International Journal of Food Microbiology 397 (2023) 110198

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Hepatitis E virus in pork meat products and exposure assessment in Belgium

Tatjana Locus [a,b,d], Ellen Lambrecht [b], Michael Peeters [a], Vanessa Suin [a], Bavo Verhaegen [c], Koenraad Van Hoorde [c], Sophie Lamoral [a], Thomas Vanwolleghem [d], Steven Van Gucht [a,*]

[a] Sciensano, Infectious Diseases in Humans, Viral Diseases, Engelandstraat 642, 1180 Ukkel, Belgium
[b] ILVO, Flemish Research Institute for Agriculture, Fisheries and Food, Technology and Food Unit, Brusselsesteenweg 370, 9090 Melle, Belgium
[c] Sciensano, Infectious Diseases in Humans, Foodborne Pathogens, Juliette Wytsmanstraat 14, 1050 Elsene, Belgium
[d] University of Antwerp, Laboratory of Experimental Medicine and Pediatrics, Viral Hepatitis Research Group, Drie Eikenstraat 655, 2650 Edegem, Belgium

ARTICLE INFO

Keywords:
HEV
Zoonosis
Foodborne
Exposure assessment
Pork products
Food safety

ABSTRACT

Zoonotic hepatitis E virus (HEV) genotype 3 infections are the predominant cause of acute viral hepatitis in Europe, mostly associated with the consumption of HEV contaminated pork meat. In this study we looked at the HEV RNA positivity rate of pork meat products readily available from Belgian supermarkets and evaluated the overall HEV consumer exposure in a Belgian context.

Two basic assessments were performed in a 'worst-case' scenario setting: one solely focusing on the contamination level of the product itself (ingredients and processing parameters) and another estimating the overall consumer exposure, taking into account consumption habits in Belgium. Non-thermal-processed ready-to-eat (i.e. ready for consumption without additional cooking step by consumer) pork meat products (e.g. raw dried sausages), had a high estimated HEV contamination level, while thermal-processed ready-to-eat pork meat products (e.g. pork liver pâté) had the highest overall consumer exposure estimates.

Following these assessments, pork liver pâtés, raw dried hams and raw dried sausages (n = 54) were purchased from Belgian supermarkets (n = 3) and analyzed for HEV RNA by RT-PCR. In total, 31 % (n = 17) products tested positive. HEV RNA was found in 65 % of the pork liver pâtés, 15 % of raw dried hams and 0 % of raw dried sausages. Phylogenetic analysis of four isolates (all gt3c) from pork liver pâté samples showed similarities with human clinical cases from Germany and Belgium.

1. Introduction

Hepatitis E virus (HEV) is a quasi-enveloped positive single stranded RNA virus (Purdy et al., 2017). It is a major cause of acute viral hepatitis, with >20,000 acute clinical cases reported over the last decade in Europe (ECDC, 2019). Eight genotypes (gt) have been identified, five of which are reported to cause human disease (Smith et al., 2020). Genotypes 1 and 2 are mainly found in developing countries, causing outbreaks of acute hepatitis, while gt3 is the primary cause of hepatitis E in Western countries (ECDC, 2019). Most of the infections remain asymptomatic, but severe acute hepatitis or chronic infection in immunocompromised patients do occur, as identified in a recent Belgian survey (Peeters et al., 2022). Host factors contributing to disease presentation are pre-existing chronic liver disease, age above 50 years, and an immunocompromised status (Peeters et al., 2022).

HEV gt3 is responsible for the majority of human hepatitis E cases in Europe and is mainly associated with the consumption of HEV contaminated meat products (ECDC, 2019). It circulates with a high prevalence in wild boar populations and has also been detected in deer, rabbits and other spillover species (Spahr et al., 2017). However, given the scale of domestic pig farming in North-Western Europe, zoonotic HEV gt3 infections have been associated mostly to pork meat consumption, although quantitative estimates are lacking. HEV RNA has been detected at pig farm level (i.e. pork liver, blood and feces) and there is a considerable risk of HEV positive pigs ending up in slaughterhouses and entering the food chain (Crotta et al., 2018, 2021; Rutjes et al., 2009; Thiry et al., 2014). However, differences in HEV seroprevalence (i.e. 66.8 % up to 88 %) in pig herds exist between farms and the effect of different factors such as farm type, slaughter age and hygiene measures is not always clear cut (Meester et al., 2022; Pellerin et al., 2022).

Pork liver and blood are mostly associated with HEV RNA, while pork muscle is much less frequently contaminated (Boxman et al., 2017, 2020; Di Bartolo et al., 2012; Feagins et al., 2007; Feurer et al., 2018).

* Corresponding author at: Sciensano, Infectious Diseases in Humans, Viral Diseases, Engelandstraat 642, 1180 Ukkel, Belgium.
E-mail address: Steven.VanGucht@sciensano.be (S. Van Gucht).

https://doi.org/10.1016/j.ijfoodmicro.2023.110198
Received 16 November 2022; Received in revised form 30 March 2023; Accepted 31 March 2023
Available online 5 April 2023

Importantly, viral RNA has been detected in food products ready for sale, such as pork liver pâté, pork liver sausages and raw dried sausages (Boxman et al., 2019; Colson et al., 2010; Pallerla et al., 2020; Szabo et al., 2015). Still, HEV RNA detection in food products differs across studies and countries and has not been determined in a Belgian context. Additionally, factors associated with HEV RNA positivity in food products are unknown and not quantified.

Despite all this, direct epidemiological links have been found between human cases and consumption of HEV contaminated food products. For example, in Japan a person became ill after the consumption of hunted wild boar meat (Li et al., 2005), while another Japanese study identified grilled pork meats as being the causative agent of HEV infection (Matsubayashi et al., 2008). In France undercooked pork meat and raw pork liver sausage were found to be the culprit of HEV infection in multiple patients (Deest et al., 2007; Renou et al., 2014).

Up to now risk assessments for foodborne HEV transmission are scarce, most often due to a lack of sufficient quantitative, detailed data on ingredients, food processing parameters and virus infectivity. Mataragas and colleagues estimated that the general European population and those considered as especially susceptible (i.e. immunocompromised patients, elderly, infants and very young) have a high risk of foodborne HEV infection (Mataragas et al., 2008). However, due to lack of literature data, this study only focused on consumption of raw pork meat and not on processed pork meat products. The latter might also pose a risk and are more frequently consumed. So up till now the cumulative risk of HEV infection for consumers is unknown.

Additionally, the infectivity of the virus in food products and transmission to consumers is incompletely understood, due to a lack of suitable viral isolation methods, in vitro assays or animal models. HEV is notoriously difficult to propagate in cell culture, often requiring an adaptation of viral strain and host cell line (Berto et al., 2013; Takahashi et al., 2012). Recent studies indicate that HEV could be resistant to fermentation, curing and salting steps used during food processing (Wolff et al., 2020a; Wolff et al., 2020b). High temperature treatment (i.e. above 72 °C) remains at present, the most promising way to reduce the amount of infectious HEV in food products (Barnaud et al., 2012; Feagins et al., 2008; Imagawa et al., 2018; Johne et al., 2016).

As the majority of cases in Europe and Belgium are locally acquired zoonotic HEV gt3 infections (Colson et al., 2010; Tei et al., 2003; Yazaki et al., 2003), it is important to address previously mentioned knowledge gaps and to identify highly contaminated products. The aim of the present study is to gain insights and increase our current knowledge on HEV contamination of Belgian food products and the possible resulting foodborne transmission to the consumer via i) HEV contamination level estimates of pork meat products and overall exposure to the consumer, ii) HEV RNA detection rate in Belgian pork meat products from retail and iii) HEV phylogenetic analyses of strains isolated from food products and human cases.

2. Materials and methods

2.1. HEV contamination and exposure assessment

Information from literature on the ingredients and products at risk for HEV contamination as well as on food processing techniques associated with virus inactivation were collected. Pork liver, blood products, diaphragm (i.e. due to residual liver potentially being attached) and ready-to-eat products are considered at high probability for HEV contamination (Bouwknegt et al., 2017; Boxman et al., 2017, 2020; Feagins et al., 2007). Thermal treatment (>72 °C) is considered to inactivate the virus in food products (Barnaud et al., 2012), but other treatments (i.e. curing, fermentation, …) seem insufficient (Wolff et al., 2020a; Wolff et al., 2020b).

Two basic theoretical assessments were performed: one solely focusing on the contamination level of the final product (i.e. ingredients and processing steps) and another estimating the overall exposure to the consumer, taking into account consumption habits in Belgium.

