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Review Article

Hepatitis E virus in pigs and the environment: An updated review of public health concerns

Tauseef Ahmad[1,2]*, Hui Jin[1,2], Kuldeep Dhama[3], Mohd. Iqbal Yatoo[4], Ruchi Tiwari[5], Muhammad Bilal[6], Manish Dhawan[7,8], Talha B. Emran[9], Jeehan H. Alestad[10,11,12], Hatem M. Alhani[13,14,15], Habib K. BinKhalaf[16] and Ali A. Rabaan[17,18,19]

[1]Department of Epidemiology and Health Statistics, School of Public Health, Southeast University, Nanjing, China; [2]Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, China; [3]Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India; [4]Sher-E-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India; [5]Department of Veterinary Microbiology and Immunology, College of Veterinary Sciences, Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura, India; [6]School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huaian, China; [7]Department of Microbiology, Punjab Agricultural University, Ludhiana, India; [8]The Trafford Group of Colleges, Manchester, United Kingdom; [9]Department of Pharmacy, BGC Trust University Bangladesh, Chittagong, Bangladesh; [10]Immunology and Infectious Microbiology, Glasgow, United Kingdom; [11]Collage of medicine, Microbiology, Jabriya, Kuwait; [12]Kuwait Chair Madam in Antimicrobial Resistance Committee, Alternative Permanent Representative of Kuwait to the United Nation Agencies, Rome, Italy; [13]Department of Pediatric Infectious Disease, Maternity and Children Hospital, Dammam, Saudi Arabia; [14]Department of Infection Control, Maternity and Children Hospital, Dammam, Saudi Arabia; [15]Department of Preventive Medicine and Infection Prevention and Control, Directorate of Ministry of Health, Eastern Region, Dammam, Saudi Arabia; [16]Department of Molecular Laboratory, King Fahad Hospital, Hofuf, Saudi Arabia; [17]Molecular Diagnostic Laboratory, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia; [18]College of Medicine, Alfaisal University, Riyadh, Saudi Arabia; [19]Department of Public Health and Nutrition, The University of Haripur, Haripur, Pakistan

*Corresponding author: tahmad@seu.edu.cn

Abstract

Hepatitis E virus (HEV) is an important public health problem and is responsible for both acute and chronic viral hepatitis. Public health implications of HEV are derived from its transmission route, either water-borne or food-borne, and its zoonotic potential. Not only in developing countries, but HEV cases are also found in a high number in developed countries. The spread of HEV to the environment might pollute surface waters, which could act as the source of infection for both humans and animals. Identification of the virus in animal products suggests the circulation of HEV within water and food chains. High seroprevalence and circulation of HEV in livestock, in particular pigs, as well as in environmental samples warrants further investigation into pig markets. HEV virulence in different environments and meat supply chains could shed light on the possible sources of infection in humans and the degree of occupational risk. The purpose of this review is to discuss HEV infections with an emphasis on livestock- and environment-related risk factors, and food-borne, water-borne, and zoonotic transmissions.

Keywords: Hepatitis E virus, livestock, zoonotic transmission, water-borne, environment

Introduction

Hepatitis E virus (HEV), one of main contributors to global acute viral hepatitis, poses a significant public health concern with high prevalence in developing countries. It was long-

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neglected, although has now also emerged as an important virus in the developed countries [1-4]. In areas with heavy seasonal rainfall, high evaporation rates, and dense population, HEV could cause outbreaks of acute hepatitis [5-7]. Based on a report from the World Health Organization (WHO), approximately 20 million cases of HEV are found annually across countries. Of the total reported hepatitis cases, an estimated 3.3 million infections were asymptomatic. In 2015, according to the WHO estimation, approximately there were 44,000 HEV-associated deaths worldwide [8]. Moreover, HEV mortality rate among women during pregnancy is possible to reach 30% [9-12].

