

REVIEW

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Recent knowledge on hepatitis E virus in Suidae reservoirs and transmission routes to human

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Abstract

Hepatitis E virus (HEV) causes self-limiting acute hepatitis in humans that can eventually result in acute liver failures or progress to chronic infections. While in tropical and sub-tropical areas, HEV infections are associated with important waterborne epidemics, in Northern countries, HEV infections are autochthonous with a zoonotic origin. In the past decade, it has become clear that certain HEV genotypes are zoonotic and that swine, and more generally Suidae, are the main reservoir. Zoonotic transmissions of the virus may occur via direct contact with infected pigs, wild boars or consumption of contaminated meat. This review describes the current knowledge on domestic and wild Suidae as reservoirs of HEV and the evidence of the different routes of HEV transmission between these animals and humans.

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1 Introduction

Hepatitis E virus (HEV) infection is highly prevalent in human worldwide, with more than 20 million infections each year (WHO). Clinical hepatitis E is usually self-limiting but some cases may evolve into fulminant hepatitis with poor prognosis. In some endemic regions, up to 30% of pregnant women infected by HEV during the 3rd trimester die from acute liver failure [1]. In Northern countries, chronic HEV infections are observed in immunosuppressive contexts, notably in solid organ transplant recipients [2]. In those patients, rapid progression toward cirrhosis is observed. Extrahepatic symptoms, such as neurological, kidney or hematological dysfunctions, have also been described. HEV is transmitted by the oral route and occasionally through the parenteral route after accidental transfusion of HEV positive blood donation. In endemic regions (tropical and subtropical areas), HEV is a waterborne disease associated with large epidemics related to accidental contamination of drinking water by sewage. In Northern countries, HEV is suspected to be mostly a foodborne disease transmitted through consumption of infected food products. HEV is unique among the hepatitis viruses since it is the only one possessing non-primate animal reservoirs and being a foodborne zoonosis. HEV is widespread in pig farms and consumption of pork products, specially containing

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pig liver, is associated with HEV infections [3]. HEV is also present in wild boars and consumption of game meat or hunting is associated with HEV exposure. Contact exposure with infected animals is a possible transmission route as well, since professional occupations with animal reservoirs (pig-farmer and -veterinarians, slaughterhouse- and forestry-workers, hunters) have a higher seroprevalence than the related general population. Interactions between wild and domestic suids occur and may contribute to the spread and maintenance of HEV in both reservoirs. This review aims at presenting the recent data on Suidae reservoirs, cross-contaminations between wild and domestic pigs and on vehicles of HEV exposure through contacts or consumption of food products from infected animals.

2 Suidae reservoirs

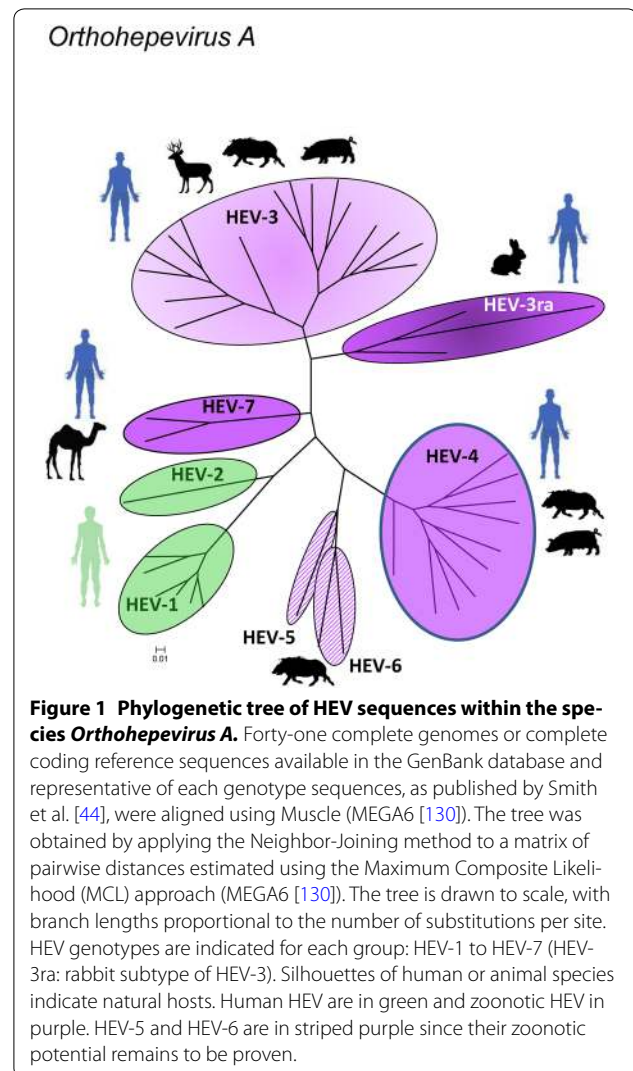
HEV belongs to the Hepeviridae family which is divided into the genera *Orthohepevirus* and *Piscihepevirus* [3]. In the *Orthohepevirus* genus, four species (A to D) can be distinguished that are divided into several genotypes. Human and zoonotic HEV are classified into the *Orthohepevirus* species A, which includes seven genotypes. HEV-1 and HEV-2 infect humans only, whereas HEV-3 and HEV-4 can be found both in human and Suidae species (Figure 1). Within the genotype HEV-3, a separate branch corresponds to the HEV present in rabbit species (HEV-3ra), which also includes a closely related human strain (Figure 1). Two other genotypes, HEV-5 and HEV-6, have been described only in wild boars from Japan [4] (Figure 1). HEV-7 was first identified in dromedary camel [5] and then in one patient with chronic hepatitis after liver-transplantation [6].