2.1.1. Data collection

Data on meat products originating from pork and/or wild boar were collected from (i) a supermarket, (ii) nine artisanal butchers, (iii) seven industrial meat companies and (iv) standard literature recipes. In total 594 products were included (Supplementary Table 1). Data on the proportion of pork and/or wild boar meat in the product, the presence of highly contaminated ingredients (i.e. liver, blood or diaphragm) and the production process were included in the list. Food products were assigned to five product categories, based on production process (Table 1).

2.1.2. Product contamination level

To estimate the potential HEV contamination level of a product, scores for each product were calculated based on (i) the percentage of pork/wild boar meat (i.e. muscles vs organs and fat), (ii) presence of highly contaminated ingredients (i.e. liver, blood or diaphragm) and (iii) ready-to-eat (RTE) status and processing steps.

Firstly, the “percentage pork/wild boar meat”, further indicated as intermediate contamination estimate 1 (I1), was estimated. A score of 1, 2, 3 or 4 was given when ≤20 %, ≤40 %, ≤60 % or >60 % pork/wild boar meat was present in a product, respectively. The effect of highly contaminated ingredients presence, intermediate contamination estimate 2 (I2), was calculated by summing the scores of following individual variables: presence of liver, added blood products and/or diaphragm. Considering I2, a score of 0 or 1 was given if the ingredient was absent or present in the product, respectively. If more than one highly contaminated ingredient was present, the total score was the sum of the individual scores. Thirdly, a sum was made of the RTE status (i.e. yes = 1, no = 0) in combination with a heating step (i.e. yes = 0, yes but below 72 °C = 1, no = 2) during production. This sum (i.e. RTE status + heating step) was defined as intermediate contamination estimate 3 (I3).

All three intermediate contamination estimates were normalized (i.e. the score of the individual product minus the lowest score in the intermediate contamination estimate divided by the total range of the intermediate contamination estimate).

Table 1
Description of the five categories used to assign pork/wild boar meat products based on the production process. Examples for each category as well as the percentage of products belonging to each category are given.

Category	Description	Examples	% of total products
Cat1	Raw RTE	Minced pork meat used as a spread on sandwiches	1 %
Cat2	Raw, intended to be heated by the consumer (‘ready to heat’)	Pork chops, sausage, cordon bleu and bacon	23 %
Cat3	RTE, which has undergone an extensive preservation step such as fermentation, smoking, acidification and/or drying and has not been heated to a high temperature (>72 °C) during production. Does not require any re-heating by the consumer	Salami, chorizo, dried sausages and dried ham	22 %
Cat4	RTE, which has been heated (>72 °C), without a preservation step as defined in Cat3. Does not require any re-heating by the consumer	Cooked bacon, cooked ham, meat salad spread, ham sausage and pork liver pâté	26 %
Cat5	Food products heated to some extent by the producer and intended to be reheated by the consumer	Blood sausage, cooked meatballs in tomato sauce, lasagna Bolognese and hotdog sausages	28 %

The importance of variables in determining the contamination level was unknown. Because of this five formulas were set-up based on different combinations of the variables as well as different weights attributed to the variables (Table 2). It was estimated that I1 would have a smaller effect on the contamination status of a product than the presence of highly contaminated ingredients (I2) and processing steps (I3), since pork liver and blood are much more frequently contaminated with HEV than pork muscle (Boxman et al., 2017; Feurer et al., 2018). Additionally, heating to 72 °C for >20 min is the only known inactivation method (Barnaud et al., 2012). For all five formulas the scores were normalized and the resulting final product scores ranged from 0 (no contamination) to 1 (highest contamination).

2.1.3. Overall consumer exposure

A second analysis was performed to evaluate the exposure of consumers to HEV through the consumption of pork/wild boar meat products using Risk Ranger, a food safety risk assessment tool (Ross and Sumner, 2002). This assessment tool has been used to evaluate the foodborne infection risk for pathogen-food combinations in several studies, i.e. Hepatitis A virus (HAV) and Norovirus in leafy greens and berries (Torok et al., 2019), *Campylobacter jejuni* in street vended poultry (Birgen et al., 2019), histamines in differently preserved fish (El Hariri et al., 2018), *Listeria monocytogenes* in RTE foods (EFSA, 2015), microbial contaminants of red meat (Sumner et al., 2005), etc. It has also been used to evaluate the HEV foodborne infection risk in raw pork products in Europe (Mataragas et al., 2008).

Using Risk Ranger has several advantages. Firstly, the software is relatively easy to use. Secondly, it offers a way to compare foodborne risks of pathogen-food combinations and to prioritize them based on their ranking. Lastly, it also offers the user the opportunity to address the different aspects that are important in determining the risk of a foodborne pathogen and in this way identify knowledge gaps.

The Risk Ranger's estimation is based on eleven questions divided over three categories: (i) susceptibility of the population and hazard severity; (ii) probability of exposure via food and (iii) likelihood of a disease causing dose being present in a meal (Supplementary Table 2).

Susceptibility to and severity of a foodborne infection with HEV differs in consumers with a different health-status. As a consequence three consumer populations were defined for analyses:

(A) extremely susceptible population: an extremely small proportion (0.1 %, predetermined by Risk Ranger) of the population in which a HEV infection would pose a severe hazard, possibly resulting in death (i.e. transplant recipients, cancer patients, …)
(B) highly susceptible population: a very small part (3 %, predetermined by Risk Ranger) of the population more susceptible to HEV infection and resulting in a more serious clinical outcome than in the general population (i.e. >50 years, alcoholic, diabetic, …)
(C) general population: the total Belgium population (including extremely and highly susceptible populations) for which on average a HEV infection rarely requires medical attention.

Probability of exposure to HEV via food (ii) was based on the latest Sciensano National Food Consumption Survey from 2014 to 2015 in Belgium (see Supplementary Tables 3 and 4). Per food category, the daily consuming population and the average amount consumed were extrapolated.

Table 2
The five formulas used to estimate the final product contamination level. I1 i.e. "percentage pork/wild boar meat", I2 i.e. presence of highly contaminated ingredients and I3 i.e. RTE status and processing steps.

Name	Formula
Formula 1	I2 + I3
Formula 2	I1 + I2 + I3
Formula 3	(0.5 * I1) + I2 + I3
Formula 4	(0.5 * I1) + (2 * I2) + I3
Formula 5	(0.5 * I1) + I2 + (2 * I3)

Detailed information on food containing a disease causing dose (iii) of HEV in Belgium (and Europe) is lacking. As a proxy for the Belgian consumer, a literature survey on HEV in pork and wild boar meat products in Europe was performed (see Supplementary Tables 5 and 6). Contamination with HEV was determined by the presence of viral RNA through RT-PCR. We decided to include all positive samples, since the HEV disease causing dose (i.e. ID50) is unknown at the moment. Similarly, we focused on viral RNA only, since studies looking at viral infectivity in food are scarce. Therefore we can assume that the estimated amount of food containing a disease causing dose will be an overestimation, i.e. the worst-case scenario. In total, information of >10,000 food samples from studies between 2010 and 2020 was available over a wide range of product types (Supplementary Tables 5 and 6).

Besides the three population types, the overall exposure was calculated for each of the five product categories (see Section 2.1.1 and Table 1) separately. This resulted in 15 analyses in total. Risk Ranger gives "Risk Rankings" as output; rankings are defined as low (<32), medium (32–48) or high (>48) risk for foodborne infection (Ross and Sumner, 2002).

2.2. Detection of HEV RNA in pork meat products

2.2.1. Sample collection

Products presenting a possible high HEV contamination level based on the previous performed assessments were purchased between February and April 2022 from various supermarkets (n = 3) in East-Flanders and Brussels, Belgium. In total 54 products were collected: 23 pork liver pâtés, 18 raw dried sausages and 13 raw dried hams. Samples were processed before the expiration date and were stored at 4 °C for short term or −20 °C for long term storage.

2.2.2. Virus extraction

The virus extraction method was based on the method described by Szabo and colleagues (Szabo et al., 2015) with some modifications. Two grams of meat product was cut into fine pieces under sterile conditions, 7 ml of TRIzol (Invitrogen) was added, samples were vortexed thoroughly and incubated (20 min, 300 rpm at room temperature), followed by centrifugation (4 °C, 12,000 *g* for 20 min) to pellet food particles. Subsequently, the supernatants were transferred to a new falcon and 200 µl of chloroform per 1 ml of sample (i.e. approximately 1.5 ml in total) was added. The mixture was vortexed thoroughly until all phases were mixed and samples were centrifuged (4 °C, 12,000 *g* for 15 min). This step was repeated once to remove residual fat (Pallerla et al., 2020). After the final centrifugation, the upper phase was collected and used for RNA extraction.

