Summary of the European Food Safety Authority (EFSA) Scientific Opinion on the Hepatitis E Virus (HEV) as a Food-borne Pathogen

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Abstract

The hepatitis E virus (HEV) is a causative agent of important infection in humans in European countries. Food-borne transmission appears to be the major pathway for human HEV infections in Europe, and domestic pigs, wild boar and probably deer are considered the main animal reservoirs of the HEV. For this reason, the European Food Safety Authority (EFSA) has published a scientific opinion on the HEV and the possibilities of its transmission across this continent. This opinion focuses on current methods for the detection, characterisation and tracing of the HEV in food-producing animals and foods; HEV reservoirs, its food-borne pathways and potential control options; the epidemiology of HEV, its occurrence and persistence in foods; and possible control measures along food chains. The aim of this paper is to summarise the reviewed data and to familiarise the reader with the main findings and conclusions presented in this scientific opinion.

Food-borne, hepatitis E virus, HEV, liver, pork, wild boar

Introduction

The hepatitis E virus (HEV) is the causative agent of an important infection in humans – viral hepatitis E (HE). The infection has been connected with more than 21 000 reported acute clinical cases of HE and 28 fatalities over the last ten years. In the process, an overall ten-fold increase in notified HE has been observed during this period. The majority (80%) of such cases were reported in France, Germany and the United Kingdom of Great Britain and Northern Ireland. More than 95% of these cases of HE were of autochthonous origin, i.e. locally acquired. Although twenty European countries have implemented surveillance systems to record the number of acute, chronic and fatal cases of HE, the infection is not notifiable in all European countries. As surveillance systems differ between these countries, the real number of HE cases is underestimated and the reported numbers of HE cases are not comparable (Adlhoch et al. 2016; EFSA 2017).

Although there are two different epidemiological profiles associated with HEV transmission, food-borne transmission of HEV appears to be a major route in Europe. Domestic pigs, wild boar and probably deer are considered the main reservoir of the virus. According to several studies, regional consumption habits (such as consumption of raw or undercooked pork, venison and offal) are risks factors of HEV infection. Several studies indicate that the human populations of Central Europe have a higher seroprevalence than inhabitants of Nordic countries (Adlhoch et al. 2016; EFSA 2017).

Based on these data, the European Food Safety Authority (EFSA) has provided a scientific opinion on the occurrence and control of HEV as a food-borne pathogen. This opinion is a critical evaluation of available information on 1) the methodologies for the detection, characterisation and quantification of HEV in food-producing animals and food; 2) the prevalence of HEV in relevant food-producing animals, foodstuffs and their environment; 3) the epidemiology and geographical distribution of HEV, the occurrence and persistence of the virus in food and consumer habits contributing to the spread of HEV infection; and 4) to investigate possible control measures along food chains. The aim of this paper is to summarise the reviewed data and to familiarise the reader with the main findings and conclusions of this scientific opinion (EFSA 2017).

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Characteristics of the hepatitis E virus focusing on strains found in humans

The HEV is classified in the family *Hepeviridae* which includes agents infecting a wide range of mammals, birds and salmonid fish. The family is comprised of two genera: *Orthohepevirus* (HEV infecting mammalian species and birds) and *Piscihepevirus* (HEV infecting salmonid fish). The genus *Orthohepevirus* is divided into four species (A, B, C and D) and contains most of the HEV strains identified to date. The HEV strains able to infect humans and other animal species such as pigs, wild boar, deer, mongoose, rabbits and camels belong to the species *Orthohepevirus* A (Smith et al. 2014).