HEV is a non-enveloped single-stranded positive-sense RNA virus with a diameter around 27—34 nm that belongs to the family *Hepeviridae*, covering two genera: *Orthohepevirus* (infect birds as well as mammals) and *Piscihepevirus* (infect fish). Further classification of genus *Orthohepevirus* results in four known species, they are *Orthohepevirus* A, B, C, and D. *Orthohepevirus* A includes at least eight distinct HEV genotypes (HEV-1 to HEV-8) infecting both humans and animals [13-15]. HEV-1 and HEV-2 are responsible for a wide-scale water-borne epidemics in developing countries [1, 6]. Meanwhile, HEV-3 and HEV-4 are more causing impacts of relatively small outbreaks in developed countries [1, 6]. The species causing HEV infection in swine belongs to the *Orthohepevirus* A [14]. The HEV-1 and HEV-2 are constrained to humans; HEV-3 circulates in humans, deer, rabbits, mongooses, and swine; HEV-4 circulates in humans, sheep, cow, goat, cattle, yak, wild boar, and pig; HEV-5 and HEV-6 are found in wild boars; HEV-7 has been identified in dromedary camel; and genotype 8 has been isolated recently in Bactrian camel [15-17]. **Figure 1** presents different HEV genotypes circulate in humans and animals.

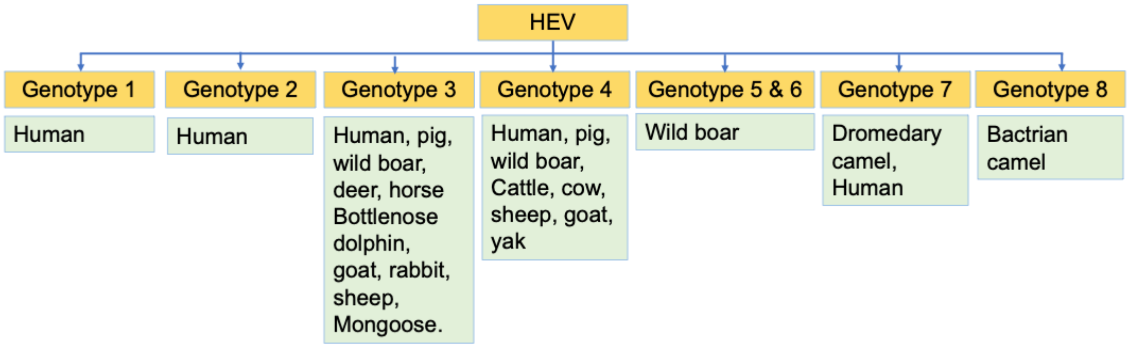

Figure 1. Orthohepevirus A genotypes and hosts

In developing countries, the HEV infection has caused 20-25% of acute hepatitis cases [18], and in the general population, the mortality ranges from 0.2 to 1% [15]. The viral infection is especially prevalent in Asia and Africa [18, 19]. In Africa, high number of HEV infection cases has been documented last year in animals with HEV genotypes closer to those infecting humans, where some animals may act as virus reservoirs suggesting the possibility of zoonotic transmission [20]. In developed countries, the HEV cases have been increased recently mainly owing to the consumption of undercooked meat, and is a silent threat which need much epidemiological investigations and appropriate mitigation strategies to be adopted [18, 21, 22]. In the present scenario, among European population, HEV is being considered as a growing zoonotic infection [23].

Autochthonous HEV causes considerable clinical problems in developed countries, where several animals including wild boar, wild deer and domestic swine act as reservoirs of HEV-3 and HEV-4 genotypes. Humans acquire HEV infections by consuming foods made of meat from infected animals that are not properly cooked, especially the liver [24]. Not much data are available with regards to HEV prevalence and contamination in food, especially in developing countries, and investigations on such issues therefore would elucidate a better understanding of food-borne impact of this important virus and its maintenance [25].

In most cases, the fecal-oral route is involved in the HEV transmission through ingestion of contaminated food and drinking water. HEV contamination of food sources and surface waters could occur through solid and liquid residues of infected animals, which act as zoonotic reservoirs. The food-borne transmission of HEV is primarily due to insufficiently cooked pig products, although living in close contact with animals or irrigation water are the known risk factors of HEV infection in both healthy as well as immunocompromised individuals [26-28]. The HEV may also transmit via other routes such as mother-to-child vertical transmission or blood transfusion [24, 29-34]. The transmission of HEV from person-to-person is infrequent [34-36]. HEV infection is an important public health issue and wide distribution of HEV in humans, animals and environmental as well as associated zoonotic concerns warrants the strengthening of One Health strategies to control and prevent HEV infection [3, 23, 25, 28-30]. This review discusses the importance of HEV infections and associated risk factors, with a particular focus on pig-associated transmission, zoonotic transmission, environmental contamination, and water-borne outbreaks.

