ($n = 34$), and (ii) acute hepatitis E cases whose serum or plasma contained HEV RNA confirmed by the reference laboratory between April and July 2014, referred to as the ‘acute cohort’ ($n = 26$).

In both cohorts, only participants with no history of travel outside the UK in the 9 weeks prior to onset of symptoms or the 9 weeks prior to donation of blood and for whom the phylotype of the HEV were available were contacted between June and August 2014. The 9 week period was used as an exclusion period for travel because this is the maximum incubation period for hepatitis E.

Recruitment to the study

All individuals had previously completed a hepatitis E surveillance questionnaire (current version available here: <https://www.gov.uk/government/publications/hepatitis-e-surveillance-form>). This surveillance form was used to confirm travel history and obtain consent to be contacted for further information and contact details (telephone number). Potential recruits were contacted by telephone between 10 June and 14 August 2014 and asked for consent to participate in the study, which involved answering a questionnaire by telephone interview. The questionnaire for this investigation was based on the risk factors identified as being associated with HEV G3 infection from a previous study on 73 donors [6]. This questionnaire (provided as supplementary information) was designed to elicit specific detailed information on purchasing and consumption of sausages and ham. Two investigators interviewed participants according to a written script. Interviews typically lasted 10 min. Questions and responses were clarified as required during the interview. Participants were asked about their ‘usual’ food buying, preparation and consumption habits to account for the fact that the exposure happened some time ago.

Serology and genotyping

Blood or serum samples received at the National hepatitis reference laboratory, (Blood Borne Virus Unit, Virus Reference Department, National Infections Service, Colindale) were tested for antibodies to HEV (anti-HEV) using the Wantai IgG and IgM ELISA detection assays (Fortress Diagnostics, Northern

Ireland). Tests were run in accordance with the manufacturer's instructions.

Samples from blood donors with detectable HEV RNA at the time of donation and viraemic anti-HEV IgM positive samples from patients presenting with acute hepatitis E were analysed. Briefly, for detection of HEV RNA, nucleic acid was extracted from serum or plasma and subjected to reverse transcription and polymerase chain reaction amplification using a modified Taqman assay as previously described [10]. Sequencing, genotypic and phylogenetic analysis across part of the ORF2, were undertaken as previously described [11].

Data entry and analysis

Data from the questionnaire was entered manually in a dedicated password-protected Access database. Data was double-checked by a second investigator verifying that all the questionnaire information was entered correctly. After validation, data were extracted to an Excel spreadsheet for analysis. All documents and files containing patient identifiable information were handled and stored in compliance with information governance protocols and Caldicott guidance.

Statistical analysis was carried out using STATA v13 (StatCorp. 2013) [12]. In the single variable analysis, Fisher's exact test was used to look for differences between the HEV G3-1 and G3-2 cohorts. Relative Risks (RR) were estimated using generalized linear modelling.

To test for possible confounding, variables with a significance of $P < 0.15$ in the single variable analysis were considered for inclusion in multivariable models. Separate analyses were carried out to examine general consumption habits and specific habits in regards to types of sausage and brands. As some variables were strongly correlated (for example, buying sausage at a particular supermarket was correlated with buying ham at the same supermarket) these variables were examined separately. Further analysis also investigated the different combinations of buying ham and sausage (buying neither, buying either sausage or ham, or buying both) at the same supermarket.

RESULTS

A total of 60 HEV infected study participants (out of a possible 64 eligible individuals identified, 94%) were recruited and interviewed. Participants from the acute cohort, who all had clinically apparent hepatitis, were

Table 1. *Demographics and infecting phylotype of hepatitis E virus (HEV) infected study participants (N = 60)*

	Donor cohort <i>n</i> = 34	Acute cohort <i>n</i> = 26	Total
Male (%)	20 (59%)	22 (85%)	42 (70%)
Age range (years)	29–68	21–87	21–87
Median age (years)	51	58	53
HEV G3-1 infection	9 (26%)	10 (38%)	19 (32%)
HEV G3-2 infection	25 (74%)	16 (62%)	41 (68%)

contacted within 3 weeks of their diagnosis. Participants from the donor cohort were contacted over a year after their diagnosis through screening. Positive responses were achieved for all questions with no apparent difference in recall between the two cohorts and participants were able to answer all questions. There were 34 participants recruited from the donor cohort (out of 36 eligible individuals) and 26 participants from the acute cohort (out of 28 eligible individuals) (Table 1). Only one individual, from the donor cohort, declined to take part and three could not be contacted (one from the donor cohort and two from the acute cohort).

