The role of pork in human infections

Andrea I Estevez-Garcia DVM PhD and her international team present research on hepatitis E viruses as emerging pathogen in Latin America and developed countries

Hepatitis E virus (HEV) is transmitted by food and through contaminated water. There is no evidence of human HEV epidemics in developed countries, but serological and molecular evidences of HEV circulation in human and pig populations are increasing as well as HEV-RNA detection in pork products. These findings raise the question about the role of pork as a possible important source for human infections. This review addresses key aspects of HEV infection and transmission and points out the need for inexpensive, accurate and rapid detection tools for usage on food matrices, particularly if viral integrity and minimal infectious dose can be assessed, which appear critical to support the global health surveillance systems.

INTRODUCTION

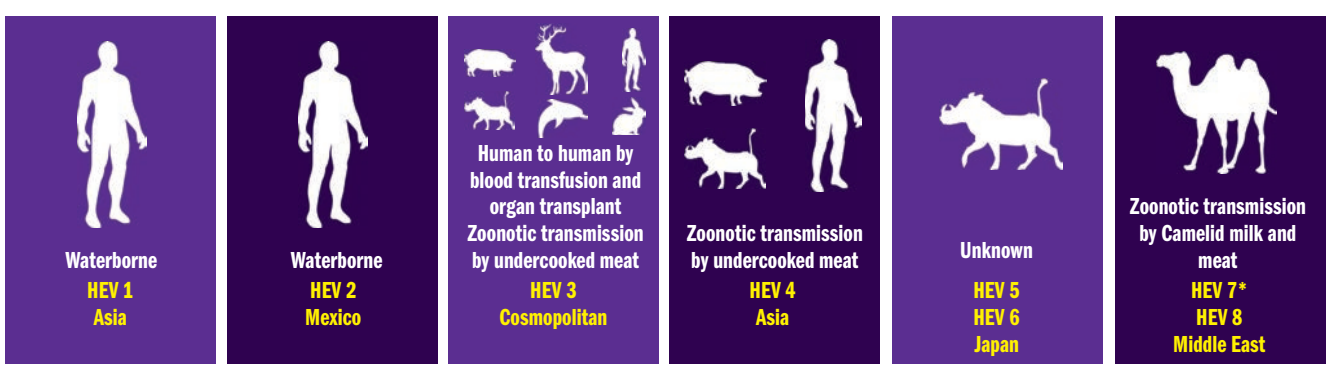
Approximately 20 million of HEV infections occur worldwide every year. While most infections remain asymptomatic, clinical cases are estimated to reach 3.3 million per year (WHO, 2017). Clinical manifestations of HEV infection in the acute form are: jaundice, anorexia, hepatomegaly, abdominal pain, vomiting, and fever. In rare cases depending on immunological host status, HEV infection induces hepatic failure (Pavio et al, 2014). In 2015, 44 000 cases were reported, among them, the mortality rate reached 3.3% (WHO, 2017). Nevertheless, in pregnant women it can reach up to 20%, presumably due to immunological/hormonal changes associated to this physiological state (Purcell and Emerson, 2008). Extrahepatic symptoms can occur ,such as the Guillain-Barré syndrome, neuralgic amyotrophy and encephalitis/meningoencephalitis/myositis (Kamar et al, 2016).
For details on taxonomy and a summary of the epidemiology of the eight genotypes that compose HEV group, please refer to figure 1.

WHAT IS KNOWN ON HEV TRANSMISSION?

The main HEV route of transmission is faecal-oral by consumption of contaminated water or food, including undercooked pork and related products (Barnaud et al, 2012; Heldt et al, 2016), oysters, mussels, cockles and shellfish (Yugo and Meng, 2013). HEV is also an environmental safety concern when liquid manure from farm animals is used to fertilize crops, thereby contaminating irrigation, drinking and coastal water (Yugo and Meng, 2013).
While HEV is endemic in industrial and developing countries, until now, epidemics have only been reported in developing countries (Yugo and Meng, 2013). Pigs and wild boars, considered the main HEV reservoirs, typically remain asymptomatic during natural or experimental infection (Meng et al, 2012). Swine farmers, veterinarians and pig handlers are at higher risk of HEV infection (Meng et al, 2002). Therefore, all biosafety measures and personal protection equipment must be applied in order to avoid any contamination when manipulating pork-derived samples.
HEV is actively circulating in Brazilian swine herds as well as in human populations; anti-HEV antibodies have been detected in the human population in several geographic regions (Carrilho et al, 2005), nevertheless until now only one human caseassociated with pork consumption has been reported to date involving HEV-3 genotype (Lopes dos Santos et al, 2010).
HEV-3 prevalence in swine feces, liver, bile and sewage from pig farms in Brazil is high (da Costa Lana et al, 2014; de Souza et al, 2012; dos Santos et al, 2011; Gardinali et al, 2012; Vasconcelos et al, 2015), thus, cross-contamination of pork meat during the evisceration process is plausible. Recently, HEV RNA was detected in 36% of Brazilian ready-to-eat products such as pork pâté and blood sausages