HEV infection in livestock in particular pigs

HEV is a zoonotic virus that can be transmitted from animals to humans. Data suggested that the most significant reservoir for HEV genotypes responsible for infection in human is pigs [37, 38]. In 1997, the HEV was identified for the first time in pigs, and pigs are recognized as the main reservoir of HEV-3 along with HEV-4 [39]. Some studies have isolated and characterized HEV from pigs to evidence the infection of HEV and determined the prevalence of anti-HEV antibodies in livestock [40-84]. HEV infection in pigs normally occurs during early life, and the virus shedding peaks at about the age of 3 months old. People occupationally exposed to pigs have increased probability of the past HEV infection by almost threefold [51]. Other than pigs, anti-HEV antibodies have been identified from a wide spectrum of species such as cattle, dogs, goats, deer, donkeys, and sheep [37, 38, 44, 45].

Anti-HEV antibodies seroprevalence

Exposure to animal feces and intake of improperly cooked pork can cause HEV infection. Due to limited data in resource-deficient countries, a study evaluated whether pigs could potentially act as a source of HEV infections among Vietnamese population [46]. Liver specimens from domestic pigs and plasma specimens from pork-meat-exposed humans and pigs were compared with unexposed controls. The study found that persons occupationally acquainted with pork or pig meat had high seroprevalence compared to unexposed individuals. Approximately 12.3% of liver tissues from pigs were positive for HEV-3 suggesting that exposure to pork meat and pigs is a risk of HEV infection.

A cross-sectional investigation in Lao People's Democratic Republic revealed the prevalence of anti-HEV IgG antibody in slaughter pigs reached 54.0% (136/252), in professionals exposed to pigs was 41.0% (57/139), and 18.1% (38/210) was in non-risk controls [52]. Furthermore, contact with young piglets was a major risk factor as compared to contact with the older slaughter pigs [52]. Another recent study published from ten US states witnessed the prevalence of HEV-IgG antibody reaching 40% of 5,033 serum samples from market-weight pigs at 25 slaughterhouses, of which the HEV RNA was found in 6.3% with the virus belonging to HEV-3 genotype [53].

European countries studies also report a high prevalence of HEV-infected pigs [54-57]. In Germany, a total of 1,072 domestic pigs' sera were evaluated against anti-HEV specific antibodies and the overall seroprevalence was 49.8% [54]. Another study from Germany reported the overall seroprevalence of anti-HEV IgG in domestic pigs' sera of 68.6%. In the Netherlands, the overall anti-HEV seroprevalence in different kind of pigs raised on organic farms (n=417, 89%) was significantly higher than those raised on conventional farms (n=265, 72%) [58]. A study from Scotland found that the seroprevalence of anti-HEV specific antibodies were IgG (29%), IgM (29%), and IgA (36.9%) in slaughter-age pigs [56]. In Switzerland, HEV seroprevalence of 58.1% was documented in domestic pigs [57]. In Bulgaria, HEV seroprevalence in domestic pigs was 60% and the seroprevalence in slaughter-aged pigs was

73.6% [21]. A study found that 43.7% of 48 samples from Iberian pigs possessed anti-HEV antibodies [59].

Studies from other countries also reported a high prevalence of HEV in the pig population [21, 51, 58]. In China, the overall seroprevalence of anti-HEV antibodies was 64.7% in swine and the prevalence of HEV was higher in swine farmers than that of the general population [78]. A systematic review and meta-analysis of studies published within the last decade (2010 to 2019) reported a high prevalence of HEV infection among domestic pig population in China [79]. The study recommended to avoid mixed feeding of different stages as an effort to reduce the infection rate of HEV in pigs which could consequently reduce the risks of zoonotic transmission from pigs to humans [79]. In Singapore, serological as well as molecular characterization study of HEV in imported pigs within the years of 2000-2019 demonstrated the presence of HEV in live pig and post-slaughter samples [80]. This study suggested the importance of regular monitoring of the prevalent HEV strains and evaluation of the genetic diversity of HEVs in the imported pigs to further evaluate the association of the role of pigs on transmitting HEV to humans [80].

