

Hepatitis E in pig-derived food products in Cape Town, South Africa, 2014

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Background. Hepatitis E virus (HEV) genotypes 3 and 4 are zoonoses, with domestic pigs being the most important reservoir. A high anti-HEV IgG seroprevalence of 26 - 28% has been found in humans in Cape Town, South Africa (SA). Studies in industrialised countries have indicated a high prevalence of HEV in pigs and their associated food products.

Objectives. To determine whether HEV could be found in pig-derived food products in Cape Town.

Methods. Pork-containing food products were purchased from supermarkets and butcheries around the Cape Town metropolitan area. HEV detection by polymerase chain reaction (PCR) was performed, and an amplified viral genome fragment was sequenced from positive samples. Phylogenetic analysis was done on the sequenced fragment.

Results. HEV was detected by PCR in 2/144 food samples – both were liver spread samples. One genome fragment sequence was obtained, which was closely related to HEV sequences obtained from humans in Cape Town.

Conclusions. HEV can be found in pork-containing meat products available for sale in Cape Town, suggesting that these products could be a potential source of HEV transmission in our geographical area. Meat of pig origin should be thoroughly cooked before being consumed.

S Afr Med J 2019;109(8):584-586. DOI:10.7196/SAMJ.2019.v109i8.13868

Hepatitis E virus (HEV) is a small, spherical, non-enveloped RNA virus belonging to the family Hepeviridae. HEV, of which four genotypes cause most human disease, is a common causative agent of acute viral hepatitis throughout the world. Genotypes 1 and 2 are associated with waterborne transmission and cause epidemics. Genotypes 3 and 4 are zoonoses, with domestic pigs being the most important reservoir.^[1] Infection is usually subclinical and self-limiting but can cause severe jaundice and acute liver failure, particularly in pregnant women and immunocompromised patients.^[1] Studies have found a high prevalence (26 - 28%) of anti-HEV IgG in humans in Western Cape Province, South Africa (SA).^[2,3] Risk factors associated with HEV seropositivity include exposure to pig meat.^[2,3] In animals, HEV infection has been identified in pigs in the Eastern Cape^[4] and Western Cape provinces^[5] of SA.

Studies in industrialised countries found a high prevalence of HEV in pigs and their associated food products. For example, HEV RNA was detected in pig-derived products sold in stores in the USA,^[6] UK^[7] and France.^[8] In a mini-outbreak of hepatitis E reported in France,^[8] 7/13 individuals who ate *figatellu* (dried sausage made from raw pig liver) had detectable IgM and/or HEV RNA, as opposed to 0/5 individuals from the same families who did not consume the sausage. In that study, HEV sequences from sausage and infected individuals were genetically similar.^[8] The risk of contracting the virus by way of pork products is higher when consuming undercooked or raw pork products.^[9] Cooking at 71°C for 20 minutes has been found to fully inactivate the virus.^[9]

The objective of the current study was to determine whether HEV could be found in pig-derived food products in Cape Town, SA. We hypothesised that HEV RNA could be found in samples from

various pig-derived meats purchased in the Cape Town area. This study points to a plausible infective source to explain the high levels of antibody reactivity against HEV seen in humans in Cape Town.

Methods

Pork products ($N=144$) were purchased from 59 vendors (45 supermarkets and 14 butcheries) around the Cape Town metropolitan area over 3 days in July 2014. Samples were kept in 'cold boxes' during acquisition and stored at 4°C overnight until processing the next day. Most of the samples collected ($n=104$) were raw pig products (Table 1). The remaining samples ($n=40$) were processed or partly processed meats (Table 1).

The samples were processed using sterile equipment to prevent cross-contamination. Prior to nucleic acid extraction, ~25 mg of meat from each sample was finely chopped with a scalpel to aid tissue lysis. Nucleic acid was extracted from each sample using the Qiagen QIAamp DNA Mini Kit (Qiagen, Germany) as per the manufacturer's instructions, and eluted in 100 µL of the buffer provided. Real-time reverse transcription polymerase chain reaction (PCR) was performed on the RotorGene 2000 as described by Garson *et al.*,^[10] using the QuantiFast Pathogen RT-PCR +IC Kit (Qiagen, Germany). Positive samples were amplified as described by Meng *et al.*,^[11] using the SuperScript One-Step RT-PCR System with Platinum Taq (Thermo Fisher Scientific, USA) for the first round of PCR, and SuperTherm Taq DNA Polymerase (Separation Scientific, SA) for the nested reaction, using the following primers:^[11] forward outer 3156, reverse outer 3157, forward inner 3158, reverse inner 3159. This region of open reading frame 2 was chosen for analysis based on detection of the four main genotypes, sufficient variability for

Table 1. Pork-containing food sample type and source

	Supermarket, <i>n</i>	Butchery, <i>n</i>
Raw		
Meat cut	19	6
Mince	2	1
Stewing pork	16	8
Tripe	0	1
Trotters	9	4
Liver	0	10
Other organs	2	6
Sausage	16	4
Subtotal (<i>n</i> =104)	64	40
Smoked/cured		
Smoked sausage	1	0
Dried sausage	2	0
Liver spread	5	0
Bacon	15	4
Ham	3	0
Ribs	6	4
Subtotal (<i>n</i> =40)	32	8
Total (<i>N</i> =144)	96	48

analysis and ability to compare with other sequences and studies, as this is the most commonly sequenced region. The resulting 348 base-pair product from open reading frame 2 was sent to Inqaba Biotec (SA) for bidirectional sequencing using the nested amplification primers. Chromatograms were edited in FinchTV (Geospiza, USA) and sequences were analysed using MEGA6 (MEGA, USA).^[12] The maximum likelihood tree was calculated in MEGA6 using an alignment of 279 nucleotide positions, and bootstrap support was calculated with 1 000 replicates.