HEV-3 is widely distributed and present in pigs from many geographic areas including Americas, Europe, Africa, Japan, south-east Asia and Oceania, whereas HEV-4 is described mainly in pigs from China, Japan and Indonesia [7].

2.1 Domestic swine

2.1.1 HEV infection in domestic pigs

Since the discovery of swine HEV in 1997 [8], numerous publications have shown high prevalence of HEV in pig herds (up to 100%) all over the world. Each continent is concerned: Asia (China, India, Indonesia, Japan, Korea, Mongolia, Philippines, Taiwan, Thailand and Vietnam), Americas (Argentina, Bolivia, Brazil, Canada, Cuba and Mexico), Africa (Cameroon, Democratic Republic of Congo, Nigeria and Madagascar), Europe (Belgium, Czech Republic, Finland, France, Germany, Hungary, Italy, the Netherlands, Romania, Spain, Sweden, Switzerland and the United Kingdom) and Oceania (Australia, New Caledonia and New Zealand) [7, 9–14].



Seroprevalences were estimated between 5 and 100% [7, 9–14]. Comparisons of HEV seroprevalences between countries are limited since sample collections (serum, occasionally meat juice) [15] and method of analysis are very diverse. Both commercial ELISA tests and in house assays were used to detect anti-HEV IgG and/or IgM. Viral prevalences are even more heterogeneous as they depend on the sampling strategies used: nature of the sample (blood, fecal, liver, other organs), age of the animals and method of analysis by conventional or real time RT-PCR that are still not standardized.

All studies converge toward an infection of pigs at an early age after the loss of maternal antibodies [16]. The peak of viral excretion in pig fecal samples is detected between 3 and 8 weeks after weaning [17], and then viral excretion decreases between 15 and 18 weeks of age [18] with the appearance of antibodies, IgM followed by IgG (seroconversion) [19]. The duration of the immunity

acquired after HEV infection has not been estimated. Possible re-infection in case of transient decrease of immunity (sow after delivery or during co-infection) cannot be excluded. A loss of protection by a decrease of antibody or cellular response over time may also happen in older animals, especially in sows [20].

The presence of HEV RNA in serum samples (viremia) is less documented and seems to be less frequent than in liver or fecal samples [21]. Thus, the search for HEV RNA in blood or serum for diagnosis and prevalence studies may not be the best method to detect acute HEV infection in natural settings. Also, experimental infection of pigs shows that viremia can be influenced by the inoculation route [22]. Viral RNA was detected more frequently in the blood of infected animals after intravenous inoculation than after oral inoculation [22].

In both natural and experimental infections of pigs, rare histological signs of hepatitis are observed and infected animals do not show weight loss or hyperthermia. Thus, HEV in pig herds is not considered as an animal health threat. The main site of HEV multiplication is the liver but HEV can be found in other pork organs after experimental infections. HEV RNA or replicative forms of HEV RNA can be detected in extra-hepatic sites such as spleen, duodenum, jejunum, colon, lung, gastro-hepatic lymph nodes and muscles [22–24]. In one study, HEV was additionally detected in the lung [22]. Bouwknecht et al. reported the detection of HEV in tissues from three different muscles (Longissimus, Biceps femoris, Iliopsoas) in most of the inoculated and contact-infected pigs [23]. Generally, the highest detection rates and amounts of HEV RNA are found in the liver, whereas other organs show lower detection rates as well as lower HEV RNA concentrations. In these experimental models, several inoculation routes were used: intravenous, oral route and contact with inoculated animals. Again, depending on the inoculation route, HEV distribution was modulated, suggesting that experimental settings influence the outcome of the viral infection and may not completely reflect natural infection. Presence of HEV in muscle or other pig organs entering the food chain is of concern for food safety. Up to now, only few studies were conducted at slaughterhouse, on small sample size, to investigate the presence of HEV RNA in the organs of naturally infected pigs. In one of these studies, HEV RNA was searched in several organs such as loin, bladder or tonsils from selected animals ($n = 43$) [25]. HEV was detected in the bladder (10/43) and tonsils (3/43). No HEV-positive loin samples were observed [25]. In another study, detection of HEV RNA was investigated in the pork production chain at several steps: production (carcass dissection and liver removal), processing or point of sale. At the production phase (slaughterhouse),

HEV RNA was found in liver, bile or fecal samples but also in pork lingual muscle with an estimated prevalence of HEV RNA of 2.7% ($n = 112$) [26]. At all steps, production, processing plant or point of sale, HEV RNA was amplified from workers' hands and gloves and working surfaces, suggesting that cross contamination may occur [26]. Thus, further studies are needed to determine HEV distribution in pork organs during slaughtering.

Hepatitis E virus can persist in the farm environment [27] but survival parameters are mainly unknown. Experiments performed with HEV in cell culture supernatant indicate that infectious HEV can be detected after one month storage at room temperature and after more than 2 months storage at 4 °C [28]. The virus is mainly excreted fecally in pigs, leading to an accumulation of HEV in the environment of infected livestock. Thus, both contact between individuals and environmental exposure can play a role in HEV transmission. This was confirmed during the controlled inoculations of animals with HEV and transmission to contact pigs [29, 30]. Experimentally, a minimum load of 10^6 copies of HEV RNA/g appears necessary to infect pigs per os and cause excretion of the virus and transmission to their congeners [29]. A mathematical model, taking into account three possible transmission pathways: direct or indirect contacts between the animals or the role played by the environment, was used to quantify HEV transmission within herds. The results obtained show that transmission by direct contact can be a factor of persistence of HEV in pig herds, however this way of transmission alone does not explain the high prevalences observed in herds. The major factor identified as playing a role in the spread and maintenance of infection in the population is the accumulation of the virus in the animal environment, leading to a continuous oro-fecal contamination process [29].