In one study conducted in Japan, of 160 serum samples collected from pigs, 72.5% (116/160) were IgG positive and IgM negative, while 23.8% (38/160) were positive for both IgG as well as IgM [81]. A recent study, anti-HEV IgG seroprevalence reached 66% in slaughterhouse staff, 51% in pig-farmers, and 38% in pork meat vendors [46] and those who had exposure to pigs or pork meat had high chances of HEV infection when compared with unexposed individuals [46]. A study from Abruzzo, Italy, a reportedly hyperendemic region having the highest HEV seroprevalence in humans, out of 233 blood samples collected from different local pig slaughterhouses between 87.3% and 100% of serum samples were found positive for anti-HEV antibodies (IgG), such high seroprevalence in pigs suggests the intense circulation of HEV in the region [82]. Previously published studies showed that pigs successively receive HEV infection on farms, where a higher prevalence was found among older pigs [26, 83, 84].

HEV RNAs prevalence

Constant contact and consumption of pig and pig products increase risk and hence warrants proper inspection and surveillance [59, 60]. Raw viscera have more feasibility to harbor HEV in comparison with pork, where ground pork and pig liver could act as a potential source of HEV [60, 61]. In one study, HEV RNAs with positivity values of 3.1% (4/129), 6.1% (7/114), and 1.2% (2/170) were found in renal, hepatic, and blood samples, respectively, suggesting higher transmission risk of consuming pig organs [61]. Similarly, investigation in Southern Italy, found that 99 pork and 63 wild boar sausages and salami collected from the market have been detected with HEV belonging to HEV-3 genotype [62]. However, the mere presence of HEV in samples may rarely matter than the infectivity of HEV and there should be a robust system to evaluate the infectivity of HEV in food samples. In this regard, cell culture-based systems are reported to have promising prospects [63].

A study concluded that HEV RNA in pigs and retail pork livers in Canada was comparable to that of Europe and the USA [64]. In Brazil, HEV RNA has been detected in 0.8% (6/713) of serum samples from backyard pigs in southern part of the county and falling in three different genotypes related to human HEV strains indicating backyard pigs as a reservoir of HEV and thus in need for infection and spillover control from backyard farms [65].

In a small study from Switzerland, HEV RNA was detected in 11.1% (10/90) meat products, 18.9% (7/37) liver sausages, and 5.7% (3/53) raw meat sausages with viral loads of up to 5.54 $\log_{10}$ genome copies per gram and the viruses belonged to HEV-3 [66]. The foregoing study indicates the HEV contamination in ready-to-eat meat products on the retail market, hence the necessity for developing efficient diagnostic methods for easy detection [66]. A study that detected and quantified HEV RNA in ready-to-eat raw pork sausages in the Netherlands found that 14.5% (46/316) samples were positive for HEV RNA with the average viral load was 2.76 to 4.5 $\log_{10}$ genome copies per 5 grams [67]. These findings act as the basis for risk assessment and risk management with respect to pork and pork product consumption and raw pork sausages as a risk factor for HEV infection among Dutch population [67]. Various ingredients related to pigs and piggeries are also considered as a possible risk for HEV transmission. Transmission of HEV also has been identified in slurry samples from swine farming activities in Italy where 75% (18/24 samples) were positive containing HEV RNA [68]. Therefore, strategies of improving the safety standard in swine farming and educating individuals involved in pork meat production on the zoonotic risk could minimize risk for HEV infection in human.

In Bangkok, HEV RNA was observed in 0.2% of fresh meat in the markets and 3.9% in the slaughterhouse samples [69]. Fecal and bile samples were found more common than liver, pork, and intestine samples hence posing a risk of zoonosis. A study in Estonia found that domestic pigs, wild boars, pig farmworkers, and hunters were infected with HEV and this indicates the important attention for direct contact between the animals and the persons or handlers [70]. Both direct contact and environmental contaminations could contribute to not only HEV transmission in livestock but also in humans [71].

