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Hepatitis E Virus: An Emerging Foodborne Pathogen

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Hepatitis E Virus (HEV) is endemic in areas with poor sanitation and has traditionally been classified as a water-borne virus. Until recently, cases of HEV in industrialized countries were associated with travel to those areas. In the last decade, locally acquired cases of HEV have increased in the European Union, leading to the investigation of potential foodborne transmission of the virus. In the mid-1990's HEV was found to be unique among other water- and food-borne viruses because of the observation of zoonotic transmission of the virus. HEV is endemic on domestic swine farms worldwide and can infect pigs of all ages. Consequently, pork liver and pork liver containing products have been identified as the source of many of the foodborne HEV outbreaks in Europe. Other pork products and game meats have also been implicated in HEV outbreaks. Finally, anecdotal evidence exists for HEV transmission via shellfish and produce. HEV disease presentation is typically a self-limiting acute hepatitis; however, chronic hepatitis and extrahepatic manifestations occur in high-risk populations. Detection and control of HEV remains challenging because an efficient cell culture system has yet to be developed. Thus, detection relies upon molecular and serological methods. No standardized method exists for the detection of HEV in foods and research on the stability of HEV in foods and the environment has been limited. This review summarizes the current knowledge available on foodborne HEV.

Keywords: hepatitis E virus, foodborne virus, foodborne illness, zoonosis, swine

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INTRODUCTION OF FOODBORNE VIRUSES

The main unifying trait of foodborne viruses is that they are non-enveloped viruses, lacking a lipid envelope. Non-enveloped viruses, in general, are resistant to environmental stress such as heat, extreme pH, desiccation, organic solvents, etc. Typical treatments used to inactivate vegetative foodborne bacterial pathogens or enveloped viruses (such as influenza virus) are not effective in inactivating non-enveloped viruses. This allows non-enveloped viruses to be maintained in foods and the environment for long periods. The stability of non-enveloped viruses also makes them more resistant to common sanitation methods and food processing technologies. Foodborne viruses, primarily noroviruses, are a major cause of foodborne disease in industrialized countries (De Aceituno et al., 2013). However, there are emerging foodborne viruses, such as hepatitis E virus (HEV) and sapoviruses, increasing in prevalence in the European Union (EU) and Asia (Ruggeri et al., 2013). Of the emerging foodborne viruses, HEV is unique because it has known animal reservoirs and is zoonotic (Meng et al., 1997). Norovirus and rotavirus have genotypes or serotypes that can infect animals, but these viruses are not known to be transmitted to humans (i.e., not zoonotic) (Bank-Wolf et al., 2010). A summary of food-borne viruses is found in **Table 1**.

TABLE 1 | Summary of major foodborne viruses.

Virus	Genome	Envelope	Disease
Norovirus	+ssRNA	No	Gastroenteritis
Adenovirus	dsDNA	No	Gastroenteritis
Rotavirus	dsRNA	No	Gastroenteritis
Sapovirus	+ssRNA	No	Gastroenteritis
Astrovirus	+ssRNA	No	Gastroenteritis
Aichivirus	+ssRNA	No	Gastroenteritis
Hepatitis A virus	+ssRNA	No	Jaundice, Hepatitis, Gastroenteritis
Hepatitis E virus	+ssRNA	No	Jaundice, Hepatitis, Gastroenteritis
Polio virus	+ssRNA	No	Poliomyelitis

+ssRNA, single-stranded positive-sense RNA virus; dsDNA, double-stranded RNA virus; dsRNA, double-stranded RNA viruses.