Ethical approval

The study was approved by the Human Research Ethics Committee of the University of Cape Town (ref. no. 379/2014).

Results

HEV RNA was detected by real-time reverse transcription PCR in two liver spread samples (PF039 and PF045) obtained from different supermarket chains in different suburbs of Cape Town. The cycle threshold values for samples PF039 and PF045 were 37.4 and 34.6, respectively. High-quality HEV sequence (Genbank accession MF503296) was obtained in one of these samples (PF045), and it clustered with other genotype 3e HEV sequences previously reported in humans in Cape Town (Fig. 1). Our HEV sequence shared 87.5 - 89.6% similarity with other HEV sequences from Cape Town, and was also closely related (90.4% similarity) to an HEV genotype 3e sequence (Genbank accession KC758126) from the Netherlands (Fig. 1).

Discussion

HEV can be found in pork-containing meat available for sale in Cape Town. This corresponds with what has been reported in industrialised countries.^[6-8] The high similarity between our genotype 3 HEV and that of human HEV cases in Cape Town^[2,13,14] suggests that pork-containing food products could be a potential source of HEV transmission in our geographical area. Another possible reservoir not yet studied locally could be filter-feeding shellfish, as they feed on sewage entering the ocean; such shellfish are regularly eaten in Cape Town. Although not yet reported in SA, further potential sources of HEV in our study area may include seafood and raw vegetables. HEV

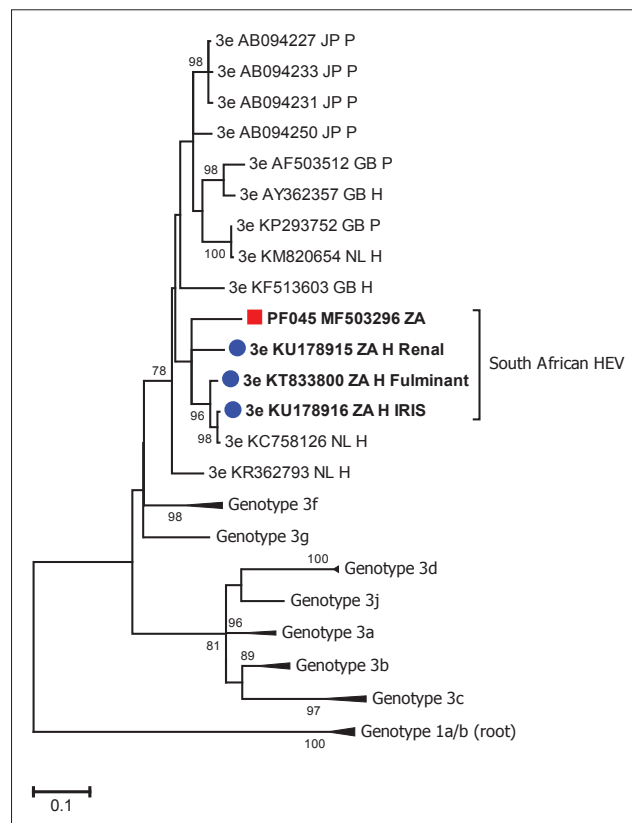


Fig. 1. Maximum likelihood tree showing hepatitis E genotype 3 with subgenotypes, rooted on genotype 1a/b. Genotypes other than 3e have been condensed. The tree was calculated in MEGA from 279 nucleotide positions in open reading frame 2, with 1 000 bootstrap replicates. Bootstrap support >70% is shown. This study's sequence is marked PF045_MF503296_ZA (red square). International Organization for Standardization (ISO) country codes JP, GB, NL and ZA refer to Japan, the UK, the Netherlands and South Africa (SA), respectively. H and P at the end of each sequence name refer to human and pig origin of the sequence, respectively. SA sequences marked renal, fulminant and IRIS (blue circles) are from humans.^[2,13,14] The scale bar shows 0.1 substitutions per site (10%). (HEV = hepatitis E virus.)

has been detected in seafood^[15] and vegetables^[16] in the UK and Italy, respectively. Finally, blood donor safety needs further consideration.^[17]

Conclusions

HEV can be found in pork-containing meat available for sale in Cape Town. Further studies are needed to investigate the foodborne transmission of HEV in SA. Public awareness around pork-containing food safety should be encouraged, especially for high-risk groups,^[17] and meat of pig origin should be thoroughly cooked before being consumed.

Declaration. None.

Acknowledgements. None.

Author contributions. MK: study outline, manuscript review; SK: study design, laboratory work, analysis, manuscript writing/review; JB, MB, AG, RPH: contributed equally to sample collection, laboratory work, manuscript writing/review. JB, MB, AG, RPH were undergraduate MB ChB students while the study was being conducted. RPH was in possession of a PhD degree before entering the MB ChB course.

Funding. The project received no specific funding for reagents or laboratory work. MK was a Wellcome Trust (UK) fellow (102429/Z/13/Z)

and is currently supported by the Carnegie Corporation of New York (USA) early-career fellowship, the Canadian Institutes for Health Research (CIHR), Canadian HIV Trials Network (CTN) international fellowship, and the US National Institutes of Health (1R01HD093578-01).

Conflicts of interest. None.

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Accepted 4 February 2019.