Environmental contaminations with HEV can also be observed in the vicinities of herds or slaughterhouse. HEV RNA was detected by nested RT-PCR in 32/452 samples from both inside and outside farm buildings, on trucks, and other objects sampled in the slaughterhouse yard, such as on a utility vehicle. According to the results of this study, the movements of trucks and utility vehicles might play an important role in HEV dissemination on a slaughterhouse site and throughout an entire network [31].

2.1.2 Risk factors associated with HEV infection within pig farms

A possible way to reduce human exposure through consumption of infected meat is to reduce the number of infected animals at slaughterhouse. To reach this goal, few studies have investigated factors associated with a high HEV prevalence at herd level. A retrospective survey

was conducted in France in 90 farms previously selected for a prevalence survey of HEV performed at slaughterhouse [32]. A high risk of HEV infection of pork livers was associated with the early slaughtering of young animal, the genetic line of the female breeding stock, lack of biosecurity measures and the use of drinking water from a nearby source. High seroprevalences at the end of the rearing period were associated with excessive mixtures of post-weaning animals and low conditions of hygiene [33]. Critical points to consider include interactions between husbandry conditions, loss of passive immunity and hygiene.

In a study performed in six European countries (Czech Republic, Italy, the Netherlands, Spain, Portugal and the United Kingdom), HEV prevalence was determined in different types of herds: weaners, growers and fatteners. HEV RNA prevalence in pig fecal samples was high in all kinds of settings: weaners 8–30%, growers 20–44% and fatteners/finishers 8–73%. These data suggest that HEV positive fattener herds are at risk of delivering HEV positive pigs at slaughterhouse [34]. This risk can arise both from livers still infected with HEV or fecal cross-contaminations of carcasses. Analysis of livestock management was not performed to identify risk factors associated with positivity. Thus, further studies are needed to define measures to prevent such high HEV presence at the fattening stage.

Another study has focused on HEV seroprevalence in pigs from different farming systems in The Netherlands, including conventional, free-range, and organic farms. HEV-specific antibodies were detected in samples from all conventional, free-range and organic pig farms, indicating equal probability of introducing HEV for the different farming types. The estimated average within-herd seroprevalence was significantly higher for pigs from organic farms (89%) than for pigs from conventional farms (72%, $P = 0.04$) and close to significant for pigs from free-range farms (76%, $P = 0.06$) [35]. It seems that a higher probability of HEV infection is present in organic farm due to possible increase in animal contacts or longer exposure through environmental contamination [35]. It would be interesting to get information on the age of the pig sampled within each herd and the excretion profile of HEV in free-range and organic farms to evaluate the probability that these rearing systems deliver HEV positive pigs at slaughterhouse.

Another concern is the presence of other pig pathogens that may modulate the time-course of natural HEV infections within herds. Frequent co-circulation of HEV with immunomodulatory viruses such as Porcine Reproductive and Respiratory Syndrome virus (PRRSV) or Porcine circovirus-2 (PCV2), may influence HEV pathogenesis. A fatal disease associated with co-infection of HEV and

PCV2 was recently described in piglets. General hyperthermia, hemorrhage, inflammatory cell infiltration and necrosis were observed in the tissues of dead animals [36]. In addition, experimental co-infection of pig with HEV and PRRSV demonstrated increased and prolonged excretion of HEV in co-infected pigs. This co-infection led to an increased length of HEV excretion estimated at 49 days compared to 9 days during infection with HEV alone. An exacerbated transmission, on average four times greater than in a single HEV infection, was also observed [37]. Multiple infections of pig by pathogens must be taken into account to prevent HEV propagation or long time excretion that may influence the presence of HEV at the time of slaughtering.

Hepatitis E virus infections have little impact on animal health, as the animals have no obvious symptoms; as a result, there is no surveillance or reporting of infected herds. The level of enzootic HEV infections is suspected to be extremely high. The persistence of the virus in farms is not yet well explained, but its resistance in the environment, the possibility of partial protection conferred by antibodies or the chronic infection of certain animals are factors that could make its eradication difficult. Drastic internal biosecurity measures as well as the control of undercurrent pathogens that may have an immunomodulatory action such as PRRSV would seem to be effective in limiting the spread of HEV to the porcine population. The effect of HEV vaccine prophylaxis in animals has been modelled and may reduce the number of HEV-positive animals at slaughter [38] and thus reduce human exposure via food consumption.

Even if control measures, such as good herd practice and vaccination against HEV, would lead to a reduced level of enzootic HEV in herds, maintaining HEV “low or free” herds would have to take into account the large presence of HEV in wildlife and in particular in wild boars.