HEPATITIS E VIRUS DISCOVERY

In 1973, a large-scale epidemic of hepatitis was observed in Kashmir affecting over 200,000 individuals in an area with very poor sanitation (Khuroo, 1980; Khuroo et al., 2016). Epidemics in India continued to be observed over the next 14 years. Patient serum samples collected during this period were tested for antibodies against hepatitis A virus (HAV) and hepatitis B virus (HBV). The serological tests ruled out HAV and HBV as the cause of the epidemics, leading to the identification of a non-A, non-B hepatitis virus (Khuroo, 1980). An outbreak at a Russian military outpost a few years later led to symptoms in patients like those observed in the 1978 Kashmir epidemic. Ingestion of pooled patient fecal samples by human volunteers led to the development of the prior observed non-A, non-B hepatitis and shedding of virus particles in the feces (Balayan et al., 1983). Immune electron microscopy revealed that the virus isolated from the subject was antigenically distinct from both Hepatitis A and Hepatitis C (Balayan et al., 1983). Following this, the physiological traits of the virus were characterized using virus collected from challenge studies conducted in non-human primates (Bradley et al., 1991). In 1991, the full-length HEV genome was sequenced and an enzyme immunoassay was developed for clinical diagnosis (Tam et al., 1991).

CLASSIFICATION AND TAXONOMY OF HEPATITIS E VIRUS

HEV is a member of the *Hepeviridae* family in the genus *Orthohepevirus* (Meng, 2013; Cossaboom et al., 2016). The genus *Orthohepevirus* is divided into 4 species, *Orthohepevirus* A-D, of which *Orthohepevirus A* includes HEVs infecting humans (Meng, 2013; Cossaboom et al., 2016). *Orthohepevirus A* contains 8 genotypes, HEV1-8, which are determined by amino acid sequence comparisons of concatenated open reading frames 1 and 2 (ORF1 and ORF2) sequences (Smith et al., 2016; Sridhar et al., 2017). HEV1 and HEV2 are specific to humans and these viruses are often the cause of waterborne HEV outbreaks (Geng and Wang, 2016) (**Table 2**). HEV3 and HEV4 infect a wide variety of hosts including humans, pigs, wild boar, deer, primates, and rabbits (Cossaboom et al., 2011; Doceul et al., 2016; Smith et al., 2016) (**Table 2**). HEV3 and HEV4 are zoonotic and are the causative agents of foodborne HEV infections in industrialized countries (Hughes et al., 2010; Meng, 2010; Teo, 2010; Yugo et al., 2014).

TABLE 2 | *Orthohepevirus A* HEV genotypes infecting humans: host range and transmission routes.

Genotype	Natural host(s)	Transmission route
1	Humans	Water
2	Humans	Water
3	Humans, pigs, wild boar, rabbits, deer, non-human primates	Food, direct contact with reservoir animals
4	Humans, pigs, wild boar, rabbits, deer, non-human primates	Food, direct contact with reservoir animals

HEPATITIS E VIRUS EPIDEMIOLOGY AND DISEASE MANIFESTATIONS

An estimated 2.3 billion people, one third of the world's population, have been exposed to HEV (Teshale and Hu, 2011; Pérez-Gracia et al., 2013). HEV is traditionally endemic in developing countries where sanitation is poor, which leads to waterborne outbreaks caused by HEV1 and HEV2 strains (Geng and Wang, 2016). Foodborne HEV outbreaks are most often associated zoonotic HEV3 and HEV4 strains. The transmission mode of HEV is generally the fecal oral route. HEV infections have been associated with direct contact with reservoir animals and consumption of contaminated water and foods (Cossaboom et al., 2016). High-risk foods for HEV contamination include raw or undercooked meat of infected animals, filter feeding bivalve shellfish, and produce (Brassard et al., 2012; Cossaboom et al., 2016; Mansuy et al., 2016; Hazards et al., 2017). In 2004, cases of HEV acquired after blood transfusion were reported in Japan and India (Khuroo et al., 2004; Matsubayashi et al., 2004). Since then, transfusion-associated HEV has been reported worldwide and seems to be highly prevalent among donors (Dreier and Juhl, 2014; Al-Sadeq et al., 2017). Foodborne HEV can also indirectly affect patients receiving blood transfusions. In Japan, a case of transfusion-acquired HEV was linked to a donor who had become infected after consuming pig liver and intestines (Matsubayashi et al., 2008).