HEV ss(+) RNA genome
Hepeviridae family
Orthohepevirus genus
Orthohepevirus A species

HEV Genotypes: hosts, geographical distribution and transmission route according to literature.



(References: Purdy et al, 2017 Lee et al, 2016; Smith et al, 2016)

(total n= 50) using RT-PCR (Heldt et al, 2016). Sequencing revealed a HEV strain belonging to the zoonotic genotype 3 (Heldt et al, 2016). Assessment of the prevalence of HEV contamination in pork products seems detrimental to determine which factors are associated with HEV contamination.

HEV has also been identified in Colombia, where the presence of HEV-RNA was detected in 110/280 faeces samples and 109/295 livers from slaughterhouses (Forero et al, 2017). In Argentina, human cases of HEV-1 were reported and five additional HEV strains related to European, American and Japanese HEV genotype 3 have been identified. The source of infection was not established and the merit of the swine reservoir still needs to be addressed (Munné et al, 2011).

Although global prevalence rates of anti-HEV antibodies in pig populations are high (almost 100% in USA, 90% in Mexico, 65% in France, 46% in New Zealand, 46% in Laos and 48% in Spain) (Riveiro-Barciela et al, 2012; Rose et al, 2011), the presence of HEV RNA in commercially available pork livers or at slaughterhouses ranges between 4-11% in several European countries (Barnaud et al, 2012; Di Bartolo et al, 2012; Rose et al, 2011). A possible explanation is that infected pigs would likely have recovered from infection by the time of slaughter and the product would not represent a health risk to the consumer (Bouwknegt, 2013).

Some studies have also detected HEV-RNA in pork meat samples or in pork sausages in the UK, Czech Republic, Italy, Spain (Berto et al, 2012; Di Bartolo et al, 2012) and Canada (Mykytczuk et al, 2017). The highest rate of HEV-RNA detected in ready to eat pork liver pate was 36 positive samples of 72, representing 50% of occurrence.(Mykytczuk et al, 2017).

The number of studies detecting the presence of HEV-RNA in pork and related products is apparently increasing, but its true significance in public health remains unexplored. In the UK, a questionnaire-based study identified the strength of association between the consumption of certain pork products and HEV infection: pork pies (OR 6.33), sausages (OR 7.59) and ham (OR 10.9; Said et al, 2014). Another study conducted in Southern France found an association between the presence of anti-HEV antibodies and the consumption of uncooked pork liver sausages, offal and mussels, by means of multivariate analysis in a blood donor cohort (Mansuy et al, 2015). During the first autochthonous HEV-3 outbreak in Australia, 16 people were hospitalised with high-hepatic enzyme. There was a significant ($p<0.05$) association with pork pate consumption. The pork livers used for pate preparation by the restaurant were subsequently traced back to a single Australian farm (Yapa et al, 2016). This evidence show a strong epidemiological association of pork and mussels as source of human contamination, also showing that exposition can be more common than expected.

HEPATITIS E, A POORLY UNDERSTOOD DISEASE

HEV-3 has been documented for decades in developing countries and occasionally autochthonous cases have been diagnosed in the United States, Canada, and Australia (Clemente-Casares et al, 2016). HEV may be endemic and under-diagnosed in industrialised countries (De Schryver et al, 2015; Wilhelm et al, 2011).

In Portugal, an increasing number of chronic hepatitis cases have been observed, all related to HEV in immunosuppressed patients (Mesquita et al, 2016).