2.2 Wild fauna

Substantial evidences have been gathered in the past 20 years indicating that wild boar is an important reservoir of HEV. It is not surprising that wild boars (*Sus scrofa*) are also susceptible to HEV as they are closely related to domestic pigs (*Sus scrofa domesticus*). The first report suggesting that HEV can also infect wild boars comes from the detection of HEV antibodies in wild-caught pigs in Australia at the end of the 1990s [39]. A few years later, the first partial sequences of an HEV isolate originating from a wild boar were obtained in Japan [40]. Comparison of ORF2 partial sequences (298 nucleotides) showed that this strain was closely related to HEV-3 isolates previously found in Japanese patients and farm pigs [40]. The first full-genome HEV

sequences recovered from a wild boar were published a few months later. Comparison with complete or near-complete HEV isolates showed that this strain belongs to HEV-3 and shares 99.7% nucleotide identity with other deer and human isolates [41]. To date, most of the HEV strains detected in wild boars belong to HEV-3. Several variants assigned to HEV-4 have also been found in wild boars in Japan [4, 42, 43]. In addition, HEV isolates (JBOAR135-Shiz09, wbJOY_06 and wbJNN_13) that have been only detected in wild boars have been characterized [42, 43]. These HEV strains have been assigned to two novel genotypes: HEV-5 (JBOAR135-Shiz09) and HEV-6 (wbJOY_06 and wbJNN_13). These 2 genotypes share less than 80% nucleotide identity with HEV-1 to -4 [42, 43]. The wbJNN_13 and wbJOY_06 isolates are 80.4% similar over their entire genome [42] and it has been suggested that they belong to two distinct subtypes within the HEV-6 genotype [44]. Up to now, no human case has been associated with these two genotypes, thus their zoonotic potential is not known.

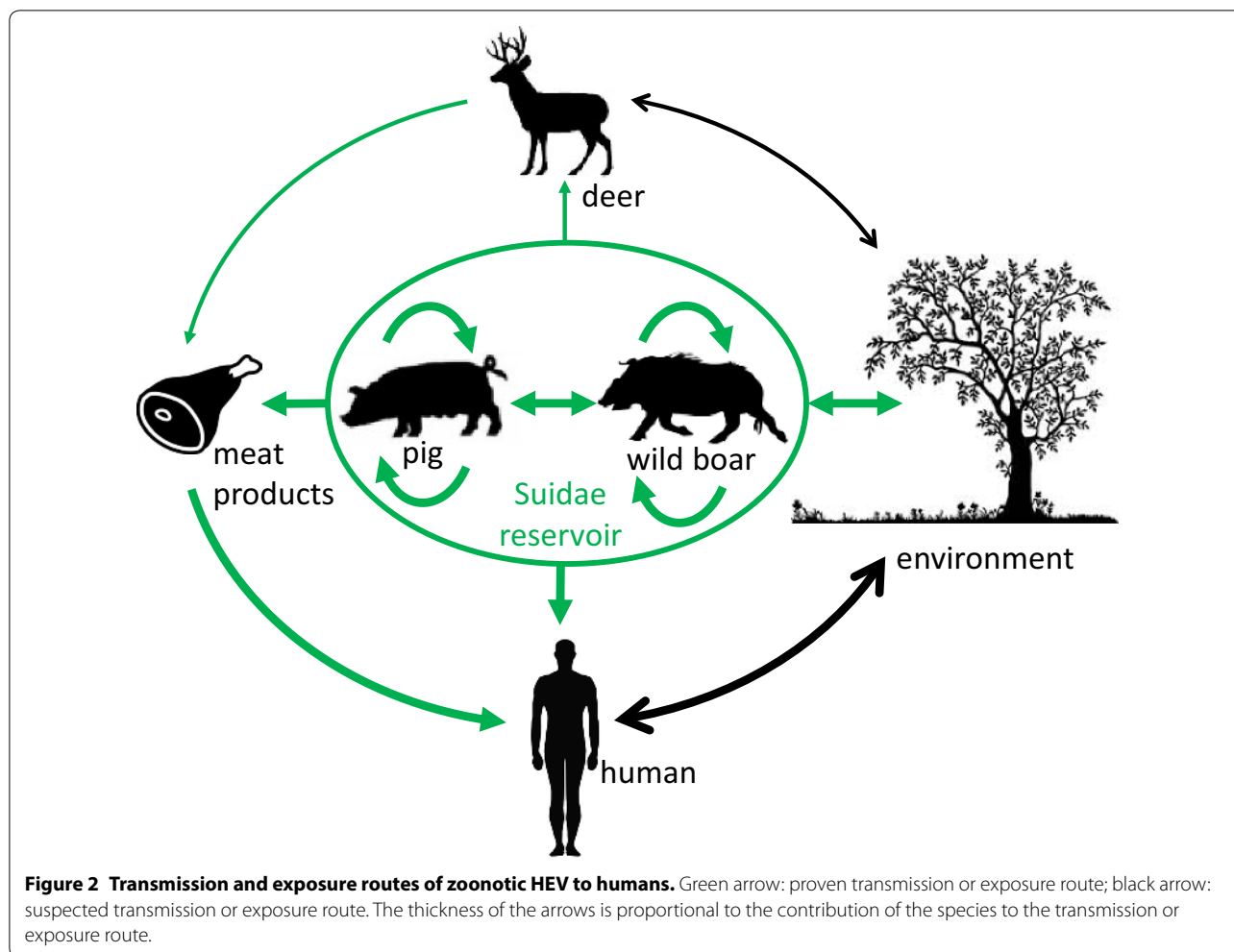
Very few data are available on clinical signs caused by HEV in wild boar. In one study, no difference was found between the biometric characteristics (body length and weight) of HEV-infected and non-infected wild boars [45]. In another report, no clinical manifestations were observed in a viremic male wild boar negative for anti-HEV IgG [40]. Experimental infection of 3-month old wild boars with a wild boar HEV-3 strain via the intravenous route or by contact caused subtle clinical symptoms such as reduced feed intake and mild diarrhea that were concomitant with increased bile acids (BA), alanine aminotransferase (ALT) and gamma-glutamyl transferase (γ GT) [46]. Mild lymphoplasmacytic hepatitis was also recorded. Higher viral loads of HEV were found in the feces of the infected wild boars in comparison to the feces of miniature pigs infected under the same conditions [46]. HEV RNA was also detected in the liver, gall bladder, small and large intestine and spleen of the infected wild boars. Chronic infections in two wild boars naturally infected with HEV-3 have also been reported [47]. In these two animals, viremia and/or viral shedding in the feces were detected for 12–16 weeks although high titres of anti-HEV antibodies were simultaneously present in their serum. No clinical signs or histopathological lesions evocative of hepatitis were recorded and HEV RNA was not detected in the liver or other tissues of the chronically-infected wild boars [47].