HEV infection is typically self-limiting with clinical presentation ranging from asymptomatic infection to acute liver failure (Debing et al., 2016). Pregnant women tend to have symptomatic infections and are at higher risk of acute liver failure and death than other infected individuals (Abravanel et al., 2013). Interestingly, the high morbidity and mortality amongst pregnant women has only been observed following HEV1 and HEV2 infections and not infection with zoonotic HEV strains (Abravanel et al., 2013). HEV was exclusively described as an acute infection until 2008 when significant numbers of chronic HEV infections were reported amongst organ transplant recipients in France (Kamar et al., 2008). Subsequently, chronic HEV infection has been reported in

other groups of immunocompromised patients including those with human immunodeficiency virus (HIV) and those with hematological cancers (Péron et al., 2006; Colson et al., 2009; Kamar et al., 2012). To date, chronic HEV infections have only been associated with HEV3 and HEV4 infections (Xin and Xiao, 2016). There is also evidence of neurological manifestations of acute and chronic HEV infection. HEV RNA has been detected in the cerebral spinal fluid of patients with peripheral neuropathy, which resolved upon clearance of the virus (Kamar et al., 2011).

MOLECULAR BIOLOGY OF HEPATITIS E VIRUS

HEV has a single stranded positive sense RNA genome (ss+RNA) that is approximately 7.2 kb in length (Cao and Meng, 2012; Emerson and Purcell, 2013; Debing et al., 2016). The genome is divided into three ORFs, has a 5′ cap structure, and is polyadenylated at the 3′ end (Cao and Meng, 2012). An overview of the HEV genome structure in presented in **Figure 1**. ORF1 encodes the nonstructural proteins, ORF2 encodes the capsid protein, and ORF3 encodes a small multifunctional phosphoprotein (Cao and Meng, 2012) (**Table 3**). Proteins translated from ORF2 and ORF3 are encoded by a biscistronic subgenomic mRNA (Graff et al., 2006).

ORF1 spans the genome from the 5′ non-coding region (NCR) to position 5,082 and encodes a 1,693 amino acid polypeptide. This polypeptide includes domains, which function as the methyltransferase (Met), papain-like cysteine protease (PCP), helicase (Hel), RNA dependent RNA polymerase (RdRp), as well as other domains with unknown functions (Y domain, X domain, and hypervariable region) (Koonin, 1991; Koonin et al., 1992; Agrawal et al., 2001; Magden et al., 2001; Zhang et al., 2001; Emerson et al., 2004; Pudupakam et al., 2009, 2011; Cao and Meng, 2012). ORF2 encodes the 660-amino acid capsid protein, which has a molecular weight of 72 kDa (Cao and Meng, 2012). The HEV virion has $T = 3$ icosahedral symmetry and contains 180 copies of the capsid protein (Guu et al., 2009; Yamashita et al., 2009; Xing et al., 2010; Cao and Meng, 2012; Emerson and Purcell, 2013). The capsid protein makes up the viral particle structure, interacts with the host cell receptor, and is immunogenic (Li et al., 1997, 2005b; Guu et al., 2009; Yamashita et al., 2009; Cao and Meng, 2012). The HEV capsid structure is more homologous to that of small plant viruses than that of Norovirus or Hepatitis A (Guu et al., 2009).

ORF3 encodes a small, 114 amino acid, cytoskeleton associated phosphoprotein (Zafrullah et al., 1997; Cao and Meng, 2012). This phosophoprotein is theorized to have a regulatory role in assembly of HEV virions, pathogenesis, and release of membrane-associated viral particles (Tyagi et al., 2002; Surjit et al., 2006; Moin et al., 2007; Ding et al., 2017).