2.2.3. Nucleic acid extraction

Nucleic acid extraction was performed using the NucliSENS® miniMAG® system (bioMérieux, France) according to the manufacturer's instructions. RNA was eluted in 50 µl and stored at −80 °C prior to RT-PCR. Negative extraction control samples (TRIzol and RNase-free H_2O) were run at a frequency of one in between each set of ten samples.

2.2.4. RT-PCR

HEV RNA was detected using an optimized in-house one-step RT-PCR protocol (Pas et al., 2012). For each reaction, 15 µl of sample RNA was mixed with 15 µl of MasterMix, consisting of 1 µM of TaqMan™ Fast Virus 1-Step Master Mix (Thermo Fisher) and the following primers and probe: forward primer HEV-AB-F (5′-CGG TGG TTT CTG GGG TGA-3′, 0.5 µM), reverse primer HEV-AB-R (5′-GCR AAG GGR TTG GTT GG-3′, 0.5 µM), and probe HEV-pr-MGB (5′-FAM-ATT CTC AGC

CCT TCG C-MGB-3′, 0.25 μM). Reverse transcription was performed at 50 °C for 5 min, followed by denaturation for 3 min at 95 °C and 45 cycles of 15 s at 95 °C and 30 s at 60 °C.

Undiluted and 1/10 diluted extracted RNA samples were tested to check for inhibition. Ct values above 39 were no longer considered positive. Viral loads of positive samples are expressed as average Ct values ± standard deviation.

2.2.5. Sequencing & phylogenetic analysis

A 493 bp fragment of ORF2 (Boxman et al., 2017) was sequenced from all HEV RNA positive RT-PCR samples with a Ct value below 33. The DNA amplicons were sequenced and analyzed with SeqMan Ultra® and MegAlign PRO Version 17.3 (DNASTAR, Madison, WI, USA). Sequences were aligned according to the MAFFT method and phylogenetic analysis was performed using the RAxML maximum likelihood method (Katoh and Standley, 2013; Stamatakis, 2014). HEV gt1, 3 and 4 reference strains (Smith et al., 2020), a selection of closely related of HEV gt3 sequences published in the NCBI database and HEV gt3 sequences obtained from human cases diagnosed at the Belgian national reference center for hepatitis E virus (Sciensano) during the pork meat sampling period (01/2022 until 05/2022) were included in the phylogenetic analysis. Sequences from pork meat samples were submitted to the HEVnet Typing Tool version 0.1 (Mulder et al., 2019) to confirm the genotype.

3. Results

3.1. HEV contamination and exposure assessment

3.1.1. Data exploration

Data on 594 pork/wild boar meat products in Belgian retail were collected (supplementary table 1). Only seven of the products contained wild boar meat, i.e. three wild boar pâtés also containing pork liver, one dried sausage, two ragouts and one roast. A low percentage, i.e. 9.6 % (n = 57) and 8 % (n = 47) of the meat products contained porcine blood and liver, respectively and none contained diaphragm. In addition, those products that contained liver were RTE and heated (>72 °C for >20 min) during processing, except for raw liver sausage/figatellu. The 594 products were divided in five categories (Cat1, Cat2, Cat3, Cat4 and Cat5) according to production process (see Section 2.1.1) and all categories were more or less equally represented in the list (Table 1). An exception was category 1, representing only 1 % of the products in the list.

3.1.2. Product contamination level

Each product was analyzed by five formulas (Table 2), resulting in five product scores ranging from zero (no contamination) to one (highest contamination). For each category the average score per formula was calculated (Fig. 1). Independent of the formula used, raw and non-thermal-processed RTE products (i.e. categories 1 and 3 respectively) had the highest scores, while non-RTE thermal-processed products (i.e.

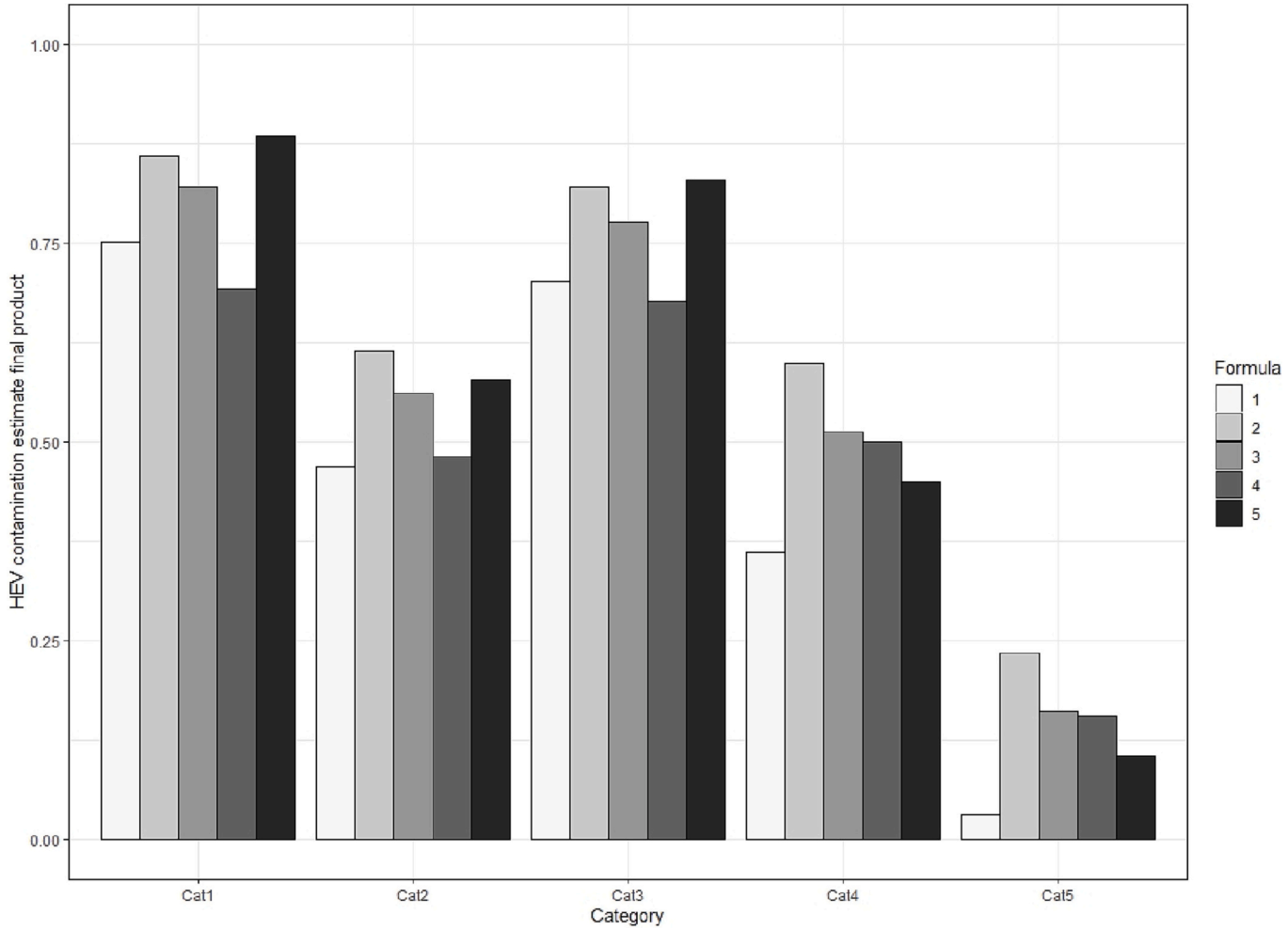

Fig. 1. Calculation of the normalized average scores by the five formulas (Table 2) for each of the five categories. Cat1: raw, ready to eat; Cat2: raw, heated by consumer; Cat3: undergone preservation step, ready to eat; Cat4: heated, ready to eat; Cat5: heated, reheated by consumer.

category 5) had the lowest scores. We concluded, with the data we have at the moment, that all five formulas give the same overall result and thus the relative importance of one variable does not outweigh one of the other variables. Looking at individual products, a very high score was noticeable for different types of salami, as well as raw liver sausage (figatellu) and chorizo.

3.1.3. Overall consumer exposure

A relative exposure analysis taking consumer habits into account was performed for each product category to estimate HEV consumer exposure (Fig. 2).