Several studies have been undertaken, mainly in Japan and Europe, to determine the prevalence of HEV antibodies and/or RNA in wild boars (Additional file 1). Seroprevalences ranging from 1.6 to 41.6% and from 4.9 to 57.4% were found in Japan and Europe, respectively. RNA prevalences of up to 10.3% in Japan and up to 68.2% in

Europe were also reported. These data clearly show that HEV infection is widely present in wild boars in Japan and in Europe and that wild swine likely represent a reservoir of HEV in these areas. Several factors such as the geographical location [4, 14, 42, 43, 45, 48–54], the year of sampling [4, 42, 48, 55, 56], the wild boar density [53, 57, 58] and the managing conditions [48, 59] can impact HEV prevalence. A higher HEV seroprevalence is usually found in adults and subadults than in juvenile [14, 48, 49, 52, 55, 60, 61]. No difference was observed between male and female wild boars [43, 45, 48, 58, 59, 61–64]. The reported HEV RNA prevalences (Additional file 1) can also vary according to the wild boar sample specimen used as HEV RNA seems to be more frequently detected in bile followed by liver and serum [50], as found for domestic pigs [65].

Comparisons of data obtained using the same serological assay have highlighted lower HEV seroprevalences in wild boars than in domestic pigs sampled in the same region [14, 54, 55, 60, 64]. In addition, HEV RNA can be found in wild boars in all age groups including animals older than 2 years [40, 45, 48, 52, 58, 59, 61, 63, 66–69] whereas in domestic pigs, it is more often detected in young animals (2–6 months) [70–73]. These data suggest that HEV circulates at lower rates and more progressively in wild boars than in pigs. The fact that domestic pigs are reared under intensive conditions and that wild boars are free-ranging and gather at lower densities could explain these differences. It is also possible that in wild boars, a weak or short-lasting protective immunity against HEV leads to chronic infections or re-infections of older animals.

Hepatitis E virus has also been detected in deer. Anti-HEV antibodies and/or HEV RNA have been found in several deer species such as red deer, roe deer, sika deer, fallow deer and white-tailed deer in America (Mexico and Canada), Asia (China, Japan) and Europe (Czech Republic, Belgium, France, Germany, Hungary, Italy, The Netherlands, Spain, Sweden) [40, 56, 60, 66, 68, 74–83]. Anti-HEV antibodies were found in up to 62% (89/142) of white-tailed deer sampled in Mexico [74] but HEV seroprevalences ranging from 0 to 14% are more commonly found in several deer species. To date, all the HEV sequences found in deer belong to HEV-3 [41, 56, 60, 78, 79, 82–84] but very few sequences are available. All these studies suggest that HEV circulates in deer. However, lower HEV seroprevalences and HEV RNA detection rates are frequently found in deer compared to wild boars sampled within the same geographical region [40, 56, 60, 77, 81]. A recent study has also shown that lower viral loads are consistently found in livers from deer in comparison to wild boar [56]. This data suggest that deer are not a true reservoir of HEV but are infected accidentally



by sharing the same habitat as wild boar. Wild swine therefore likely represent a source of spillover HEV infections for other wild animals and subsequently for humans (Figure 2).

2.3 HEV transmission between domestic and wild Suidae

Experimental infections have shown that HEV can cross the domestic pig-wild boar barrier. Oral and intravenous inoculation of wild boar HEV strains to miniature or domestic pigs can lead to viremia, excretion of HEV in the feces and seroconversion [22, 46]. In addition, wild boars naturally infected or inoculated intravenously with HEV are able to transmit the virus by contact to domestic or miniature pigs [46, 47]. These data strongly suggest that transmission via the fecal-oral route between domestic and wild swine is possible and can occur naturally when pigs and wild boars are reared in close contact (Figure 2). Such interactions between domestic pigs and wild boars are common as

some breeds of domestic pigs are raised outdoors in semi-open or open spaces in close contact with wildlife. These managing conditions exist worldwide, including in areas such as Corsica and Tuscany where a high HEV seroprevalence is found among wild boars [55, 61]. It is also important to consider the role of hybrid pigs in the epidemiology of HEV infection in swine. Wild boar can interbreed with domestic pigs, resulting in the birth of feral hybrid pigs that circulate freely but have a behavior that is more similar to domestic pigs (diurnal activity, more frequent and larger litter). These hybrid pigs are more likely to interact with domestic pigs and to gather at higher densities than wild boars. A study has shown that in Corsica, a high percentage of hybrid pigs (43.5%) has anti-HEV antibodies. This seroprevalence is lower than in domestic pigs (88%) but higher than in pure wild boars (26%) [55]. Hybrid pigs could then play an intermediate role in the transmission of HEV between domestic and wild swine.