As is typical for HEV infection the majority (70%) of the participants were male and the overall median age was 53 years (Table 1). The median age of the donor cohort in comparison to the acute cohort was younger (51 years vs. 58 years) and none had clinical hepatitis. In both cohorts, the emerging HEV G3-2 was the predominant cause of infection (68%).

Single variable analysis

The single variable analysis of possible exposures compared the differences between HEV G3-1 ($n = 19$) and G3-2 ($n = 41$) infected recruits; only variables for which the Fisher's exact test was $P < 0.15$ are shown in Table 2. Age, cohort (donor or acute), cooking method and shopping in any supermarket were not significant by Fisher's exact test (results not shown).

As there was a strong relationship between some variables (see the section 'Methods'), the results for these variables are shown both separately and in combination in Table 2 under 'Specific variables'.

All 60 participants had indicated that they typically consumed pork products. The majority ($n = 59$) ate both sausages and ham. Only one person in the study did not eat sausages and also did not buy ham

Table 2. Single variable analysis comparing the differences between HEV G3-1 and G3-2: Exposures which were considered for inclusion in multivariable analysis ($P < 0.15$)

Exposure		G3-1 (%)	G3-2 (%)	RR	P-value	95% CI
General variables						
Buys fresh sausages	No	4 (21)	2 (5)	2.17	0.074	0.69–6.80
	Yes	15 (79)	39 (95)			
Buys sausages at any supermarket	No	6 (32)	4 (10)	1.85	0.059	0.85–4.02
	Yes	13 (68)	37 (90)			
Buys thick sausages	No	3 (16)	1 (2)	2.86	0.089	0.52–15.73
	Yes	16 (84)	40 (98)			
Buys a particular sausage brand	No	11 (58)	13 (32)	1.44	0.088	0.96–2.16
	Yes	8 (42)	28 (68)			
Eats baked sausages	No	15 (79)	21 (51)	1.43	0.051	1.03–1.99
	Yes	4 (21)	20 (49)			
Specific variables						
Buys sausages at Supermarket X	No	14 (74)	11 (27)	1.95	0.002	1.23–3.09
	Yes	5 (26)	30 (73)			
Buys various brands of sausage	No	12 (63)	37 (90)	0.48	0.027	0.22–1.07
	Yes	7 (37)	4 (10)			
Buys Supermarket X own brand sausage	No	17 (89)	23 (56)	1.57	0.017	1.16–2.12
	Yes	2 (11)	18 (44)			
Buys ham in Supermarket X	No	12 (63)	9 (22)	1.92	0.003	1.14–3.20
	Yes	7 (37)	32 (78)			
Buys neither ham nor sausage from Supermarket X		11 (58)	7 (17)	1.00	0.002	1.14–3.78
Buys ham and/or sausage from Supermarket X		8 (42)	34 (83)	2.08		

RR, Relative Risk.

from Supermarket X. This person was infected with HEV G3-1. None of the participants who typically bought sausages exclusively from Supermarket X had a G3-1 infection. Of the 41 G3-2 infections, 30 (73%) usually bought sausages from supermarket X (15 of the 30 bought exclusively from Supermarket X). The results of the single variable analysis shows that there was a significant association between HEV G3-2 and buying ham and sausages in Supermarket X ($P = 0.002$).

Multivariable analysis

In the multivariable models, none of the general consumption variables were associated with HEV phylo-type. However, specific variables were found to be significantly associated with HEV G3-2 in the multivariable models (Table 3). As variables were strongly inter-related it was not possible to put all variables into one model. As an indication of model fit the Pearson's goodness of fit test was not significant for all models, suggesting the models presented in Table 3 are a good fit.

In the sausage consumption specific analysis: 'buys sausages at Supermarket X' was the only significant variable (RR 1.73; 95% CI 1.02–2.93; $P = 0.04$).

In the brand specific analysis 'buys Supermarket X own brand sausages' was the only significant variable (RR 1.42, 95% CI 1.06–1.92, $P = 0.02$).