Consumption of non-RTE thermal-processed products (i.e. category 5) by the general population and highly susceptible population (i.e. a very small part (3 %) of the population more susceptible to HEV infection and resulting in a more serious clinical outcome than in the general population) as well as consumption of raw non-RTE products (i.e. category 2) by the general population was considered as 'medium risk' (Risk ranking of 43, 48 and 46 respectively), all the other risk rankings scored above 48 (i.e. 'high risk' of foodborne infection with HEV through consumption as defined by the authors of the tool) (Fig. 2). Independent of the population type analyzed, thermal-processed RTE products (i.e. category 4) always had the highest risk ranking compared to the other categories.

3.2. Detection of HEV RNA in pork meat products

In total, 54 RTE pork meat samples were analyzed. HEV RNA was detected in 31 % (n = 17/54) of the total samples. Specifically, 65 % of liver pâtés (n = 15/23, Ct value = 32.11 ± 4.79) and 15 % of raw dried hams (n = 2/13, Ct value = 35.20 ± 0.37) tested positive for HEV RNA (Table 3). No HEV RNA was detected in raw dried sausages.

3.3. Sequencing & phylogenetic analysis

Out of the 31 HEV positive samples, Sanger sequencing was performed on 9 pork liver pâté samples with moderate to high HEV RNA loads, based on a Ct-value below 33. Sequencing was successful in 4 cases; these were the samples with the lowest Ct-values and highest viral

Table 3
HEV RNA detection in tested RTE pork meat products from Belgium supermarkets.

Product type	Total	Positive (%)	Average Ct value (± sd)
Liver pâté	23	15 (65)	32.11 ± 4.79
Raw dried sausage	18	0 (0)	/
Raw dried ham	13	2 (15)	35.20 ± 0.37
All	54	17 (31)	32.48 ± 4.60

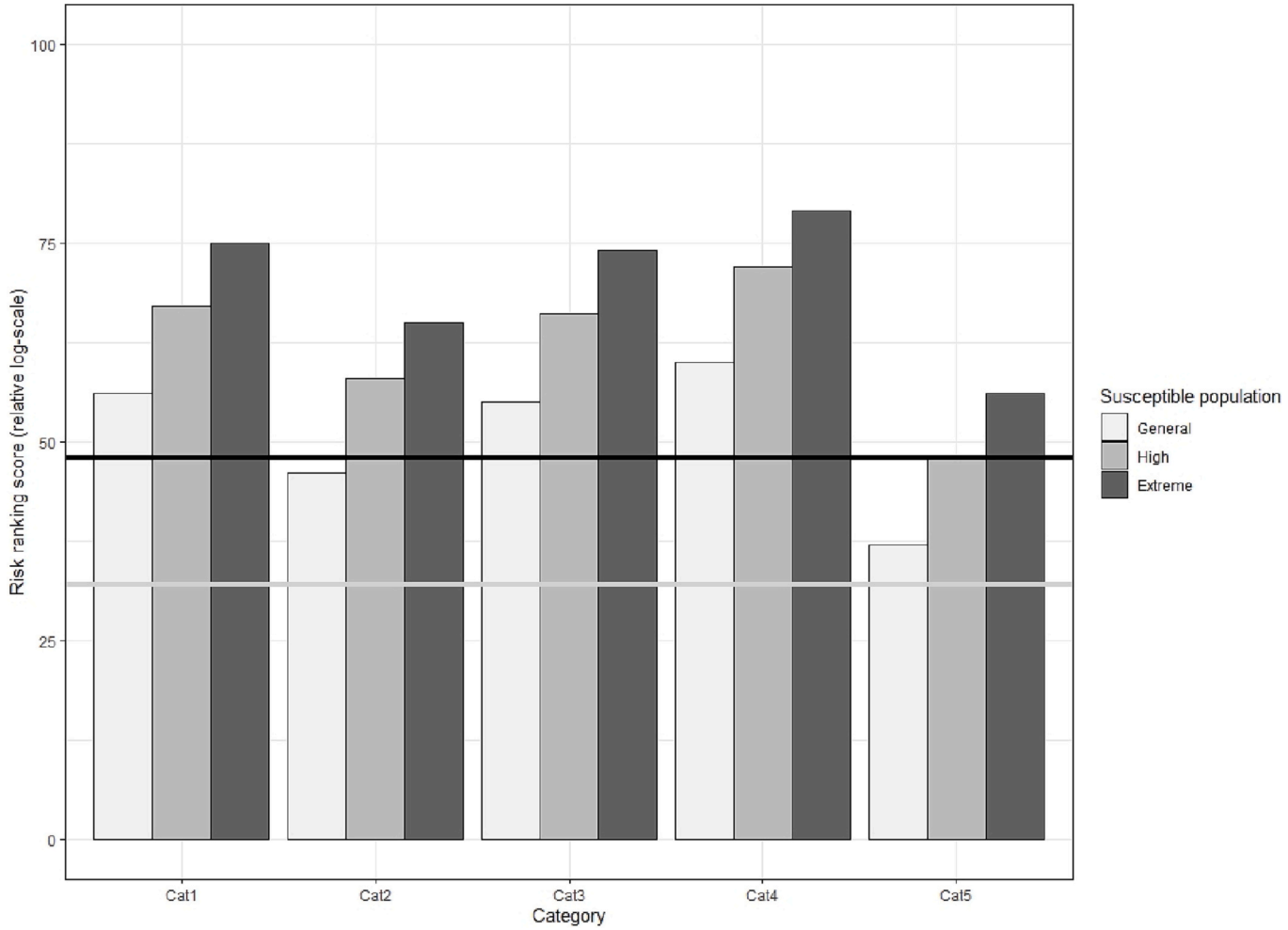

Fig. 2. Risk rankings of the 15 Risk Ranger analyses for three susceptible populations. Cat1: raw, ready to eat; Cat2: raw, heated by consumer; Cat3: undergone preservation step, ready to eat; Cat4: heated, ready to eat; Cat5: heated, reheated by consumer. Risk ranking scores are characterized as low (<32) (below grey line), medium (32–48) or high (>48) (above black line) risk for HEV foodborne infection as defined by Ross and Sumner (2002). The "Risk Ranking" value is scaled logarithmically between 0 and 100, where 0 represents no risk, and 100 represents maximum risk for foodborne infection as defined by Ross and Sumner (2002).

load. All were typed as gt3c. Phylogenetic analysis revealed sequence similarity of 97 %, 97.6 %, 98 % and 98.4 % between sequences from food products (P17_FOOD_Belgium, P9_FOOD_Belgium, P11_FOOD_-Belgium and P14_FOOD_Belgium) and human cases (MZ814618_HUMAN_Germany_2019, 01257_HUMAN_Belgium_2022, 00588_HUMAN_Belgium_2022 and MZ814751_HUMAN_Germany_2019) respectively (Fig. 3).

4. Discussion

Here we identify RTE pork meat products as having a high chance of being contaminated with HEV, based on the two assessments we performed, i.e. product contamination level and overall consumer exposure. RT-PCR analysis of selected RTE pork products from Belgian supermarkets revealed 31 % to be HEV RNA positive. Specifically, our analysis identified pork liver pâté to be highly contaminated, as 65 % of analyzed samples proved to be HEV RNA positive. Found HEV sequences in RTE pork meat products clustered with those identified in human cases in Belgium and abroad, corroborating the product contamination and consumer exposure assessments.

Globally, raw RTE products not heated at >72 °C for >20 min (Barnaud et al., 2012) during processing had the highest final product contamination estimate. More specifically when we look into detail of the 594 analyzed products (supplementary table 1), different types of raw dried sausages, i.e. salami, chorizo and raw liver sausage (figatellu), scored high. This is because of the presence of highly contaminated ingredients, i.e. pork blood or liver, the absence of a heating step during production and the RTE status in these kind of sausages. The products identified here correspond to what has been found in literature. Figatelli are notoriously contaminated with HEV RNA and have been linked to foodborne HEV infections in France and Italy (Colson et al., 2010; Garbuglia et al., 2015). More recently, HEV RNA has also been detected in raw dried pork sausages that do not contain pork liver (Boxman et al., 2020; Moor et al., 2018; Szabo et al., 2015). Unlike with figatellu, the presence of infectious HEV particles in raw dried pork sausages has not yet been demonstrated.

The second assessment with the Risk Ranger software revealed a high risk ranking for nearly every product category-and population combination. More specifically, all food categories investigated had a high risk ranking (i.e. >48) for the extremely susceptible population, while four out of the five categories and three out of the five categories had a high risk ranking for the highly susceptible and general population, respectively. When we look at the product categories, we see that thermal-processed RTE products (e.g. pork liver pâté, cooked ham, …) had the highest scores for all populations.