Several studies have identified pig and wild boar strains of HEV-3 and HEV-4 sharing 90–98% homology based on entire/almost entire [58, 69] or partial [45, 55, 59, 63, 66, 67, 79, 85] sequences. For example, the comparison of full-length sequences has highlighted an identity of 96.9% between the sequences of a wild boar HEV-3 strain identified in Germany and a Mongolian pig strain [58]. A homology of 97.5% (partial ORF2 sequence) has been found between a wild boar and a domestic pig HEV-3 strain both isolated in Corsica [85]. Two wild boar HEV-3 strains isolated in Hungary were shown to be closer to pig strains than to each other (97–98% identity based on partial ORF2 sequences) [79]. In addition, a molecular evolutionary analysis has suggested that a subtype of HEV-3 has become endemic in Japan after the importation of infected pigs from Europe in the 1960s and was then transmitted from pigs to wild boars [86]. All these data suggest that dynamic exchanges of HEV-3 and HEV-4 between domestic pigs and wild boars have occurred. However, direct transmission of HEV between domestic pigs and wild boar in natural settings will only be proven by the identification of identical or near-identical (higher than 99%) strains in both animals. Moreover, the transmission potential of HEV-5 and HEV-6 between domestic and wild swine remains unknown as to date, these genotypes have only been detected in wild boars and attempt of experimental transmission of these two genotypes to pigs has not been reported yet.

As of now, it is still unclear whether cross-infection of HEV occurs between domestic and wild swine and contributes to the prevalence and dynamics of HEV infection in the swine population. Many studies need to be undertaken in order to better understand the implication of the wild boar reservoir in the epidemiology of HEV and its importance as a source of infection. It would be critical for example to determine the origin of HEV-5 and HEV-6 and whether these two genotypes are able to infect and cause diseases in domestic pigs, humans and other wild species such as deer. Other routes of HEV transmission between pigs and wild boars such as the contamination of surface water and the environment by wild boar droppings should also be considered and further explored (Figure 2). In addition, more studies are needed to determine whether chronic or recurrent HEV infections are common in wild boars as long-term shedding could facilitate the persistence of the virus in the wild boar population and environment, thus increasing the risk of transmission to nearby outdoor pig breeding and other wild species such as deer. It is still unclear whether HEV can be transmitted between swine and deer. Evidence of transmission between these species are rare and rely mainly on the identification of near-identical sequences in both species [41, 56].

3 HEV in food products from Suidae and deer

Many reports describe the detection of HEV RNA in animal liver intended for human consumption and the data are summarized in Table 1. This includes reports from many European countries, but also from North, Central and South America as well as from Africa and Asia. As different methods with different sensitivities have been applied for analysis of the samples in these studies, a direct comparison of detection rates and genome concentrations is not possible. Nevertheless, the data may give some indications on the distribution of HEV in different types of food in the distinct geographical areas. The reported detection rates in pig liver ranged from 0 to 21%; however, the majority of studies report detection rates between 2 and 8%. Using quantitative analysis, HEV genome concentrations between 20 and 10^7 RNA copies/g are described. For wild boar liver, studies from Europe and Japan are available, reporting detection rates between 2 and 38%. The HEV genome concentrations were between 40 and 10^8 RNA copies/g. Investigations on liver samples from other animal species intended for human consumption are mainly available for several deer species. This includes successful HEV detection in red deer (detection rates 2–10%) and roe deer (detection rates 0–22%), whereas investigations of liver samples from fallow deer, Yezo deer and sika deer remained mostly negative so far. The HEV RNA amounts in liver of deer ranged between 12 and 2000 copies/g. Therefore, livers of pigs and wild boars have to be considered at high risk of containing the HEV genome and some of these livers can contain high viral genome amounts. Deer liver can also contain HEV RNA; however, the prevalence as well as the amount of viral genome in deer liver samples is markedly lower than for pigs and wild boars.

Some studies have investigated the presence of HEV RNA in muscle and meat samples. The detection rates ranged from 0 to 6% for pig muscle, from 0 to 12% for wild boar muscle, and from 0 to 5% for deer muscle. Only very few quantitative data are available, which report HEV genome amounts between 500 and 4000 copies/g in wild boar and deer muscles. In conclusion, a lower percentage of muscle samples of pigs, wild boars and deer, which nevertheless can reach up to 12%, can contain HEV RNA, in comparable low concentrations.

Meat products have also been analyzed for the presence of the HEV genome. Almost all of these studies have investigated products from pigs. Consistent high detection rates between 16 and 47% have been described for sausages or meat products containing pig liver [87, 88]. The observed detection rates, which are higher than those determined for pig livers, are mainly explained by the use of livers from a high number of animals for the production of sausages and the resulting mixing effect

Table 1 Summary of reports on detection of HEV RNA in liver, meat and meat products from animals intended for human consumption