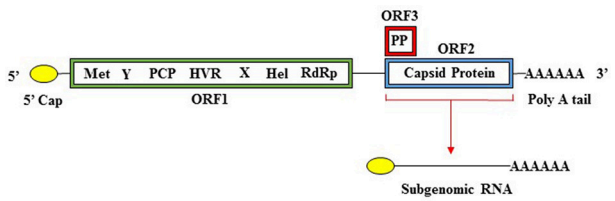

FIGURE 1 | Organization of the HEV genome. The HEV genome has a 5′ cap structure, followed by three open reading frames (ORFs), and is polyadenylated at the 3′ end. ORF1 encodes a polyprotein containing the nonstructural domains (Met, methyltransferase; Y, Y domain; PCP, papain-like cysteine protease; HVR, hypervariable region; X, X domain; Hel, helicase; RdRp, RNA-dependent RNA polymerase. ORF2 encodes the capsid protein and ORF3 encodes a phosphoprotein (PP). ORF1 is translated from full length genomic RNA while ORF2 and ORF3 are translated from a 2kb bicistronic subgenomic RNA.

CELL ENTRY AND REPLICATION

HEV replication follows the basic scheme of other ss+RNA viruses. The host translational machinery is exploited to translate the viral non-structural polyprotein encoded by ORF1. Evidence suggests that the ORF1 polyprotein does not undergo proteolytic processing to carry out viral replication functions (Suppiah et al., 2011; Perttilä et al., 2013). The RdRp, translated from the 3′ end of ORF1, synthesizes a complimentary negative-strand RNA (Debing et al., 2016). Genomic RNA is then synthesized from the negative-strand RNA and the two strands are unwound by the HEV helicase (Karpe and Lole, 2010a). In addition, a single bicistronic subgenomic mRNA is synthesized from the complementary negative-strand RNA and is used as a template to translate ORF2 and ORF3 (Zhang et al., 1999; Graff et al., 2006). The formation of the 5′ cap of the genomic RNA begins with the cleavage of 5′-gamma-phosphate by the helicase-associated 5′-triphosphatase (Magden et al., 2001; Karpe and Lole, 2010b). The P110 enzyme facilitates the methylation of guanosine triphosphate to form 7-methylguanosine, which is transferred to the 5′ end of the genome (Magden et al., 2001).

TABLE 3 | HEV proteins and function.

Protein	Function
Methyltransferase	Capping of 5′ end of genome Cap required for translation initiation Cap aids in immune evasion
Y domain	Unknown function
Papain-like cysteine protease	Post-translational processing
Hypervariable region	Unknown function
X domain	Unknown function
Helicase	Nucleoside triphosphatase RNA duplex unwinding
RNA dependent RNA polymerase	RNA genome replication Sub-genomic RNA synthesis
Capsid protein	Viral capsid structure Virus particle assembly Host receptor binding Immunogenic
Cytoskeleton-associated multifunctional phosphoprotein	Regulation of viral assembly Viral pathogenesis Particle release

Due to the lack of an efficient cell culture system, little else is known about the replication process (von Nordheim et al., 2016). A RNA element located within ORF2 may also play a role in genomic RNA synthesis but further research is needed to determine the exact function (Emerson et al., 2013). This element likely does not play a role in HEV host adaptation since HEV-1 could replicate in porcine liver cells when only ORF1 was replaced with that of HEV-4 (Chatterjee et al., 2016). The exact location of replication has yet to be determined but evidence suggests that at least part of the process may take place in the endoplasmic reticulum-Golgi intermediate compartment (Perttilä et al., 2013).

Recently, quasi-enveloped HEV particles were observed in blood despite HEV being classified as a non-enveloped virus (Takahashi et al., 2008; Nagashima et al., 2017). The concept of quasi-enveloped viruses was first described for HAV (McKnight et al., 2017). HAV has evolved to be released from cells in exosomes following infection. The HAV particles are covered by a cellular lipid bilayer upon release from the cell and therefore are not recognized by the host immune system (Feng et al., 2013). HAV is shed in the feces in its non-enveloped form but the virus released from the cell appears to be exclusively quasi-enveloped (eHAV) (Feng et al., 2013). HAV found in the feces is derived from eHAV (Hirai-Yuki et al., 2016). Some eHAV is released across the apical membrane, which leads to the biliary tract where bile acids degrade the quasi-envelope. Despite being phylogenetically unrelated, HEV could plausibly utilize this process prior to being shed in the feces. Regardless of the mechanism, quasi-enveloped HEV likely plays a role HEV evasion of the host immune system.