In the multivariable model, which examined the consumption of any pork product from Supermarket X, although the P -value was borderline significant, there was an almost twofold increased risk (RR 1.85; 95% CI 0.97–3.53; $P = 0.06$) associated with 'buys ham and/or sausage from Supermarket X' relative to buying neither of these at Supermarket X.

DISCUSSION

The ongoing increase in human hepatitis E infections seen in England and Wales is mainly linked to the emerging phylotype of HEV G3-2 [3], a virus not typically seen in the UK pigs [13]. Previously HEV G3 infections have been linked to the consumption of processed pork products [5, 6]. This focused study showed that individuals infected with HEV G3-2 were more likely to have bought ham and/or sausage at a major supermarket.

Earlier studies, undertaken in England and Wales between 2011 and 2013, identified a significant association between the consumption of pork products from

Table 3. *Multivariable analyses of risk factors for HEV G3-2 compared to G3-1 infections in study participants (N = 60)*

Variable		RR	P-value	95% CI
General consumption habits				
Buys fresh sausages	No	1.00	0.70	0.39–4.10
	Yes	1.26		
Buys sausages at any supermarket	No	1.00	0.46	0.61–2.96
	Yes	1.35		
Buys thick sausages	No	1.00	0.41	0.37–11.46
	Yes	2.06		
Buys a particular sausage brand	No	1.00	0.42	0.78–1.81
	Yes	1.19		
Eats baked sausages	No	1.00	0.43	0.82–1.61
	Yes	1.15		
Specific sausage consumption habits				
Buys fresh sausages	No	1.00	0.79	0.33–4.26
	Yes	1.19		
Buys sausages at Supermarket X	No	1.00	0.04	1.02–2.93
	Yes	1.73		
Buys thick sausages	No	1.00	0.55	0.27–11.23
	Yes	1.74		
Buys various brands of sausage	No	1.00	0.96	0.67–1.53
	Yes	1.01		
Eats baked sausages	No	1.00	0.92	0.73–1.42
	Yes	1.02		
Brand or type of sausages				
Buys fresh sausages	No	1.00	0.51	0.46–4.86
	Yes	1.49		
Buys Supermarket X own brand sausage	No	1.00	0.02	1.06–1.92
	Yes	1.42		
Buys thick sausages	No	1.00	0.45	0.33–11.89
	Yes	1.99		
Increased risk from consumption of any pork product from Supermarket X				
Buys fresh sausages	No	1.00	0.90	0.27–4.41
	Yes	1.09		
Buys thick sausages	No	1.00	0.46	0.30–14.64
	Yes	2.09		
Eats baked sausages	No	1.00	0.50	0.83–1.47
	Yes	1.11		
Buys neither ham nor sausage from Supermarket X		1.00	0.06	0.97–3.53
Buys ham and/or sausage from Supermarket X		1.85		

RR, Relative risk; CI, Confidence interval.

Supermarket X and HEV infection [5, 6]. The present study shows that the emerging phylotype HEV G3-2 is associated with the same supermarket but the previously predominant HEV G3-1 is not. The implicated products are pork sausages, which require cooking prior to consumption, and ready-to-eat pre-packed sliced ham. Even though most participants stated that they typically cooked their sausages well prior to consumption. There is uncertainty as to whether current food processes and cooking temperatures are sufficient to inactivate the virus [14, 15]. The possibility that

ready-to-eat products are implicated is also of public health concern. It is striking that only the HEV G3-2 infections in the human host were consistently associated with purchasing and consuming sausages and/or ham from Supermarket X. In addition, buying neither ham nor sausage at Supermarket X is protective against HEV G3-2 infection. Examining the relationship with different brands of sausage showed that, once again, only Supermarket X, especially own brand, was significantly associated with the dominant phylotype HEV G3-2. Only one person did not eat

sausages or buy ham at Supermarket X and this individual was infected with HEV G3-1. Whilst not conclusive evidence, this is consistent with the findings reported.