However, a note of caution is due here since we evaluated only the 'worst-case' scenario. The predictions of the Risk Ranger software are a simplification and likely give an overestimation (Ross and Sumner, 2002). Still it offers a rather simple way to prioritize pathogen-food product/susceptible population combinations and has been used in the past to evaluate the foodborne infection risk for certain pathogens (i.e. HAV, norovirus, *Campylobacter jejuni*, *Listeria monocytogenes*) (Birgen et al., 2019; EFSA, 2015; Torok et al., 2019).

Furthermore, it enables users to identify knowledge gaps, several of which were identified here. Firstly, as mentioned before, data on infectious virus present in food products is lacking. Due to this, data on HEV RNA detection in food products was used as an alternative. However, current molecular methods cannot discriminate between viral RNA derived from infectious or inactivated HEV particles. Consequently, it is unclear whether this data represents food products with infectious virus. Moreover, the minimal disease causing dose of HEV and the correlation between presence of viral RNA and infectious virus are unknown.

Secondly, the effect of processing techniques on HEV infectivity is understudied and factors such as initial viral load, viral strain and food matrix may have an effect on the inactivation efficiency.

This highlights the need for reliable HEV infectivity tests. These will be essential in determining the HEV minimal disease causing dose and in gaining more insight on the inactivation parameters in food matrices. These gaps need to be addressed first before a reliable quantitative risk assessment can be performed and subsequently risk management strategies can be implemented.

Finally, our assessment focused on HEV gt3, but did not take into account the individual gt3 subtype differences, since data on subtype distribution in food products is missing. It was recently found that infections with HEV clade efg subtype are associated with a more severe disease presentation than infections with HEV clade abchijklm (Peeters et al., 2022). In contrast, clade abchijklm infections were more associated with chronic infections in immunocompromised patients. If subtype specific data on food products would become available in the future, it would be interesting to include it in the analysis.

In the current study, for the first time, HEV RNA has been detected in pork meat products ready for consumption on the Belgian market. A high percentage (i.e. 31 %) of our RTE pork meat products were found to be positive for HEV RNA. However, as mentioned before it should be noted that current molecular methods can not differentiate between infectious and damaged viral particles and thus currently it is not possible to draw a conclusion on the presence of infectious virus in these HEV RNA positive products.

Nevertheless, 65 % of the pork liver pâtés tested positive for HEV RNA. This is in line with Boxman et al. (2019), who found that in the Netherlands 71 % and 69 % of pork liver sausage and pâté respectively, contained HEV RNA. In comparison, data from Germany and France, revealed a lower presence of HEV RNA in liver sausages and pâté (13 %–58 %) (Colson et al., 2010; Mansuy et al., 2011; Martin-Latil et al., 2014; Pallerla et al., 2020; Pavio et al., 2014). The seroprevalences of HEV in pigs across Belgium, the Netherlands, France, and Germany have been observed to be similar, ranging from 65 % to 73 % (Boxman et al., 2022; Krumbholz et al., 2013; Thiry et al., 2014; Walachowski et al., 2014). This suggests a comparable circulation of the virus within pig herds across these countries. Furthermore, given the similarity in pig breeding practices, including slaughter age, it can be assumed that a similar proportion of viremic pigs are entering slaughterhouses in these countries. Therefore differences in HEV RNA detection in pork liver products are probably due to (i) different viral extraction methods or (ii) different pâté/liver sausage compositions.

Another type of product examined in this study, i.e. raw dried hams, had a HEV RNA prevalence of 15 %. These products also had a high theoretical contamination level in our first assessment. Raw dried ham is made by curing pork thigh and rump muscle tissue. HEV contamination of pork muscle tissue is reportedly lower than liver tissue, ascribed to the inability of HEV to replicate in muscular cells or tissues (Di Bartolo et al., 2012; Feurer et al., 2018; García et al., 2020; Williams et al., 2001). However, muscles can be contaminated through the blood of viremic pigs and remain contaminated even after bleeding (Crotta et al., 2021). Additionally, co-infection with another virus (i.e. porcine reproductive or respiratory syndrome virus) could also increase the risk for pork muscle to be contaminated with HEV (Salines et al., 2019). The effect of dry curing on the virus has not been well investigated, but it is assumed to be highly stable at conditions applied in raw meat product preservation processes (Wolff et al., 2020b). All the previous data indicate that attention should be given to these types of products in the future.

None of the raw dried sausages tested in our study were positive for HEV RNA. In other European countries varying degrees of HEV RNA detection in raw dry sausages (i.e. 0 % to 20 %) have been observed (Boxman et al., 2020; Giannini et al., 2018; Montone et al., 2019; Moor et al., 2018; Pallerla et al., 2020; Szabo et al., 2015). These variations could be explained by the use of highly contaminated ingredients, i.e. diaphragm and porcine blood (Boxman et al., 2017, 2019) in raw dried sausages in some countries, other compositions and processing techniques, other methodologies for detecting HEV RNA in food products or the small sampling size in this study. Despite the fact that we do not detect HEV RNA in raw dried sausages, we cannot exclude

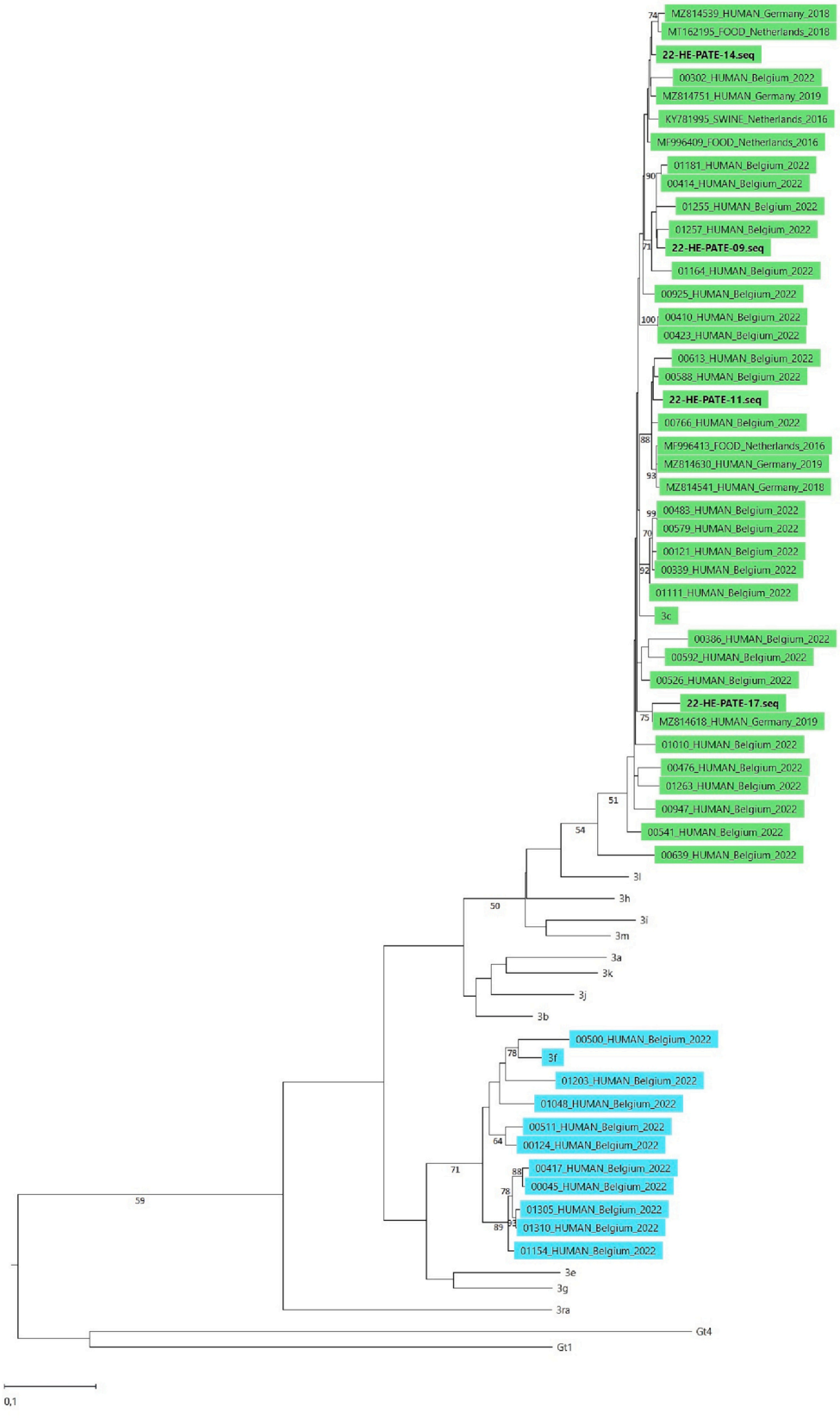

Fig. 3. Phylogenetic tree of a 493 base pairs fragment from ORF2 of HEV gt3. The tree is at scale, with branch lengths measured as the number of substitutions per site. HEV gt3 reference strains used are 3a, 3b, 3c, 3e, 3f, 3g, 3h, 3i, 3j, 3k, 3l, 3m and 3ra according to Smith et al., 2020. Gt1 (MH918640) and Gt4 (AB369688) reference strains were added as outgroup. Gt3c and 3f strains are colored in green and blue respectively. Bootstrap values >50 are displayed on the tree. The names of the sequences are composed of the GenBank accession numbers or the internal number at Sciensano combined with the source (i.e. FOOD = food products, SWINE = pig serum samples, HUMAN = human clinical serum samples) the location and year of sampling. Names indicated in bold are the four sequences isolated from pork liver pâté products identified in current study.

contamination of these kind of products.