HEPATITIS E VIRUS ZOONOSIS

Unlike common foodborne viruses such as HAV and norovirus, HEV3 and HEV4 strains are zoonotic. As previously mentioned, humans, pigs, wild boar, deer, primates, and rabbits are all infected by HEV3 and HEV4 strains (Cossaboom et al., 2011; Smith et al., 2016). The first zoonotic HEV strain belonged to genotype 3 and was a swine strain that was transmitted to humans (Meng et al., 1998; Schlauder et al., 1998). This seminal discovery lead to the subsequent finding that in addition to pigs, wild boar and rabbits are also reservoirs for zoonotic HEV strains (Doceul et al., 2016).

HEV has also been shown to be transmitted between different animal species. Pigs inoculated with a rabbit strain developed viremia and fecal shedding (Cossaboom et al., 2012). Virus shed from rabbit HEV-infected pigs produced viremia and fecal shedding in rabbits, which confirms the infectivity of rabbit HEV in pigs (Cossaboom et al., 2012). Rabbit HEV has also been shown to be infectious in cynomologus macaques, which suggests that this virus may also be infectious to humans (Liu et al., 2013).

HEPATITIS E VIRUS PREVALENCE IN SWINE

HEV is considered endemic on conventional swine farms worldwide. A nationwide survey conducted in France in 2008–2009 found that 31% of pigs tested (1,069/6,565) were seropositive for HEV and 65% of pig farms (137/186) had at least one HEV seropositive pig (Rose et al., 2011). In 2011, 50.21% (714/1,422) of pigs tested in Northern Italy were HEV seropositive and 97.43% (38/39) of farms sampled had least one seropositive pig (Martinelli et al., 2011). Similar farm-level results were obtained in Spain, Norway, and New Zealand (Garkavenko et al., 2001; Seminati et al., 2008; Lange et al., 2016). Seroprevalence levels tend to vary between different types of farms and different age groups. Farms involved in breeding (farrow to finish or farrow to weaning) had higher HEV seroprevalence than fattening farms (Martinelli et al., 2011). Between individual pigs, seroprevalence was highest among sows and lowest among recently weaned pigs (weaners), which indicates that the circulation of HEV is influenced by maternal immunity (Martinelli et al., 2011). Because weaners no longer receive maternal antibodies, they are more vulnerable to HEV infection. Therefore, seroconversion is likely to occur shortly after weaning. While seroprevalence studies can provide information about HEV exposure and predict age of infection, this type of study does not provide information on whether the virus itself is present in the pig.

HEV RNA has been detected in pigs of all ages and the prevalence in pigs close to slaughter age is of particular interest. In a survey of pig farms in Portugal, 32% (16/50) of fattener pigs were shedding the virus in their feces (Berto et al., 2012c). A similar prevalence was found in a study of pigs between 2 and 4 months of age from 6 US states (Huang et al., 2002). A six-country (United Kingdom, Czech Republic, Italy, Portugal, Spain, and the Netherlands) study in Europe found HEV prevalence ranging from 8 to 73% among fattener pigs (Berto et al., 2012a). The wide variation could be attributed to the characteristics of the farms (number of animals, farrow to finish vs. fattening, etc.) and the predominating practices (organic vs. conventional, biosecurity measures, etc.) of the farms in each country (Salines et al., 2017). The sample size may be a major factor as well because the prevalence data for Spain and the Netherlands came from one farm in each country. Such a small sample size can skew the results, thus future studies should include a much larger number of farms. Extensive national surveys of near slaughter age pigs in the United States and Europe should be utilized to elucidate the true prevalence of HEV infection.