Animal species	Organ	Geographical area/Country	Detection rate	RNA log copies/g	References		
Pig	Liver	Brazil	2/118 (2%)		[115]		
		Burkina Faso	1/157 (1%)		[116]		
		Cameroon	3/345 (1%)		[117]		
		Canada	2/19 (10%)	1.3–1.6	[90]		
		Canada	25/283 (9%)	3–6.7	[118]		
		Canada	9/43 (21%)	3–7	[25]		
		China	4/114 (4%)		[119]		
		Czech Republic	2/40 (5%)		[26]		
		France (Corsica)	2/24 (8%)		[55]		
		France	128/3715 (4%)		[32]		
		Germany	8/200 (4%)		[120]		
		Hong Kong	7/479 (2%)		[121]		
		India	2/240 (1%)		[122]		
		Italy	2/33 (6%)		[26]		
		Japan	12/243 (4.9%)		[123]		
		Japan	0/110 (0%)		[124]		
		Japan	4/390 (1%)		[125]		
		Mexico	26/127 (20%)		[126]		
		Spain	1/39 (3%)		[26]		
		Thailand	3/1090 (1%)		[127]		
		The Netherlands	4/62 (6%)		[98]		
		United Kingdom	1/40 (3%)		[92]		
		USA	14/127 (11%)		[97]		
			Meat (muscle)	Canada	0/599 (0%)		[118]
				Canada	0/43 (0%)		[25]
				Czech Republic	1/40 (3%)		[26]
				Italy	2/33 (6%)		[26]
				Spain	0/39 (0%)		[26]
				Thailand	2/559 (1%)		[127]
				United Kingdom	0/40 (0%)		[92]
			Sausages (and other products) containing liver	Canada	36/76 (47%)	0.6–2.7	[90]
				France	68/394 (17.3%)	2.2–6.3	[87]
	France	22/70 (31%)		1.6–6.2	[91]		
	Germany	11/50 (22%)			[89]		
	Italy	11/68 (16%)		3.4–5.3	[88]		
	Sausages without liver	Germany	14/70 (20%)		[89]		
	Sausages (not specified)	Canada	0/35 (0%)		[90]		
		Czech Republic	0/92 (0%)		[26]		
		Italy	0/128 (0%)		[26]		
		Spain	6/93 (6%)		[26]		
		United Kingdom	6/63 (10%)		[92]		
Wild boar		Liver	Belgium	4/61 (7%)		[60]	
			Czech Republic	50/437 (11%)	7.3	[81]	
	France		7/285 (3%)		[128]		
	France		5/86 (6%)	1.6–8.1	[80]		
	Germany		39/232 (17%)	7.4	[56]		
	Germany		4/22 (18%)		[111]		
	Germany		22/148 (15%)		[58]		

Table 1 continued

Animal species	Organ	Geographical area/Country	Detection rate	RNA log copies/g	References
		Germany	48/126 (38%)		[50]
		Hungary	8/75 (11%)		[78]
		Italy	7/372 (2%)		[62]
		Italy	55/164 (33%)		[63]
		Japan	19/552 (3%)		[4]
		Japan	7/39 (18%)		[129]
		Japan	1/33 (3%)		[40]
		The Netherlands	2/102 (2%)		[66]
	Meat (muscle)	Germany	29/232 (12%)	3.6	[56]
		Germany	1/22 (5%)		[111]
		The Netherlands	0/64 (0%)		[66]
	Sausages without liver	Germany	1/10 (10%)		[89]
Red deer	Liver	Belgium	1/29 (3%)		[60]
		France	2/62 (3%)	1.1-3.1	[80]
		Germany	2/83 (2%)	3.3	[56]
		Hungary	3/30 (10%)		[78]
		The Netherlands	1/39 (3%)		[66]
	Meat (muscle)	Germany	2/83 (2%)	2.7	[56]
		The Netherlands	2/39 (5%)		[66]
Roe deer	Liver	Belgium	0/27 (0%)		[60]
		Germany	5/78 (6%)	3.3	[56]
		Hungary	9/41 (22%)		[78]
		Italy	0/30 (0%)		[62]
		The Netherlands	0/8 (0%)		[66]
	Meat (muscle)	Germany	4/78 (5%)	2.7	[56]
		The Netherlands	0/6 (0%)		[66]
Fallow deer	Liver	Germany	0/22 (0%)		[56]
	Meat (muscle)	Germany	0/22 (0%)		[56]
Yezo deer	Liver	Japan	0/79 (0%)		[125]
Sika deer	Liver	Japan	0/132 (0%)		[40]

[89, 90]. The number of HEV genome in the liver-containing products ranged from 4 to 2×10^6 copies/g [91]. HEV RNA was also found in sausages containing no liver (and in sausages where the presence or absence of liver was not known), but with more varying detection rates ranging from 0 to 20% [26, 92].

Detection of HEV RNA does not necessarily indicate the presence of infectious virus. Therefore, attempts have been made to demonstrate the infectivity of food items previously tested positive for HEV RNA. However, the measurement of HEV infectivity is difficult due to the lack of efficient cell culture models for HEV propagation [93]. Therefore, only a few studies are available investigating the presence of infectious HEV in organ or meat products from animals intended for human consumption. An A549 cell culture system was successfully used to demonstrate infectivity of HEV present in pig liver sold at retail in Japan [94]. Using a 3D cell culture model,

HEV was isolated from porcine liver sausages from retail in France thus demonstrating the presence of infectious virus [95]. The presence of infectious HEV in commercially sold pig livers was also demonstrated by experimental inoculation of sample homogenates into pigs [96–98].