The number of viral particles existing in the environment has been considered significant during the transmission process [71]. Transmission via porcine blood ingredient during meat production is found feasible [72]. Positive results for HEV RNA were observed in 91.6% (33/36) batches of liquid products from a non-heating process and 29.1% (7/24) batches of powder products from a spray-drying process [72].

A study from Finland reported that 11.9% of domestic pigs were HEV RNA positive. HEV contamination was found in fattening pigs and weaning pigs, where the occurrence was more common in the first than the latter (14.8% vs. 12.5%) [73]. A study in Italy found that 64.6% pigs were positive for HEV RNA in at least one sample, and the HEV genome was detected in 51.1%, 33.3%, and 20.8% of bile samples, feces and livers, respectively [74]. Another study from four abattoirs in Italy revealed a high seroprevalence (76.8%) of anti-HEV antibodies in pigs using ELISA and 3.6% (21/585) were found HEV RNA positive in either fecal or hepatic samples by real-time RT-PCR [75]. This study also found circulation of HEV-3 and a novel unclassified HEV subtype was noticed from phylogenetic analyses [75]. A study from Denmark reported fecal samples of 49.5% Danish pig population to be positive for HEV RNA [76]. In France, the farm-level HEV seroprevalence was 65%, and 31% in the slaughter-aged pigs [77]. Furthermore, 4% of pig livers were HEV RNA positive [77]. The prevalence of HEV RNA in different samples from previous studies is presented in **Table 1**.

The cross-species transmission is of importance since it dominates the cases of HEV infection in developed countries [49, 50]. The involvement of other animal species in HEV transmission complicates control of the infection. In Spain, more than 10% samples from wild boar and 16% samples from red deer were positive for HEV RNA and approximately 57% samples of wild boar and 12.8% samples from red deer were positive for anti-HEV antibodies [59].

Altogether, there are strong evidence that indicate the prevalence of HEV in the livestock in particular pigs is high in both developed and developing countries. This poses high risk of zoonotic transmission from pigs to humans. Therefore, the regulations of farm, slaughterhouses, pork meat industries need to be strengthened. In addition, animal models for HEV infection might be important for further studies [85-87].

HEV in environmental samples

The persistence and transmission of HEV in the environment are currently underreported [88]. HEV-1 and HEV-2 are mostly found in places with limited resources as well as low sanitation leading to contamination of water supplies and food [1, 16, 17]. In India, for example, the HEV outbreaks were caused by highly polluted water between 1978 and 2013 [24]. Previously, the role of water in the HEV transmission has only been suspected without confirmation. However, detection of HEV-3 in shellfish and multiple shellfish-related outbreaks have recently triggered the discourse on water-borne HEV outbreak among scientists [89, 90].

Table 1. The prevalence of IgG, IgM and HEV RNA in different pig samples around the globe

Antibody	Country	Animal	Sample	Positive	Total tested	Prevalence (%)	Reference
IgG							
	Lao PDR	Slaughter pigs	Serum	136	252	54.0	[52]
	US	Market-weight pigs at slaughterhouses	Serum	2014	5,033	40.0	[53]
	Germany	Pigs	Serum	354	516	68.6	[55]
	Germany	Pigs	Meat-juice	134	198	67.7	[55]
	Scotland	Slaughter-age pigs	Serum	51	176	29.0	[56]
	Bulgaria	Domestic pigs	Blood	260	433	60.0	[21]
	Bulgaria	Slaughter-aged pigs	Blood	245	333	73.6	[21]
	Spain	Iberian pigs	Serum	21	48	43.8	[59]
	Germany	Domestic pigs	Serum	534	1072	49.8	[54]
	Netherlands	Pigs raised on organic farms	Serum	37	417	8.9	[58]
	Netherlands	Pigs raised on conventional farms	Serum	191	265	72.1	[58]
	Netherlands	Pigs raised on free-range	Serum	124	164	75.6	[58]
	Switzerland	Domestic pigs	Serum	1163	2,001	58.1	[57]
IgM							
	Germany	Pigs	Serum	36	516	7.0	[55]
	Scotland	Slaughter-age pigs	Serum	51	176	29.0	[56]
RNA							
	Vietnam	Domestic pigs	Pig liver tissues	26	210	12.4	[46]
	US	Market-weight pigs at slaughterhouses	serum	318	5,033	6.3	[53]
	Scotland	Slaughter-age pigs	Serum	72	162	44.4	[56]
	Spain	Iberian pigs	Serum	0	48	0.0	[59]