HEPATITIS E VIRUS PREVALENCE IN PORK PRODUCTS

Pork liver and pork liver products are the most obvious source of foodborne HEV and have been extensively studied in Europe. In a survey of foods containing raw pork liver sold in France, 68 out of 394 (17.3%) samples tested positive for HEV with prevalence for various products ranging from 3% (dried salted liver) to 30% (figatelli) (Pavio et al., 2014). In a year-long study in the United Kingdom, HEV was detected in 6 of 63 pork sausages (10%) collected from 3 retail outlets with 5 of the 6 positive samples coming from a single batch (Berto et al., 2012b). HEV has also been detected in sausages sold in Spain (Di Bartolo

et al., 2012) and Italy (Di Bartolo et al., 2015), in livers and sausages sold in Germany (Wenzel et al., 2011; Szabo et al., 2015), and livers sold in the Netherlands (Bouwknegt et al., 2007). Although fewer studies have been conducted in North America, HEV has been detected in pork liver and pork liver products sold in Canada and the United States (U.S.). Forty-seven percentage of pork pâtés (36/76) and 10.5% of pork livers (2/19) purchased from grocery stores in Ottawa between March 2014 and September 2015 tested positive for HEV (Mykytczuk et al., 2017). In 2007, HEV was detected in 11% (14/127) of pork livers purchased from grocery stores in Virginia and Iowa (Feagins et al., 2007).

Pork liver products are not as commonly consumed in the US as they are in Europe. Therefore, other pork products need to be considered as a source of foodborne HEV. Cossaboom et al. (2016) specifically targeted non-liver pork products and detected HEV in 25% (3/12) of pork chitterling packages purchased in southwestern Virginia grocery stores. Sausages, skeletal muscle, and nervous tissue were also tested but were negative for HEV. This survey, along with the previously mentioned pork liver survey conducted in Virginia and Iowa, had a relatively small sample size and covered a small region of the US. Larger and more expansive surveys are needed to truly understand the prevalence of HEV in pork products.

HEPATITIS E VIRUS ASSOCIATED FOODBORNE OUTBREAKS

The number of HEV cases in the EU each year has been rising since 2005 with the largest increase occurring between 2011 and 2015 (Aspinall et al., 2017). 98.5% of cases with known travel history were locally-acquired. Clusters of cases linked to raw or undercooked pork have been reported in France. In 2013, seventeen people contracted HEV after consuming spit-roasted piglet stuffed with raw stuffing made from the liver: three developed hepatitis while the other 14 were asymptomatic (Guillois et al., 2016). Figatelli, a type of raw pork liver sausage, has been linked to two clusters of HEV cases in Marseille (Colson et al., 2012) (Colson et al., 2010) and two cases (1 symptomatic, 1 asymptomatic) in a small French resort town (Renou et al., 2014). In Spain, an outbreak within a family was traced to wild boar meat the family had consumed (Rivero-Juarez et al., 2017). Several outbreaks occurred in Germany but no food source of infection was identified (Hazards et al., 2017). HEV surveillance programs exist in 20 EU countries including France, Spain, and Germany (Aspinall et al., 2017).

According to a 2014 epidemiological survey, 6% of the US population is HEV seropositive (Ditah et al., 2014). Data on outbreaks and the number of cases occurring each year is not readily available due to the lack of a surveillance program. Cases of acute hepatitis not caused by Hepatitis A, B, or C viruses may not be investigated further for a viral causative agent. Although HEV is not considered a reportable disease in the US, cases should be documented so that the disease burden of HEV can be elucidated. HEV should be ruled out in cases of non-A, B, or C hepatitis and in cases of unexplained chronic hepatitis.