Phylogenetic analysis revealed that all four sequences from pork liver pâté samples belonged to gt3c, one of the two genotype clades most found in Belgian clinical cases (Peeters et al., 2022). In addition, the viral strains isolated from pork liver pate showed phylogenetic relationships and similarities with sequences from German and Belgian human clinical cases.

Phylogenetic analyses of strains from food products and human clinical cases could serve as an indirect indication of foodborne HEV transmission. In fact, 99–100 % sequence similarities from food and human cases have been found in direct epidemiological investigations (Li et al., 2005; Purdy and Khudyakov, 2011; Takahashi et al., 2012; Tei et al., 2003). Within pig farms, sequences from infected animals can be highly similar as well, up to 99–100 % (Bouquet et al., 2011; Boxman et al., 2022). However, in random sampling studies, 100 % similarities between pigs, pork products and human sequences are rare (Bouquet et al., 2011; Boxman et al., 2017; Boxman et al., 2019; Rutjes et al., 2009, Yazaki et al., 2003). In France for example, sequence similarities of 68.4–99.3 % were found between pig liver (n = 43) and human sequences (n = 106) (Bouquet et al., 2011). In the Netherlands 100 % similarity was found between one pair of pork and human sequences, but lower similarities (i.e. 87.2–99.3 %) were found for the other tested sequences (i.e. 16 strains from human cases and 46 from swine and environment) (Rutjes et al., 2009). It should be noted however, that these studies used a shorter segment of the HEV genome, i.e. 204–306 nt and 148 nt of ORF2 respectively, compared to our study (i.e. 493 nt of ORF2). Nevertheless, more recent studies using the same methodology found >99 % sequence similarity between strains from human cases and strains from pork liver, blood and pork liver products (Boxman et al., 2017; Boxman et al., 2019; Boxman et al., 2022). However, not all cross-sectional sampling studies comparing sequences from food and human cases find these high similarities (Boxman et al., 2020; Pallerla et al., 2020). In our study none of the compared the sequences matched 100 %. This might be due to the low number of sequences tested (i.e. n = 4) compared to other studies, the high diversity of HEV gt3 strains and the fact that the majority of included human cases are solely those exhibiting symptoms (Bouquet et al., 2011, Boxman et al., 2019, Rutjes et al., 2009, Pallerla et al., 2020). Nevertheless, the clustering of related sequences and the presence of HEV RNA positive food products suggest a potential for foodborne transmission.

Given the contamination and exposure assessments and documented related viral strains in food products and human cases, we would advise immunocompromised patients, e.g. solid organ transplant recipients, to avoid pork liver products, especially if these are RTE.

Declaration of competing interest

All authors disclose no commercial associations that might create a conflict of interest in connection with this study.

Data availability

Data will be made available on request.

Acknowledgements

Research that yielded these results was funded by the Belgian Federal Public Service for Health, Food Chain Safety and Environment through the contract RT 18/6 DECHAHEV. We would like to thank lab technicians of the viral diseases and foodborne pathogens unit at Sciensano for their help, Isabelle Moyersoen for her aid in guiding us through the Sciensano National Food Consumption Survey from 2014 to 2015 in Belgium and the artisanal butchers and industrial producers that cooperated in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2023.110198.

References

Barnaud, E., Rogée, S., Garry, P., Rose, N., Pavio, N., 2012. Thermal inactivation of infectious hepatitis E virus in experimentally contaminated food. Appl. Environ. Microbiol. 78, 5153–5159. https://doi.org/10.1128/AEM.00436-12.

Berto, A., Grierson, S., Hakze-van der Honing, R., Martelli, F., Johne, R., Reetz, J., Ulrich, R.G., Pavio, N., Poel, W.H.M.Van Der, Banks, M., 2013. HEV in pork liver sausages, France. Emerg. Infect. Dis. 19, 3–5. https://doi.org/10.3201/eid1902.121255.

Birgen, B.J., Njue, L.G., Kaindi, D.M., Ogutu, F.O., 2019. Qualitative risk assessment of Campylobacter jejuni in street vended poultry in informal settlements of Nairobi County. Eur.J. Nutr. Food Saf. 11, 28–37.

Bouquet, J., Tessé, S., Lunazzi, A., Eloit, M., Rose, N., Nicand, E., Pavio, N., 2011. Close similarity between recovered from humans and swine, sequences of hepatitis E virus france, 2008−2009. Emerg. Infect. Dis. 17, 2018–2025.

Bouwknegt, M., Koppen, K., Rietveld, H., Straatsma, G., Heres, L., van't Hooft, B.J., 2017. Ordinal QMRA to Prioritize Pork Products That May Contribute to Foodborne Hepatitis E Virus Transmission. Abstract T3-H.2, Society for Risk Analysis Annual Meeting, Presented 13 December 2017. SRA, Arlington (VA), USA.

Boxman, I.L.A., Jansen, C.C.C., Hägele, G., Zwartkruis-Nahuis, A., Cremer, J., Vennema, H., Tijsma, A.S.L., 2017. Porcine blood used as ingredient in meat productions may serve as a vehicle for hepatitis E virus transmission. Int. J. Food Microbiol. 257, 225–231. https://doi.org/10.1016/j.ijfoodmicro.2017.06.029.

Boxman, I.L.A., Jansen, C.C.C., Hägele, G., Zwartkruis-Nahuis, A., Tijsma, A.S.L., Vennema, H., 2019. Monitoring of pork liver and meat products on the Dutch market for the presence of HEV RNA. Int. J. Food Microbiol. 296, 58–64. https://doi.org/10.1016/j.ijfoodmicro.2019.02.018.

Boxman, I.L.A., Jansen, C.C.C., Zwartkruis-Nahuis, A.J.T., Hägele, G., Sosef, N.P., Dirks, R.A.M., 2020. Detection and quantification of hepatitis E virus RNA in ready to eat raw pork sausages in the Netherlands. Int. J. Food Microbiol. 333, 108791 https://doi.org/10.1016/j.ijfoodmicro.2020.108791.

Boxman, I.L.A., Verhoef, L., Dop, P.Y., Vennema, H., Dirks, A.M., Opsteegh, M., 2022. High prevalence of acute hepatitis E virus infection in pigs in Dutch slaughterhouses. Int. J. Food Microbiol. 379, 109830 https://doi.org/10.1016/j.ijfoodmicro.2022.109830.

Colson, P., Borentain, P., Queyriaux, B., Kaba, M., Moal, V., Gallian, P., Heyries, L., Raoult, D., Gerolami, R., 2010. Pig liver sausage as a source of hepatitis E virus transmission to humans. J. Infect. Dis. 202, 825–834. https://doi.org/10.1086/655898.

Crotta, M., Lavazza, A., Mateus, A., Guitian, J., 2021. Viraemic pigs entering the food chain are the most likely source of hepatitis E virus (HEV) in pork meat: modelling the fate of HEV during slaughtering of pigs. Food Control 121, 107662. https://doi.org/10.1016/j.foodcont.2020.107662.

Crotta, M., Lavazza, A., Mateus, A., Guitian, J., 2018. Quantitative risk assessment of hepatitis E virus: modelling the occurrence of viraemic pigs and the presence of the virus in organs of food safety interest. Microb. Risk Anal. 9, 64–71. https://doi.org/10.1016/j.mran.2018.02.001.