Studies from developed countries also have showed a high HEV seroprevalence in individuals exposed to water or those who consume shellfish [91, 92]. Besides the evidence of HEV infection in humans via the intake of seafood (mussels and shellfish), captive dolphins, which generally feed on fish, have been reported to be positive for anti-HEV antibody and HEV RNA (genotype 3) [93], in which this finding raises concerns of environmental contamination of food or wastewater as a source of HEV.

To date, the waterborne HEV-3 transmission remains to be elucidated. A current study in Germany recognized that work-related contact with wastewater can be associated with autochthonous hepatitis E, indicating the possible role of water in transmitting HEV-3 [94]. Animal and human hosts of HEV-3 might pollute wastewater matrices by the secretion of feces. The release of HEV particles to the environment can pollute surface waters, which could possibly be an HEV infection source for animals and humans [88].

HEV has been identified in urban sewage samples in various countries such as in Spain, Italy, and the Netherlands [95-97]. Recently, several studies also have been conducted to determine HEV in urban sewages in European countries [98-106]. In Germany, wastewater samples were found positive by RT-qPCR for genotypes HEV-3c and 3f, where HEV-3c was identified as the most dominant genotype [107]. In addition, approximately 75% of samples from the urban river showed the detection of HEV RNA [107]. In another study, HEV prevalence was monitored in effluent and influent water in drinking and wastewater treatment plants (WWTPs) [108]. The data suggested that the prevalence of HEV in inflowing water samples varied based among WWTPs but no HEV was identified in effluent water [108]. A large-scale of nine years-study (2011-2019) was conducted that covered 48 different WWTPs in 20 distinct regions of Italy [106]. Out of 1,374 sewage samples, among 74 samples detected with HEV RNA, 56 and 18 of which belonged to HEV-3 and HEV-1, respectively. HEV-3 strains were detected throughout the investigation period while HEV-1 strains was detected only in 2011-2012 suggesting that HEV-3 was the prevalent genotype [106]. In a 5-year integrated surveillance that included environmental and human, 169 HEV cases were confirmed with an annual occurrence of 0.72 cases/1,000,000 [105]. Among 65 HEV RNA-positive samples, 66%, 32%, and 1% were detected to be HEV-3, HEV-1, and HEV-4, respectively [105]. A study of water samples from the Arias–Arenales River in Salta city, Argentina found that HEV RNA was detected in 1.6% of the tested samples where the viruses belonged to HEV-3 while the prevalence of anti-HEV IgG was 9% [109]. A study analyzing 250 water and 68 sediment samples from the Sinos River, Brazil along with 50 pork products sold around the river found that HEV-3 was identified in 36% of food samples and HEV was not identified in water or sediment [110].

Altogether, there are robust evidence to demonstrate that HEV is widespread in both developed and developing countries indicating a significant threat to public health worldwide. Zoonotic-transmission has caused HEV infections in humans in the developed nations and this requires the development of vaccines to prevent the spread to humans [111]. A study since the 90s in European countries clearly indicated that HEV frequently infects the European communities, and some animal species such as deer, wild boars, and pigs serves as reservoirs for the virus [22]. Identification of virus in polluted pork products and mussels suggesting the transmission of HEV strains from water to food chains.

HEV in water and water-borne outbreaks

Genotypes 1 and 2 of HEV are mainly reported in Asia including Afghanistan, India, Bangladesh, China, Nepal, and Pakistan, some African countries such as Chad, the Central African Republic, Nigeria, and Sudan, as well as Mexico [112]. Analysis of drinking and sewage water samples from Faisalabad, Pakistan showed the presence of HEV-1 strain [113]. In Italy, HEV-1 and HEV-2 were identified in sewage water samples from WWTPs [96]. In Spain, HEV genome was detected from slaughterhouse sewage mainly from pigs and the viruses had 92-94% nucleotide similarity with those strain detected from the humans [114]. A high positivity rate of 20/46 (43.5%) of HEV RNA was also reported in sewage samples from Barcelona, Spain [115].