There is only one report of liver failure due to HEV in an immunocompetent patient, a Canadian woman, who acquired the virus in India. Her liver function was severely compromised, requiring a transplant (Chris and Keystone, 2016). Cases of chronic HEV infection have also been reported in immunocompetent individuals (Grewal et al, 2014; Tallón et al, 2011) and the presence of HEV-RNA has been reported in blood or blood products from asymptomatic donors in several European countries (Baylis et al, 2012; Hewitt et al, 2014; Slot et al, 2013). The impacts of these findings on public health still needs to be addressed.

The source and transmission routes of HEV-3 autochthonous infections in industrialised nations are not clear. Long-term cohort and case-control studies are necessary to define risk factors and conditions leading to human exposure and infection (De Schryver et al, 2015).

It is also necessary to evaluate the frequency and quantity of HEV-viral loads in pork at slaughter and retail to better assess its potential risk to public health.

HEV DETECTION IN FOOD: THE CHALLENGES

RNA viruses incriminated in food-borne infections are not easily cultivable due to several factors, including lack of suitable cell lines for in vitro cultivation, instability of the viral RNA, commonly low viral load, and compromised viability (Ceuppens et al, 2014; Payne et al, 2012). Viral detection in different food matrices always relies on reverse transcription-PCR or quantitative real time PCR (Payne et al, 2012). Food components can increase false negative results since they inhibit RNA amplification, and most viral RNA is prone to fast degradation during sample handling and storage (Elizaquível et al, 2014). The acid adsorption/elution/concentration process, proteinase K treatment and direct RNA extraction have all been utilised to remove potential inhibitors of reverse transcription and subsequent PCR (Stals et al, 2012). Direct extraction of RNA with guanidine thiocyanate, or silica membrane kits, or both, in meat products has proven to be effective for HEV detection (Rodríguez-Lázaro et al, 2015). PCR primers selected for HEV detection typically target open reading frames (ORFs) 1, ORF2 or ORF3 (Jothikumar et al, 2006), using nested RT-PCR assays. PCR methodologies do not provide information regarding viral integrity/viability (Stals et al, 2012). The 'viability-RT-qPCR' is a promising new method capable to overcome the problems of regular PCR assays. With this method, samples are treated with photoactivated propidium monoazide (PMA) and ethidium monoazide (EMA) dyes. The concept of this technique is the dependency of foodborne viruses on the integrity of the viral capsid and of the viral genome for infectivity. The

covalent ligation of intercalants with the viral RNA only takes place in viral particles with damaged capsids or free nucleic acid fragments and the resulting intercalated dye interferes with DNA amplification (Elizaquível et al, 2014). A second method is called 'binding based-RT-qPCR' where a ligand/reaction takes place prior to the RT-qPCR in order to exclude free nucleic acids and damaged virus particles (Li et al, 2011). The most common method used is based on quantitative RT-PCR (Elizaquível et al, 2014).
All techniques aforementioned are expensive, onerous and required specialised equipment to be performed, the search for accurate, cheap, easy and portable tests is still open.

CONTROL OF VIRUSES IN FOOD-PROCESSING ENVIRONMENTS

It is not known how long HEV remains infectious in food processing environments or in food matrices (Cook and Poel, 2015). Previous research indicated that heating pork liver pâté to an internal temperature of 71°C for 20 minutes is sufficient to inactivate HEV. The HEV detection in processed ready-to-eat food (Heldt et al, 2016) reinforces the need of further research to assess thermal resistance of this agent on food matrices as well as cross-contamination routes in food processing environments. There is no data on the oral infectious dose of HEV currently available (Barnaud et al, 2012). Nevertheless, the efficacies of heat on enterically transmitted viruses in different food matrices would be expected to differ, since food components are known to influence the thermal inactivation of viruses (Bozkurt et al, 2015). Data on efficacy of hand-sanitisers depend on the study design and the type of virus. HEV has been shown to be susceptible to chlorine disinfection (Girones et al, 2014); however, more experimental data is needed to support evidence-based policies, actions on water safety and ultimately aimed at protecting vulnerable populations (Girones et al, 2014).

CONCLUSION

In order to formulate HEV-control measures, it is necessary to investigate cross-transmission at the slaughter/pork processing areas, monitoring sewage, establish suitability of sanitisers, identify critical points of viral contamination. Other requirements are: evaluate HEV persistence, harmonisation of detection methods, working on get a better understanding of HEV epidemiology in swine herds.

AUTHORS

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