HEPATITIS E VIRUS VACCINE

HEV 239 (Hecolin; Innovax; Xiamen, China), a recombinant vaccine produced using a genetically modified strain of *Escherichia coli*, has been available in China since 2012 and is approved for use in people over 16 years old (Park, 2012; Li et al., 2015). No vaccine is available elsewhere. The immunogenic constituent of Hecolin is derived from the ORF2 protein of an HEV1 strain (Li et al., 2005a; Wen et al., 2016). Vaccination with Hecolin confers protection against genotypes 1 and 4 for up to 4.5 years (Zhu et al., 2010; Zhang et al., 2015). However, a recent study showed that sera from people vaccinated with Hecolin produced a stronger antibody response to HEV1 and HEV2 than to HEV3 and HEV4 (Wen et al., 2016). Antibody titers induced by the HEV p179 vaccine, derived from the ORF2 protein of HEV4, were 2-fold higher for HEV3 and HEV4 compared to HEV1 and HEV2 (Wen et al., 2016). HEV p179 was shown to be safe and well tolerated in a phase 1 randomized open-label study (Cao et al., 2017) and could potentially be protective against HEV3. Future vaccine design could include ORF1 proteins as they have been shown to induce T-cell responses in patients with acute, resolved and chronic hepatitis E (Al-Ayoubi et al., 2018).

No vaccine is currently available for animal use. Theoretical models have been used to test the effects of HEV vaccination in pigs. In scenarios where the HEV is not eliminated from the pig population, reduced transmission rates resulted in an increase in the rates of infectious pigs at slaughter age while a shortened infectious period resulted in a decrease (Backer et al., 2012). More research is needed to determine whether vaccination would be an effective strategy to control HEV in the domestic pig population. Future work should take passive immunity, husbandry practices, and other pathogens into consideration (Salines et al., 2017).

INACTIVATION OF HEPATITIS E VIRUS IN FOOD

Without an efficient cell culture system, study of inactivation methods has been severely limited. Using a swine bioassay, where HEV contaminated pork liver was injected into the ear veins of naïve pigs, Feagins et al. (2008) found that cooking pork liver to an internal temperature of 71°C (161°F) was sufficient to fully inactivate HEV. However, heating to an internal temperature of 56°C (132°F) for 1 h lead to subsequent infection in inoculated pigs. In another study, it was found that HEV RNA persisted in liver suspension stored for 50 days at 4°C and 70 days at 22°C and 37°C, indicating that the virus is highly stable in foods during long-term storage (Schielke et al., 2011). Heating the HEV contaminated liver suspension at 56°C for 30 min only resulted in an approximate 2-log reduction in viral genomic equivalents (Schielke et al., 2011).

Identifying an appropriate surrogate virus for HEV studies will also aide in advancing knowledge of HEV stability and inactivation in foods. Cutthroat trout virus (CTV) is also a member of the *Hepeviridae* family, has a similar genome organization to HEV, and is quasi-enveloped (von Nordheim et al., 2016). CTV replication in cell culture is robust and

is a promising model for study of the molecular biology of HEV (von Nordheim et al., 2016). These features could also be useful for testing the effectiveness of various inactivation strategies.

Advancements are being achieved in adapting HEV to cell culture. Several available immortalized cells lines have been shown to support increases in HEV RNA following infection, however these increases have not been sustained in serial passages (Tanaka et al., 2007; Okamoto, 2013). Cell lines shown to support HEV genome replication included A549 (human lung cell cancer) and PLC/PRF/5 (human hepatocellular carcinoma) (Tanaka et al., 2007; Okamoto, 2013). The swine kidney epithelial cell line, LLC-PK1, was also shown to support the replication of a HEV3 strain (Shukla et al., 2011; Okamoto, 2013). A subsequent study using PLC/PRF/5 cells grown in 3-D culture for HEV infection showed increases in HEV RNA genome, release of viral particles, and subsequent infection in serial passages (Berto et al., 2013). Optimization of these *in vitro* HEV cultivation techniques will greatly advance our understanding of the replication and pathogenesis of the virus, as well as aid in the development of mitigation strategies for this virus.