Another study from Spain showed the presence of both HEV-1 and HEV-3 genotypes in water samples from WWTPs [116], and sewage [117]. In Colombia, of the total sixty environmental samples, the HEV genome was detected in 23.3% (7/30) of drinking water plants/creek and in 16.7% (5/30) of sewage samples [118]. HEV-1 and possibly HEV-2 have caused several water-borne outbreaks in many parts of the world [3, 24, 119].

HEV prevention strategies

Considering the importance and wide circulation of HEV in humans and animals, global public health priority needs to be given for enhancing monitoring and surveillance as well as adopting adequate mitigation strategies for evading and managing HEV and its associated zoonotic significance [120]. HEV 239, Hecolin® vaccine (Xiamen Innovax Biotech, China) is purchasable in private market in China, but it has not received WHO approval for its use in endemic settings and disease outbreaks worldwide. In HEV outbreaks, the two important preventive approaches comprise the provision of drinking clean water and improving the sanitary disposal of human waste. Implementing these approaches in a timely manner in regions where the HEV epidemic occurs is a challenging issue [121].

Globally, the anti-HEV antibodies (IgM and IgG) seroprevalence has been recorded both in humans and animals, and HEV RNA also has been identified from environmental samples. Some contributing factors on the increasing HEV infection rate include low socioeconomic status, poor hygiene, low access to clean water, lack of proper sanitation, and the lack of commercial access to a hepatitis E vaccine especially in the high endemic regions. Global availability of the effective vaccine to tackle future HEV outbreaks, larger analysis of magnitude of the worldwide burden, improving diagnostics and epidemiological methodologies, improving standards of water quality, hygiene, and sanitation in endemic regions along with implementation of One Health approach are needed for effective prevention and control of HEV. Awareness with regards to the prevalence and spatial distribution of HEV in livestock animals, especially in pigs and strengthening of HEV testing in boars, along with controlling environmental contamination of the virus could play vital role in implementing appropriate prevention and control strategies to avoid the animals-to-humans transmission of HEV.

Conclusion and future prospects

In the past, the HEV was considered an infection of developing countries only. But after the discovery of new HEV genotypes, different animal reservoirs, the cross-species transmission have changed the understanding of HEV worldwide and it became a significant global public health problem. Since a few years ago, the cases of HEV have been increased in developed countries, which are mainly transmitted due to the consumption of uncooked food. However, other modes of transmissions also contribute significantly.

Genotype 1 and 2 of HEV are responsible for large outbreaks in low-income countries, while HEV-3 and HEV-4 are mainly responsible for sporadic cases in the developed countries. In developed countries, exposure to animal feces and consumption of undercooked pork meat could have cause significant number of hepatitis E. Therefore, the cross-species transmission is considered the leading cause of HEV infection in the developed countries. Identification of virus in polluted pork products and mussels indicate the circulation of HEV strains from water to food chain, and thus necessitate a careful assessment of swine herds and food safety. Although the waterborne HEV-3 transmission remains uncertain, the occupational contact with wastewater might be associated with autochthonous hepatitis E, suggesting the possible role of water in transmitting HEV-3. HEV has been identified in urban sewage samples in countries around the globe.

A better understanding of the viral dynamics and disease progress by developing models that are amicable to natural host or disease pathology to support future research and minimize risks. Special attention should be paid to vulnerable and high-risk groups including pregnant women. In the absence of specific knowledge and lack of interventions, general preventive measures can be helpful. Safety measures and disease awareness are of worth importance while traveling to HEV endemic areas. There is a dire need for effective treatment and commercially available vaccines to prevent and control HEV infection with particular emphasis on low-income countries as well as adopting the concept of One Health approach.

Ethics approval

Not required.

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Conflict of interest

All the authors declare that there are no conflicts of interest.

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Underlying data

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