There are 4 major known genotypes infecting humans (HEV-1, HEV-2, HEV-3 and HEV-4). These genotypes display different epidemiological profiles and geographical locations. HEV-1 is mostly associated with large water-borne outbreaks and epidemics in tropical and subtropical countries of Asia and Africa. HEV-2 has been detected in Mexico, Nigeria and Chad. The relative conservation of these two genotypes corresponds to their primary circulation within humans. Detection of these two genotypes in clinical samples from Europeans suggests HEV infection related to travel to the above-mentioned countries (i.e. imported infection). In contrast, the diversity of HEV-3 and HEV-4 is related to their zoonotic transmission (transmission from animals to humans and *vice versa*). HEV-3 has been found in clinical samples originating from human patients with acute HE as well as from various animal species, such as domestic pigs (*Sus scrofa* f. *domestica*), wild boar (*Sus scrofa*) and cervids (*Cervidae*: deer) worldwide. HEV-4 contains human and animal strains, particularly originating from Asian countries. Recently, HEV-4 was also detected in clinical samples from humans with autochthonous HE and domestic pigs in Europe. These two genotypes are causative agents of sporadic or epidemic cases connected to zoonotic and food-borne transmission of HEV in European countries. If HEV-3 and HEV-4 are detected in human patients suffering from HE in Europe, autochthonous (local) origin of the infection can be considered. Based on these data, the main interest of the EFSA is focused on HEV-3 and HEV-4 (Lu et al. 2006 and Smith et al. 2014).

Methods of detection of the hepatitis E virus

Numerous methods for HEV detection depending on the analysed matrices (foodstuffs, the environment or food-producing animals) and required testing value (detection of viral genome, infectivity of the virus or specific antibodies against HEV) are available. Molecular methods rely on direct detection of the HEV genome. For this purpose, several conventional reverse transcription polymerase chain reaction (RT-PCR) or real time RT-PCR (RT-qPCR) have already been described. Conventional RT-PCR assays have been available for many years. In addition to their use for HEV genome detection, they are increasingly being applied for subsequent characterisation of specific parts of the HEV genome by sequencing. They can, therefore, be used as a tool in molecular epidemiology/epizootology (Schlauder et al. 1999; Huang et al. 2002 and Preiss et al. 2006). RT-qPCR assays represent a more progressive method. Comparisons of conventional assays for HEV detection with RT-qPCR using human and pig samples have consistently indicated the higher sensitivity of RT-qPCR (Jothikumar et al. 2006; Zhao et al. 2007 and Son et al. 2014). In addition, quantification of the viral load in analysed matrices can be readily performed by RT-qPCR if appropriate standards are used. Although these assays can be used for the analysis of human, animal, food and environmental matrices, no standardised method for the detection and quantification of HEV in food is available so far. For this reason, the International Organisation for Standardisation (ISO/TC34/SC9) launched an enquiry in April 2015 that resulted in the development of standardised ISO methods for HEV detection in the future. However, these methods provide no information about the infectivity of the detected virus (EFSA 2017).

Methods for the detection of infectious viral particles are based either on the experimental

inoculation of the animal or on cell culture techniques. To date, several animal species have been tested for susceptibility to experimental HEV infection. Although the experimental inoculation of animals is generally useful for HEV infectivity assessment, the limits of these methods are obvious, e.g. ethical aspects and the limitation of sample numbers (experiments with large animals are laborious, time-consuming and expensive) (Cook et al. 2016). Despite the numerous reports of successful HEV-1, HEV-2, HEV-3 and HEV-4 isolation in cell cultures - particularly from clinical specimens (Okamoto 2011 and 2013; Johne et al. 2014 and Cook et al. 2016), only two studies have described the successful isolation of HEV from food samples (Takahashi et al. 2012 and Berto et al. 2013). To date, no standardised or validated methods for preparation of food samples before inoculation into a cell culture are available. In addition, the HEV culture systems appear to have a high detection limit and variable reproducibility (Cook et al. 2016).

Serological methods detect exposure of the host organism (i.e. food-producing animal) to the virus. The early production of anti-HEV IgM after infection with its relatively short self-life could be used as a marker of recent infection. In contrast, the late development but longer duration of the presence of anti-HEV IgG is a sign of prior infection and can be used to estimate the exposure to HEV in a population (Meng et al. 1997 and Takahashi et al. 2005). Although it is believed that all mammalian HEV strains recognised to date belong to one serotype, both commercially available and "in-house" serological assays reveal significant variability in analytical sensitivity and specificity. As these test characteristics vary between the different serological assays, it is important to consider these when the results are compared or interpreted (EFSA 2017).