METHODS OF DETECTION FOR HEPATITIS E VIRUS

Because HEV and other foodborne viruses are difficult to culture, molecular methods are used for detection. Even so, molecular detection in food can be challenging due to low levels of virus in contaminated sample and the presence of inhibitory compounds in the food matrix (De Aceituno et al., 2013). While other foodborne viruses likely are restricted to the food surface, HEV is also present inside the cells of meat thus adequate breakdown of the food matrix is critical for detection (Szabo et al., 2015). Common molecular methods used include RNA detection using PCR based methods and viral antigen detection using ELISA based methods.

Although no standard method currently exists for the detection of HEV RNA in foods, all protocols follow three general steps: (1) virus elution and concentration; (2) extraction of viral RNA; (3) RNA detection (De Aceituno et al., 2013; Martin-Latil et al., 2014; Hazards et al., 2017). A variety of methods have been described but few comparison studies have been done to optimize the elution-concentration and RNA extraction processes for HEV detection. Martin-Latil et al. (2014) found that homogenization in distilled water by stomacher yielded the highest HEV recovery rates from pork liver sausages compared to phosphate buffered saline (PBS) and Tris-HCl, glycine, beef extract (TGBE). Also, purification using the organic solvent choroform:butanol before or after concentration by polyethylene glycol (PEG) did not result in significantly higher recovery rates. When compared with homogenization in TRIzol® reagent, homogenization in distilled water and concentration with PEG resulted in a lower mean HEV recovery rate from pork liver sausages (Szabo et al., 2015). Ultrafiltration was shown to be more efficient in concentrating HEV from pork liver than PEG (Son et al., 2014). Commercial kits are widely used for RNA extraction but have occasionally been shown to be less efficient in extracting viral RNA than protocols developed by individual laboratories (Bouwknegt et al., 2007; Martínez-Martínez et al., 2011). The elution-concentration and extraction method chosen also influence the resulting prevalence data, thus it is important that a standard protocol for HEV elution-concentration and extraction RNA be developed to combat this emerging foodborne virus. Larger studies encompassing multiple laboratories should be conducted to optimize the conditions and develop a validated method for HEV detection in foods.

Reverse transcriptase polymerase chain reaction (RT-PCR) is commonly used to detect viral RNA in food. One complication is developing cross-reactive primer sets that can detect all HEV genotypes. Several groups have developed degenerate primers or genotype specific primers for use in HEV RT-PCR assays. These primer sets typically target the ORF2/3 overlap region as it is conserved between genotypes. However, no standardized reagents are yet available commercially for the detection of HEV RNA. The RT-PCR method itself has the disadvantages of being time consuming and providing only qualitative results. Real-time RT-PCR is faster, provides quantitative results, and has been proven to be more sensitive than RT-PCR for the detection of HEV specifically (Son et al., 2014). However, RT-PCR has not been eliminated completely. DNA fragments generated by real-time RT-PCR are not long enough for sequencing and typing, which are useful for determining genotype and determining relatedness among HEV strains (Hazards et al., 2017).

CONCLUSION

HEV is an emerging foodborne pathogen, which needs to be researched more extensively. The lack of an efficient cell culture system has hindered study of HEV molecular biology. The receptor used to enter cells has yet to be identified and may be a vital clue to the wide tissue tropism of HEV. Many of the prevalence studies in pigs and pork products have been small-scale, which can skew results. Large scale studies are needed to assess the true prevalence of HEV in pigs and pork products. Lack of standardized detection methods is also an obstacle for HEV research. Methods can vary among individual labs, which can result in wide variation in virus recovery rates. Developing a gold standard method will ensure accuracy and precision, which will enhance the quality of information used to determine prevalence in foodborne HEV worldwide. Overall, more research is needed to understand and combat this emerging foodborne pathogen.

AUTHOR CONTRIBUTIONS

LH was responsible for writing the text of the majority of the manuscript. ED revised the manuscript text, prepared tables and figures.

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