Deest, G., Zehner, L., Nicand, E., Gaudy-Graffin, C., Goudeau, A., Bacq, Y., 2007. Autochthonous hepatitis E in France and consumption of raw pig meat. Gastroenterol. Clin. Biol. 31, 1095–1097. https://doi.org/10.1016/S0399-8320(07)78342-2.

Di Bartolo, I., Diez-valcarce, M., Vasickova, P., Kralik, P., Hernandez, M., Angeloni, G., Ostanello, F., Bouwknegt, M., Rodríguez-lázaro, D., Pavlik, I., 2012. Hepatitis E virus in pork production chain in Czech Republic, Italy, and Spain, 2010. Emerg. Infect. Dis. 18, 1282–1289.

ECDC, 2019. Options for National Testing and Surveillance for Hepatitis E Virus in the EU/EEA - Operational Guidance.

EFSA Panel on Biological Hazards, 2015. Scientific opinion on the development of a risk ranking toolbox for the EFSA BIOHAZ Panel. EFSA J. 13, 3939. https://doi.org/10.2903/j.efsa.2015.3939.

El Hariri, O., Bouchriti, N., Bengueddour, R., 2018. Risk assessment of histamine in chilled, frozen, canned and semi-preserved fish in Morocco; implementation of risk ranger and recommendations to risk managers. Foods 7, 157. https://doi.org/10.3390/foods7100157.

Feagins, A.R., Opriessnig, T., Guenette, D.K., Halbur, P.G., Meng, X.J., 2007. Detection and characterization of infectious hepatitis E virus from commmercial pig livers sold in local grocery in the USA. J. Gen. Virol. 88, 912–917. https://doi.org/10.1099/vir.0.82613-0.

Feagins, A.R., Opriessnig, T., Guenette, D.K., Halbur, P.G., Meng, X.J., 2008. Inactivation of infectious hepatitis E virus present in commercial pig livers sold in local grocery stores in the United States. Int. J. Food Microbiol. 123, 32–37. https://doi.org/10.1016/j.ijfoodmicro.2007.11.068.

Feurer, C., Le Roux, A., Rossel, R., Barnaud, E., Dumarest, M., Garry, P., Pavio, N., 2018. High load of hepatitis E viral RNA in pork livers but absence in pork muscle at French slaughterhouses. Int. J. Food Microbiol. 264, 25–30. https://doi.org/10.1016/j.ijfoodmicro.2017.10.013.

García, N., Hernández, M., Gutierrez-Boada, M., Valero, A., Navarro, A., Muñoz-Chimeno, M., Fernández-Manzano, A., Escobar, F.M., Martínez, I., Bárcena, C., González, S., Avellón, A., Eiros, J.M., Fongaro, G., Domínguez, L., Goyache, J.,

Rodríguez-Lázaro, D., 2020. Occurrence of hepatitis E virus in pigs and pork cuts and organs at the time of slaughter, Spain, 2017. Front. Microbiol. 10, 1–10. https://doi.org/10.3389/fmicb.2019.02990.

Garbuglia, A.R., Alessandrini, A.I., Pavio, N., Tessé, S., Grignolo, S., Viscoli, C., Lapa, D., Capobianchi, M.R., 2015. Male patient with acute hepatitis E in Genoa, Italy: figatelli (pork liver sausage) as probable source of the infection. Clin. Microbiol. Infect. 21, e4–e6. https://doi.org/10.1016/j.cmi.2014.07.007.

Giannini, P., Jermini, M., Leggeri, L., Nüesch-Inderbinen, M., Stephan, R., 2018. Detection of hepatitis e virus RNA in raw cured sausages and raw cured sausages containing pig liver at retail stores in Switzerland. J. Food Prot. 81, 43–45. https://doi.org/10.4315/0362-028X.JFP-17-270.

Imagawa, T., Sugiyama, R., Shiota, T., Li, T., Yoshizaki, S., Wakita, T., Ishii, K., 2018. Evaluation of heating conditions for inactivation of hepatitis E virus genotypes 3 and 4. J. Food Prot. 81, 947–952.

Johne, R., Trojnar, E., Filter, M., Hofmann, J., 2016. Thermal stability of hepatitis E virus as estimated by a cell culture method. Appl. Environ. Microbiol. 82, 4225–4231. https://doi.org/10.1128/AEM.00951-16.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Krumbholz, A., Joel, S., Neubert, A., Dremsek, P., Dürrwald, R., Johne, R., Hlinak, A., Walther, M., Lange, J., Wutzler, P., Sauerbrei, A., Ulrich, R.G., Zell, R., 2013. Age-related and regional differences in the prevalence of hepatitis E virus-specific antibodies in pigs in Germany. Vet. Microbiol. 167, 394–402. https://doi.org/10.1016/j.vetmic.2013.10.001.

Li, T., Chijiwa, K, Sera, N., Ishibashi, T., Etoh, Y., Shinohara, Y., Kurata, Y., Ishida, M., Sakamoto, S., Takeda, N., Miyamura, T., 2005. Hepatitis E virus transmission from wild boar meat. Emerg. Infect. Dis. 11, 1958–1959.

Mansuy, J., Bendall, R., Legrand-Abravanell, F., Sauné, K., Miédouge, M., Ellis, V., Rech, H., Destruel, F., Kamar, N., Dalton, H.R., Izopet, J., 2011. Hepatitis E virus antibodies in blood donors,France. Em. Inf. Dis. 17, 2309–2312. https://doi.org/10.3201/eid1712.110371.

Martin-Latil, S., Hennechart-Collette, C., Guillier, L., Perelle, S., 2014. Method for HEV detection in raw pig liver products and its implementation for naturally contaminated food. Int. J. Food Microbiol. 176, 1–8. https://doi.org/10.1016/j.ijfoodmicro.2014.01.016.

Mataragas, M., Skandamis, P.N., Drosinos, E.H., 2008. Risk profiles of pork and poultry meat and risk ratings of various pathogen/product combinations. Int. J. Food Microbiol. 126, 1–12. https://doi.org/10.1016/j.ijfoodmicro.2008.05.014.

Matsubayashi, K., Kang, J., Sakata, H., Takahashi, K., Shindo, M., Kato, M., Sato, S., Kato, T., Nishimori, H., Tsuji, K., Maguchi, H., Yoshida, J., Maekubo, H., Mishiro, S., Ikeda, H., 2008. A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route. Transfusion 48, 1368–1375. https://doi.org/10.1111/j.1537-2995.2008.01722.x.

Meester, M., Bouwknegt, M., Hakze-van der Honing, R., Vernooij, H., Houben, M., van Oort, S., van der Poel, W.H.M., Stegeman, A., Tobias, T., 2022. Repeated cross-sectional sampling of pigs at slaughter indicates varying age of hepatitis E virus infection within and between pig farms. Vet. Res. 50, 1–12. https://doi.org/10.1186/s13567-022-01068-3.

Montone, A.M.I., De Sabato, L., Suffredini, E., Alise, M., Zaccherini, A., Volzone, P., Di Maro, O., Neola, B., Capuano, F., Di Bartolo, I., 2019. Occurrence of HEV-RNA in Italian regional pork and wild boar food products. Food Environ. Virol. 11, 420–426. https://doi.org/10.1007/s12560-019-09403-2.

Moor, D., Liniger, M., Baumgartner, A., Felleisen, R., 2018. Screening of ready-to-eat meat products for hepatitis E virus in Switzerland. Food Environ. Virol. 10, 263–271. https://doi.org/10.1007/s12560-018-9340-x.

Mulder, A.C., Kroneman, A., Franz, E., Vennema, H., Tulen, A.D., Takkinen, J., Hofhuis, A., Adlhoch, C., Aberle, S., Subissi, L., Suin, V., Midgley, S., Kuznetsova, T., Izopet, J., Pavio, N., Baechlein, C., Baylisa, S.A., Corman, V.M., Fabera, M., Johne, R., Kamp, C., Wenzel, J.J., Coughlan, S., Di Bartolo, I., Bruni, R., Ciccaglionea, A.R., Garbuglia, A.R., Suffredini, E., Boxman, I., Hogema, B., van der Poel, W., Zaaijera, H., de Sousaa, R., Velebit, B., Avellóna, A., Buti, M., Girones, R., Quer, J., Widén, F., Norder, H., Nyström, K., Bachofen, C., Sahli, R., Ijaza, S., Treagus, S., Kulka, M., Rizzi, V., 2019. HEVnet: a one health, collaborative, interdisciplinary network and sequence data repository for enhanced hepatitis e virus molecular typing, characterisation and epidemiological investigations. Eurosurveillance 24, 1–6. https://doi.org/10.2807/1560-7917.ES.2019.24.10.1800407.