Hepatitis E virus infection and disease in humans

The clinical features of HE are similar to those of acute viral hepatitis caused by other hepatotropic viruses, for which reason a HE diagnosis should be confirmed by the detection of anti-HEV IgM in the serum and/or the HEV genome in the serum or stool samples of the patient. The majority of HEV-3 and HEV-4 infections are asymptomatic (> 70%) and people merely seroconvert, i.e. the specific antibodies against HEV appear (Guillois et al. 2016). Symptomatic cases may show an acute self-limiting hepatitis with fatigue, asthenia, nausea and fever, which may be followed by jaundice, elevated liver enzymes (ALT, AST and GGT) and bilirubin, abdominal pain and hepatosplenomegaly. The patients are usually older (middle aged and elderly people) and predominantly male (Lewis et al. 2010 and Adlhoch et al. 2016). Most humans with acute infection recover completely within a couple of weeks. The incubation period is estimated to be between two and six weeks, up to 60 days (Lhomme et al. 2016). Although the lowest infection dose for transfusion-transmitted infection via plasma products has been identified (20 000 IU), the minimum number of infectious particles capable of causing food-borne HEV transmission is still unknown (Tedder et al. 2017).

HEV infection in patients with pre-existing chronic liver disease can lead to a fatal outcome due to liver failure (Festa et al. 2014). Chronic infection caused by HEV-3 and HEV-4 has been described in patients with underlying chronic diseases or immunosuppressive conditions - solid organ transplantation, pre-existing liver disease or haematological malignancy (Netzler et al. 2016). Various neurological symptoms and haematological disorders have also been defined in these patients recently (Woolson et al. 2014 and Kamar et al. 2015).

Transmission of the hepatitis E virus focusing on autochthonous cases of hepatitis E in Europe

Epidemiological studies have identified several main routes of HEV transmission in Europeans countries: 1) foods originating from infected animals (domestic pigs and wild

boar); 2) foods contaminated with the excreta of infected animals or humans; 3) indirect transfer through contamination of the environment (water); and 4) direct contact with infected animals. Humans and animals are characterised as sources of HEV (reservoir) and food and environmental elements are considered vehicles within the transmission pathways of the virus. Several studies have identified regional consumption habits (such as consumption of raw or undercooked pork, venison, offal and products thereof) as risk factors of HEV infection. There are indications that the human populations of Central European countries have a higher seroprevalence than, for example, Nordic countries. Groups occupationally exposed to reservoir animals (e.g. farmers, slaughterhouse and meat-processing plant workers, veterinary surgeons and hunters) also show a higher seroprevalence in comparison with the general population (Carpentier et al. 2012; Dremsek et al. 2012 and Krumbholz et al. 2012). According to present data, all these people are at increased risk of HEV infection (EFSA 2017).

Transfusion and transplantation-transmitted HEV infection has been observed sporadically in Europe as well as vertical transmission of HEV from infected mother to foetus. Direct person-to-person contact is considered to be too inefficient to represent a significant risk of HEV transmission (EFSA 2017).

Hepatitis E virus occurrence in animal species

Although domestic pigs are the main animal reservoir of HEV worldwide, the HEV genome and/or anti-HEV antibodies have also been detected in a wide range of other animal species, including wild boars, deer, moose, rats, dogs, cats, mongooses, cows, sheep, goats, avian species, rabbits, bats and horses. Transmission from animals to humans is documented in many countries. However, many animal species carry strains of HEV that are unrelated to those associated with zoonotic infection, e.g. moose, rats, bats and avian species (Montalvo Villalba et al. 2013; Pavio and Bouquet 2014; Khuroo et al. 2016 and Roth et al. 2016).

Molecular evidence of the zoonotic transmission and relationship of HEV is provided by studies that have compared virus sequences and subtypes derived from human cases with sequences of the virus found in pigs, wild boar and deer or consumed products thereof. These HEV strains show significant genetic similarities (in some cases even 100% identity), so it is clearly not possible to distinguish and classify them as human or animal HEV strains (Smith et al. 2014). Table 1 shows the results of various studies focusing on the occurrence of HEV in meat, organs and products thereof originating from the major food and wild game animal species that may result in zoonotic HEV transmissions in European countries.