Stronger evidence for transmission of HEV by ingestion of food of animal origin comes from case reports and outbreak investigations. Colson et al. reported an outbreak of hepatitis E involving 7 persons in France, who ate liver sausages named *figatelli* [99]. Closely related HEV sequences were detected in the patients and local sausage samples. A similar case where identical HEV sequences were identified in a French hepatitis E patient and the leftover *figatellu* has been reported later [100]. In another outbreak that occurred on a French coastal island, 3 persons diseased with hepatitis E [101]. The epidemiological investigations pointed towards a spit-roasted piglet as the

infection source. Identical HEV sequences were detected in the patients and in a liquid manure sample from the farm where the piglet was born. Investigation of an acute hepatitis E case in an immunodeficient patient identified pork meat as the source of infection by sequence comparison between HEV strains detected in the patient and in the meat [102]. Two case reports from Japan identified grilled meat from wild boar as the source of infection by demonstrating identical HEV sequences in the patient and the meat sample [103, 104]. Similarly, a wild boar liver was shown to contain HEV sequences that were identical to the ones found in two patients from Japan who ate the liver [105]. Four hepatitis E cases in Japan could also be linked to the consumption of meat from sika deer as identical HEV sequences were identified in the patients and the leftover meat [84].

4 Contact exposure with Suidae

Transmission of HEV via contact with pigs or wild boars has been repeatedly suggested in Europe, Asia, Africa and Southern America. Most evidence for this transmission pathway comes from serological studies analyzing persons with occupational contact with animals compared to a control group (Additional file 2). For the investigation of HEV transmission from pigs, the exposed groups usually included pig farmers, slaughterers, butchers and swine veterinarians. In most of the studies, these groups show a higher HEV-specific antibody prevalence as compared to non-exposed persons. However, a direct comparison of the data from the different studies is not possible as different assays were used. In addition, factors other than the contact with pigs could differ between the exposed and non-exposed groups (e.g. their nutritional behavior) and may have influenced the results. Some of the studies found no significant differences in the seroprevalences between exposed and non-exposed people [106, 107]. However, as the majority of the studies demonstrate higher prevalences in persons who had been in contact with pigs, HEV transmission through this pathway seems to be common.

To investigate the transmission pathway of HEV from wild animals to humans, a number of serological studies with forest workers and hunters has been conducted (Additional file 2). Two studies investigating forest workers in Germany and France found higher HEV-specific antibody prevalences in these groups compared to the control groups [108, 109]. A study performed in Japan has identified a markedly higher seroprevalence in wild boar hunters (25.3%) as compared to a group of residents (5.5%) from the same geographical area [110]. In a similar study carried out in Germany, the HEV seroprevalence found in hunters was only slightly increased in comparison to the general German population [111]. However,

a more detailed analysis of the data revealed that hunters who frequently used gloves during disemboweling of wild boars, had a significantly lower anti-HEV antibody prevalence as compared to hunters using gloves never or infrequently. The data of these studies indicate that transmission of HEV by contact with wild animals and especially with wild boars is likely.

In contrast to numerous studies describing the presence of antibody as indicator of virus transmission from pigs or wild boars to humans, evidence for induction of disease after transmission of HEV by contact is rare. Renou et al. have described a case of acute hepatitis E in a patient in France who kept a pet pig and had frequent contact with it and with its excretions [112]. By comparison of HEV strains detected in the patient and in the pig, nucleotide sequence identities between 92 and 98% were determined for different genome regions. Although the strains were not identical, both strains were more closely related to each other than to other HEV strains found in the same geographical region. The authors suspected that a distinct (minor) quasispecies of HEV from the pig was transmitted to the patient by direct contact or contact with the excretions. HEV transmission has also occurred during the surgical training of a surgeon on pigs, through contact with infected inner organs [113]. Direct contamination of a slaughterhouse worker has been reported as well, confirming that hepatitis E can be an occupationally acquired illness by means of the manipulation of infected organs from pigs [114].

5 Conclusions

In the past decade, many studies have provided evidence that Suidae are the main reservoir of zoonotic HEV and that the virus is endemic in pig farms worldwide. A few studies have started to identify risk factors associated with HEV infection in pig herds. Nevertheless, many factors remain undetermined. For example, the influence of swine genetic backgrounds on the host susceptibility to HEV infection is still unknown. It is also unclear whether chronic infections can occur in swine and whether chronicity is associated with co-infections with other common pig pathogens or certain host factors linked to immunity. More efforts are then needed to determine more thoroughly the parameters involved in the accumulation and persistence of HEV in the breeding environment. Studies on the efficiency of disinfection regimes and vaccination to restrict HEV spread in the pig population would also be useful. Such investigations will help to understand the dynamics of HEV infection in pig herds and to identify control measures able to limit the appearance and persistence of the virus in breeding farms. A better characterization of the role of wildlife as a persistent source of contamination for the environment, other wild animals like deer, and pig herds is also required. In addition, the

zoonotic potential of wild boar strains HEV-5 and HEV-6 need to be assessed experimentally to determine whether domestic swine and potentially humans are susceptible to these strains and might develop diseases. Prevention of zoonotic HEV infection will also benefit from a better monitoring of the presence of infectious HEV in pork-derived foods. In the past few years, cell culture models have been developed that allow an efficient replication of HEV. Such systems should be exploited to determine which meat products (produced or not with liver) are at risk of containing infectious viruses. Better recommendations could then be provided to consumers regarding handling and cooking of such foods.

Additional files

Additional file 1. Prevalence of HEV-specific antibodies and HEV RNA among wild boars. Data from the literature (as referenced) are summarized in the table according to the geographic location, year of sampling, type of specimen used and the genotype detected.

Additional file 2. Summary of reports on serological studies in people with occupational contact to animals. Data from published studies (as referenced) investigating the prevalence of anti-HEV antibodies in different types of exposed and control groups in different countries are summarized in the table.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NP designed the structure of the review. NP, VD, EB and RJ wrote the different sections of the manuscript. All co-authors revised the manuscript. All authors read and approved the final manuscript.

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