Pallerla, S.R., Schembecker, S., Meyer, C.G., Linh, L.T.K., Johne, R., Wedemeyer, H., Bock, C.T., Kremsner, P.G., Velavan, T.P., 2020. Hepatitis E virus genome detection in commercial pork livers and pork meat products in Germany. J. Viral Hepat. 28, 196–204. https://doi.org/10.1111/jvh.13396.

Pas, S.D., de Man, R.A., Mulders, C., Balk, A.H.M.M., van Hal, P.T.W., Weimar, W., Koopmans, M.P.G., Osterhaus, A.D.M.E., van der Eijk, A.A., 2012. Hepatitis E virus infection among solid organ transplant recipients, the Netherlands. Emerg. Infect. Dis. 18, 869–872. https://doi.org/10.3201/eid1805.111712.

Pavio, N., Merbah, T., Study, T., France, I., 2014. Frequent hepatitis E virus contamination in food containing raw pork liver, France. Emerg. Infect. Dis. 20, 1925–1927. https://doi.org/10.3201/eid2011.140891.

Peeters, M., Schenk, J., Somer, T.De, Roskams, T., Locus, T., Klamer, S., Subissi, L., Suin, V., Delwaide, J., Willems, P., Colle, I., Hoof, M.Van, Acker, J.Van, Steenkiste, C.Van, Moreno, C., Janssens, F., Reynders, M., Steverlynck, M., Verlinden, W., Lasser, L., Galocsy, C.De, Geerts, A., Maus, J., Gallant, M., Outryve, S. Van, Marot, A., 2022. Viral clade is associated with severity of symptomatic genotype 3 hepatitis E virus infections in Belgium, 2010–2018. J. Hepatol. 77.

Pellerin, M., Trabucco, B., Capai, L., Laval, M., Maestrini, O., Jori, F., Falchi, A., Doceul, V., Charrier, F., Casabianca, F., Pavio, N., 2022. Low prevalence of hepatitis E virus in the liver of Corsican pigs slaughtered after 12 months despite high antibody seroprevalence. Transbound. Emerg. Dis. 69, e2706–e2718. https://doi.org/10.1111/tbed.14621.

Purdy, M.A., Khudyakov, Y.E., 2011. The molecular epidemiology of hepatitis E virus infection. Virus Res. 161, 31–39. https://doi.org/10.1016/j.virusres.2011.04.030.

Purdy, M.A., Harrison, T.J., Jameel, S., Meng, X.J., Okamoto, H., Van der Poel, W.H.M., Smith, D.B., ICTV Report Consortium, 2017. ICTV virus taxonomy profile: Hepeviridae. J. Gen. Virol. 98, 2645–2646 https://doi.org/10.1099%2Fjgv.0.000940.

Renou, C., Roque-Afonso, A.M., Pavio, N., 2014. Foodborne transmission of hepatitis E virus from raw pork liver sausage,France. Emerg. Infect. Dis. 20, 1945–1947. https://doi.org/10.3201/eid2011.140791.

Ross, T., Sumner, J., 2002. A simple, spreadsheet-based, food safety risk assessment tool. Int. J. Food Microbiol. 77, 39–53. https://doi.org/10.1016/S0168-1605(02)00061-2.

Rutjes, S.A., Lodder, W.J., Lodder-Verschoor, F., Van Den Berg, H.H.J.L., Vennema, H., Duizer, E., Koopmans, M., De Husman, A.M.R., 2009. Sources of hepatitis E virus genotype 3 in the Netherlands. Emerg. Infect. Dis. 15, 381–387. https://doi.org/10.3201/eid1503.071472.

Salines, M., Demange, A., Stéphant, G., Renson, P., Bourry, O., Andraud, M., Rose, N., Pavio, N., 2019. Persistent viremia and presence of hepatitis E virus RNA in pig muscle meat after experimental co-infection with porcine reproductive and respiratory syndrome virus. Int. J. Food Microbiol. 292, 144–149. https://doi.org/10.1016/j.ijfoodmicro.2018.12.023.

Smith, D.B., Izopet, J., Nicot, F., Simmonds, P., Jameel, S., Meng, X.J., Norder, H., Okamoto, H., van der Poel, W.H.M., Reuter, G., Purdy, M.A., 2020. Update: proposed reference sequences for subtypes of hepatitis E virus (species Orthohepevirus A). J. Gen. Virol. 101, 692–698. https://doi.org/10.1099/jgv.0.001435.

Spahr, C., Knauf-Witzens, T., Vahlenkamp, T., Ulrich, R.G., Johne, R., 2017. Hepatitis E virus and related viruses in wild, domestic and zoo animals: a review. Zoonoses Public Health 65, 11–29. https://doi.org/10.1111/zph.12405.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Sumner, J., Ross, T., Jenson, I., Pointon, A., 2005. A risk microbiological profile of the Australian red meat industry: risk ratings of hazard–product pairings. Int. J. Food Microbiol. 105, 221–232.

Szabo, K., Trojnar, E., Anheyer-Behmenburg, H., Binder, A., Schotte, U., Ellerbroek, L., Klein, G., Johne, R., 2015. Detection of hepatitis E virus RNA in raw sausages and liver sausages from retail in Germany using an optimized method. Int. J. Food Microbiol. 215, 149–156. https://doi.org/10.1016/j.ijfoodmicro.2015.09.013.

Takahashi, H., Tanaka, T., Jirintai, S., Nagashima, S., Takahashi, M., Nishizawa, T., Mizuo, H., Yazaki, Y., Okamoto, H., 2012. A549 and PLC/PRF/5 cells can support the efficient propagation of swine and wild boar hepatitis E virus (HEV) strains: demonstration of HEV infectivity of porcine liver sold as food. Arch. Virol. 157, 235–246. https://doi.org/10.1007/s00705-011-1153-2.

Tei, S., Kitajima, N., Takahashi, K., Mishiro, S., 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 362, 371–373. https://doi.org/10.1016/S0140-6736(03)14025-1.

Thiry, D., Mauroy, A., Saegerman, C., Thomas, I., Wautier, M., Miry, C., Czaplicki, G., Berkvens, D., Praet, N., van der Poel, W., Cariolet, R., Brochier, B., Thiry, E., 2014. Estimation of hepatitis E virus (HEV) pig seroprevalence using ELISA and Western blot and comparison between human and pig HEV sequences in Belgium. Vet. Microbiol. 172, 407–414. https://doi.org/10.1016/j.vetmic.2014.06.004.

Torok, V.A., Hodgson, K.R., Jolley, J., Turnbull, A., McLeod, C., 2019. Estimating risk associated with human norovirus and hepatitis A virus in fresh Australian leafy greens and berries at retail. Int. J. Food Microbiol. 309, 108327 https://doi.org/10.1016/j.ijfoodmicro.2019.108327.

Walachowski, S., Dorenlor, V., Lefevre, J., Lunazzi, A., Eono, F., Merbah, T., Eveno, E., Pavio, N., Rose, N., 2014. Risk factors associated with the presence of hepatitis E virus in livers and seroprevalence in slaughter-age pigs: a retrospective study of 90 swine farms in France. Epidemiol. Infect. 142, 1934–1944. https://doi.org/10.1017/S0950268813003063.

Williams, T.P.E., Kasorndorkbua, C., Halbur, P.G., Haqshenas, G., Guenette, D.K., Toth, T.E., Meng, X.J., 2001. Evidence of extrahepatic sites of replication of the hepatitis E virus in a swine model. J. Clin. Microbiol. 39, 3040–3046. https://doi.org/10.1128/JCM.39.9.3040-3046.2001.

Wolff, A., Günther, T., Albert, T., Schilling-Loeffler, K., Gadicherla, A.K., Johne, R., 2020. Stability of hepatitis E virus at different pH values. Int. J. Food Microbiol. 325, 108625 https://doi.org/10.1016/j.ijfoodmicro.2020.108625.

Wolff, A., Günther, T., Albert, T., Johne, R., 2020. Effect of sodium chloride, sodium nitrite and sodium nitrate on the infectivity of hepatitis E virus. Food Environ. Virol. 12, 350–354. https://doi.org/10.1007/s12560-020-09440-2.

Yazaki, Y., Mizuo, H., Takahashi, M., Nishizawa, T., Sasaki, N., Gotanda, Y., Okamoto, H., 2003. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. J. Gen. Virol. 84, 2351–2357. https://doi.org/10.1099/vir.0.19242-0.