Although the main animal reservoirs of HEV are domestic pigs, wild boar and probably deer, closely-related HEV strains have also been found in rabbits, cattle and camels. More data are required to clarify the reservoir status of these animal species for HEV zoonotic strains (EFSA 2017).

Hepatitis E virus occurrence and persistence in food

Food-borne transmission can be divided into food made of raw material originating from an animal infected with HEV and food contaminated with HEV during its production (cross-contamination via a contaminated environment). Food may be contaminated by HEV at various steps in the food chain from farm to processing plants and point of sale. The most risky food is the raw or insufficiently heat-treated liver of reservoir animals and products thereof. Since the presence of HEV has been detected in the blood of infected animals, the raw or insufficiently heat-treated meat, offal and blood (and products derived from blood) of infected animals should also be included in the group of risky food associated with HEV infection (La Rosa et al. 2011 and Mansuy et al. 2016). In some countries

(e.g. Japan), eating undercooked deer game has been determined as a potential risk of HEV infection, although cervids are not such a significant reservoir of HEV as domestic pigs and wild boar in European countries (EFSA 2017). The HEV genome has been detected throughout the food chain (from farm to market) of both pork meat products and venison (Doceul et al. 2016). Table 1 summarises the results of several European studies focusing on the detection and characterisation of HEV in meat, organs of the main animal HEV reservoirs and products thereof whose consumption without proper heat treatment may result in zoonotic transmissions.

Contamination of the environment (water) with HEV from human and animal faecal waste may lead to contamination of bivalve molluscs, fruit and vegetables. Bivalve molluscs are known to concentrate viruses during the process of filter feeding and may accumulate HEV. Although several studies have reported detection of HEV-3 and HEV-4 in shellfish, the contribution made by these molluscs to HEV infection in humans is still unclear (Koizumi et al. 2004 and Said et al. 2009). Recently, the HEV genome has been found in food of non-animal origin: leafy greens (Kokkinos et al. 2012), berries - strawberries and frozen raspberries (Brassard et al. 2012 and Maunula et al. 2013), herbs and spices (Loisy-Hamon and Leturnier 2015). In these cases, the vehiculum of the virus could be fertiliser of animal origin or contaminated water. However, vegetarianism has been identified as a protective factor against HEV infection in several studies (Cossaboom et al. 2016; Tedder et al. 2016).

The lack of an efficient cell culture system has made it difficult to achieve a clear knowledge about HEV persistence in food matrices and the effect of the decontamination procedures used (inactivation kinetics of HEV). Recent results indicate that the resistance of HEV is variable depending on the strain or genotype, the studied matrices (e.g. meat, sausages, water and oysters), as well as the number of infectious viral particles contaminating the matrix (Cook and Van der Poel 2015). Thermal treatment to reduce virus load is a common strategy in food industries. Data on both long-term and short-term storage of HEV were obtained by treatments of HEV with various combinations of temperature and time. The results showed that the susceptibility of HEV to heat differed between HEV strains, where some were inactivated almost completely when maintained at 56 °C for 1 hour, whereas in others 20% remained infective after being maintained at 60 °C. HEV-contaminated liver blocks boiled (100 °C) or stir-fried (191 °C), with an internal temperature of 71 °C being achieved for 5 minutes, showed no residual infectivity. However, incubation at 56 °C for 1 hour did not inactivate the virus (Feagins et al. 2008). During testing of the effect of low temperatures on HEV in a liver suspension, the HEV genome was detected after 70 days of storage at 4 °C (Emerson et al. 2005; Feagins et al. 2008 and Schielke et al. 2011). It was demonstrated that infectious HEV-3 can be detected for up to 21 days at 37 °C and up to 28 days at room temperature, and a decrease (2.7 log) of the infectious virus could only be observed after 56 days at 4 °C (Johne et al. 2016).