Human infections in England and Wales remain predominantly due to HEV G3-2, accounting for around 80% of all infections. Based on an HEV RNA prevalence currently of 0.04% in 600 000 English donors, a total of around 150–200 000 infections may occur each year in England and Wales (NHSBT/PHE Epidemiology Unit, 2016/17). The 2013 pig abattoir survey identified the phylotype found in the UK pigs to be HEV G3-1 [13] a source possibly accounting for some of the residual non-G3-2 human infections at that time. Virus infections with HEV G3-2 phylotype have also been identified in patients on continental Europe (personal communication Zaajer H and Hogema B). Conversely HEV 3-2 is likely found in European domestic pig herds, although current data on HEV pig sequences in Europe is not available and this is therefore based on limited published information [2, 16, 17]. The inference is that emergence of HEV G3-2 infection in England and Wales is unlikely to have originated in the UK pigs. Pork and pork-products are imported into the UK from a number of European countries and processed products such as the ones implicated in this study potentially have a mixture of meat from different sources. Therefore, it remains plausible that the G3-2 associated infections may be as a result of consumption of imported pork meat or products and that the increase is a reflection of an emerging epizootic on mainland Europe. The question that arises is whether this observation should trigger an intervention. HEV is globally an enzootic infection of pigs. For transmission to occur from the natural host to humans a pig entering the food chain will only represent a source of human infection if it is viraemic at the time of slaughter. It is known that pigs can be viraemic at the time of slaughter [7] however the source of the pigs giving rise to the incidence of hepatitis E in the UK remains undefined. Further investigations are needed to establish what is driving the ongoing increase in hepatitis E cases. The factors associated with the emergence of HEV G3-2 as an epizootic in European pigs are unclear. There are potentially multiple influences, for example changes to husbandry practices in countries from which pork is imported. These may inadvertently affect the age at which pigs become infected and potentially the prevalence of viraemic pigs at slaughter. Further research on this subject is required to establish

interventions targeting husbandry practices that could achieve a reduction in meat containing HEV. There is increasing evidence from existing European collaborative studies of a changing phylogeny of HEV G3 [2, 13]. Whether this is simply the result of a change in husbandry practices or represents changes in the pathogenicity or transmissibility of the virus is not known. Interventions in the food chain, from animal husbandry through to the societal issue of how processed pork products are cooked and eaten has the potential to reduce this infection burden.

There were a number of limitations of this study. The study design was purposely focused on processed pork products that were identified as risk factors for HEV G3 infection in previous studies. This meant that other factors were not considered in this particular investigation and analysis. There was selection bias as potential participants were only included if they had completed a surveillance form and agreed to be contacted. To reduce further selection bias every effort was made to accommodate timing of interviews to participants requirements, including evenings for example. The fact that the demographics of the study population in being older and male, was similar to the known characteristics of hepatitis E cases provides some reassurance that this was a representative group. As hepatitis E has a long incubation period of up to 9 weeks, recall bias is always an issue. Although we contacted cases, as soon as possible many participants from the donor cohort were contacted long after their diagnosis. As their infection was detected through screening and not prompted by investigations because of clinical symptoms timing of infection is more uncertain. Therefore, to minimise possible recall bias at interview recruits were asked to report on their usual shopping and consumption habits. Questionnaires were completed comprehensively by telephone interview and there is no apparent differential recall between either of the two cohorts or between those infected with the different phylotypes. It is acknowledged that by asking for participants typical or usual shopping and eating habits it is possible that a rare event such as the purchase of a contaminated food from a diverse mix of markets, other shops, food brought by friends or other rare, less typical routes, may be missed.

In conclusion, this study provides additional evidence for the association of hepatitis E infection in England and Wales with the consumption of sausages or ham purchased at a major supermarket. The investigation concludes that if a participant bought ham

and/or sausage at Supermarket X they were almost twice as likely to have HEV G3-2 infection (RR1.85, $P = 0.06$, CI 0.97–3.53). This association was evident for the emerging HEV G3-2 phylotype but not for the previously dominant HEV G3-1. As the predominant type in the UK pigs remains G3-1 this suggests that current HEV G3-2 human infections are likely the result of consumption of pork products made from pork originating from outside the UK. This does not imply that the supermarket is in any way to blame and is likely to reflect the complex animal health practices within Europe which result in the changing epidemiology of human HEV infections.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at <https://doi.org/10.1017/S0950268817001388>

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CONFLICTS OF INTEREST

None.

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