All viruses are more resistant to heat treatment when embedded in tissues or other food matrices that afford protection against the effect of heat or other noxious agents. Pâté-like preparations (48% fat) treated with different time/temperature combinations, ranging between 62 °C and 71 °C and 5 and 20 min., showed residual infectivity. Infectious viruses were still observed when the pâté was heated at 62 °C for 120 min., at 68 °C for up to 20 min., and at 71 °C for up to 10 min. Only treatments at 71 °C for 20 min. resulted in the total loss of infectivity (Barnaud et al. 2012). No data are available on the resistance of HEV under food-processing technologies such as curing, drying and smoking, which are processes involved in the production of some foods of animal origin that are consumed raw and therefore place consumers at higher risk. More studies are needed to evaluate the residual infectivity and inactivation kinetics of HEV, even during the preparation procedures for common kinds of foodstuffs (EFSA 2017).

Table 1. Examples of hepatitis E virus (HEV) presence in organs for food production and in food of animal origin, in Europe (modified according to EFSA 2017).

Country	Animal species	Place	Sample	Results*	Reference
Belgium	Wild boar	Hunting	Liver	4/61 (6.5)	Thiry et al. (2015)
	Red deer		Liver	1/29 (3.4)	
France	Deer	Hunting	Liver	2/62 (3.2)	Lhomme et al. (2015)
	Wild boar		Liver	5/86 (5.8)	
	Domestic pig	Retail	Figatelli	42/140 (30)	Pavio et al. (2014)
			Liver quenelle	14/85 (16.5)	
			Liver sausages	49/169 (29)	
	Domestic pig	Slaughterhouse	Liver	128/3715 (4)	Rose et al. (2011)
Germany	Wild boar	Hunting	Liver and/or serum	39/232 (16.8)	
	Roe deer			5/78 (6.4)	
	Red deer			2/83 (2.4)	
	Wild boar		Muscle+	29/35 (82.3)	Anheyer-Behmenburg et al. (2017)
	Roe deer			4 (100)	
	Red deer			2 (100)	
	Domestic pig	Retail	Raw sausages	24/100 (24)	Szabo et al. (2015)
	Wild boar		Raw sausages	1/10 (10)	
	Domestic pig	Slaughterhouse	Liver	34/251 (13.5)	Baechlein et al. (2013)
	Domestic pig	Retail	Liver	8/200 (4)	Wenzel et al. (2011)
Hungary	Wild boar	Hunting	Liver	8/75 (10.7)	Forgach et al. (2010)
	Red deer		Liver	3/30 (10.0)	
	Roe deer		Liver	9/41 (21.9)	
Italy	Domestic pig	Retail	Raw and dry	10/45 (22.2)	
			liver sausages	1/23 (4.3)	Di Bartolo et al. (2015)
	Wild boar	Hunting	Liver	55/164 (33.5)	Montagnaro et al. (2015)
	Domestic pig	Slaughterhouse	Liver	2/33 (6)	Di Bartolo et al. (2012)
			Lingual muscles	2/33 (6)	
Portugal	Wild boar	Hunting	Liver	20/80 (25)	Mesquita et al. (2014)
Spain	Domestic pig	Slaughterhouse	Liver	1/39 (3)	Di Bartolo et al. (2012)
		Retail	Sausages	6/93 (6)	
Switzerland	Domestic pig	Slaughterhouse	Liver	2/160 (1.3)	Muller et al. (2017)
Czech Republic	Wild boar	Hunting	Liver	50/438 (11.4)	Kubankova et al. (2015)
	Domestic pig	Slaughterhouse	Liver	2/40 (5)	Di Bartolo et al. (2012)
			Lingual muscles	1/40 (3)	
Netherlands	Wild boar	Hunting	Liver	2/102 (2)	Rutjes et al. (2010)
	Red deer		Liver	1/39 (3)	
			Muscles	2/39 (5)	
	Domestic pig	Retail	Liver	4/62 (6.5)	Bouwknegt et al. (2007)
UK	Domestic pig	Retail	Liver	1/76 (1.3)	Banks et al. (2010)
	Domestic pig	Slaughterhouse	Liver	1/40 (2.5)	Berto et al. (2012)
		Retail	Sausages	6/63 (9.5)	

* - number of HEV positive/total number of analysed samples (%), the results were obtained by reverse transcription real time polymerase chain reaction (RT-qPCR)

+ - muscle from animals HEV-positive in liver

UK - United Kingdom of Great Britain and Northern Ireland

Possible control measures along food chains of animal origin

The most effective control measure relies on avoiding the production of HEV-contaminated food. The prevention of HEV introduction into pig production herds and the associated reduction in the number of HEV-infected pigs at the time of slaughter could be of major benefit if there are sufficient HEV-free sources of pigs to establish infection-free networks. Although several studies indicate the presence of some HEV-free herds in intensive pig farming areas (Krumbholz et al. 2013), experience with other pathogens has shown this option to be difficult to implement or unfeasible. The transmission of HEV between pigs is strongly influenced by faecal contamination of the environment which suggests the possibility of reducing the prevalence of infected pigs by appropriate farm management, hygiene (including effective disinfection of pig housing and equipment between batches) and biosecurity measures (Rose and Pavio 2014). Another potential control option could be vaccination of pigs (Krain et al. 2014), but the efficiency of pig vaccination to prevent human disease requires further investigation. Besides, no vaccine for pigs is currently commercially available (EFSA 2017).

The current control measures for food of animal origin rely on EU legislation; Regulations EC No. 853/2004 and No. 854/2004. As infected animals often do not show symptoms of infection, animals harbouring pathogens such as HEV cannot be recognised during visual-only (*ante-mortem* and *post-mortem*) inspections. Therefore, potentially contaminated organs and meat can enter the food supply chain. Presently, the only efficient control option for HEV infection from the consumption of meat, liver and products derived from animal reservoirs is sufficient heat treatment (EFSA 2017). Measures preventing or reducing the faecal contamination of carcasses reduce surface contamination. Therefore, adherence to good practices during slaughter, processing and storage should reduce the risk of cross-contamination. Nevertheless, these measures involving improved hygiene will have a lower impact on the spread of HEV since infectious viral particles may be present in the blood, offal or meat of infected pigs at the time of slaughter. Consequently, HEV should be inactivated during the subsequent processing of meat products (Brien et al. 2015). Testing of meat and offal to be eaten raw or lightly cooked, in which HEV will not be inactivated during subsequent processing, should be considered (EFSA 2017).

Limited information is available on the effect of biocidal treatments and disinfection applied in the food industry on the infectivity of HEV. However, the use of chlorination and UV treatments for water can inactivate HEV because the dose level required to obtain consistent inactivation is below that required by international guidelines for water disinfection. These measures can also be used to minimise cross-contamination by means of treatment of food-contact surfaces or decontamination of water (EFSA 2017).

In particular, the provision of information to abattoir staff and food handlers, as well as to consumers, may help prevent HEV infections. Hunters and others handling carcasses should be educated about HEV transmission associated with game mammals. In order to minimise the risk of HEV infection, consumers should thoroughly cook pork and wild boar meat products in particular. This recommendation should particularly be applied where vulnerable groups are concerned, e.g. persons with a weakened immune system, pre-existing liver injury (EFSA 2017).

Conclusions

- The validation and standardisation of methods for the detection and quantification of HEV from meat and meat products, as well as the development of efficient cell culture methods, should be encouraged and included among the priorities of current research.
- Studies are needed to estimate quantitatively the level of contamination in foods of

animal origin, including foods other than those containing pig liver that have rarely been investigated, and to determine the correlation of HEV genome detection with infectivity of the virus.

- Data on the survival of HEV in meat products as well as in bivalve molluscs, fruit and vegetables and their production and processing environment are needed. The risk of transmission of HEV from contaminated water to food should be determined.
- The level of awareness of HEV risks associated with pork products and other reservoirs and sources is low despite considerable research in recent years. Therefore, dissemination of knowledge and advice to consumers as well as those working with potential sources of HEV infection should be optimised. In particular, information about the risk of consumption of raw or undercooked pork, wild boar and deer products by vulnerable groups (e.g. persons with a weakened immune system or pre-existing liver damage) should be provided. The risks of the most serious HEV infections may be prevented in this way.
- Due to the high concentration (viral load) of HEV found in pork liver, producers and sellers of liver and products thereof should take preventive measures to minimise the risk of HEV transmission to consumers.
- Consumers should thoroughly cook meat and offal (especially of pork, wild boar and deer origin) to minimise the risks of an HEV infection (EFSA 2017).

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