Risk Assessment Studies
Report No. 44

Microbiological Hazard Evaluation

HEPATITIS E VIRUS IN FRESH PIG LIVERS

December 2010
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EXECUTIVE SUMMARY

A rising trend of hepatitis E cases was noted in recent years and local data on the potential food sources are scarce. There is growing evidence suggesting that hepatitis E virus (HEV) can be transmitted from animals, particularly pigs. Presence of HEV in pigs has been reported in both developed and developing countries. This study aims to give an overview of the prevalence of HEV in fresh pig livers available in local market. An attempt was made to see if pigs were potential sources of local human hepatitis E cases. Partial sequences of HEV isolated from pig livers were compared with those isolated from human cases.

Study on HEV in fresh pig liver

During mid-January to May 2009, the Centre for Food Safety (CFS) obtained a total of 100 fresh pig liver samples from pigs slaughtered in local slaughterhouse. Around half of them were collected from roaster pigs (around four months old) and the other half were collected from porker pigs (around six months old). The majority of live pigs imported to Hong Kong are porker pigs. Detection of HEV was conducted by the Public Health Laboratory Services Branch of the Centre for Health Protection (CHP), Department of Health.

Among the collected samples, 16 out of 51 (31%) roaster liver samples were found positive for HEV test, whilst none of the 49 porker liver samples were found positive. The positive rates were 22% (6/27) and 42% (10/24) for roaster pigs sourced from farms in two regions in Mainland China respectively. Partial ORF2 sequences of some HEV isolates from roaster pigs were the same as those from seven local human

cases with onset date within January to July 2009 as well as local cases recorded in the past. This suggests the possibility of pigs as one of the sources of human hepatitis E cases. On the other hand, isolates from seven out of 48 human cases were found to have same sequences as HEV isolates from pig samples. For these seven human cases, only three persons could recall the consumption of pig offal during the incubation period. Other sources for these local hepatitis E cases may exist.

Repeated occurrence of HEV positive samples was observed in some farms collected with multiple samples. The farm environment may be the source of HEV in pigs. However, specific risk factors for on-farm and between farm transmission of HEV are not available for interpretation. Inspecting every pig from farms positive for HEV may not be useful as infection by HEV is not uncommon and adult pigs have usually recovered from HEV infection. Recommendation on farm control for HEV is not available from World Organisation for Animal Health (OIE).

Conclusion and Recommendations

Majority of the pigs imported from Mainland China were not found with HEV; the virus was only detected from liver of roaster pigs, i.e. around four months old. Apart from contaminated water or food such as raw or undercooked shellfish which are known to be the main sources of hepatitis E infection, the present study suggests that roaster pigs are one possible source of local human hepatitis E infection. While there is evidence suggesting the transmission of HEV from pigs, food safety measures may help to prevent the HEV infection.

It is recommended to cook the pig meat and offal thoroughly to minimise the risk of contracting HEV as well as other foodborne pathogens. In addition, members of the trade and the public should always observe good personal and food hygiene.

Advice to trade

- Wash utensils and worktops with hot water and detergent after each use.
- Use separate utensils to handle raw food (including raw meat and offal) and cooked food or ready-to-eat food e.g., use different colours codes for different utensils (including cutting boards and knives).
- Cook pork and pig offal thoroughly before consumption.
- Wash hands thoroughly with running water and soap for 20 seconds before handling food and often during food preparation, after handling raw meat or offal and after handling soiled equipment or utensils.

Advice to public

- Keep raw food including raw meat and offal separate from other food items in your grocery cart and shopping bags to prevent their juices from contaminating other food items.
- Wash utensils and worktops with hot water and detergent after each use.
- Use separate utensils to handle raw food (including raw meat and offal) and cooked food or ready-to-eat food e.g., use different colours codes for different utensils (including cutting boards and knives).
- Cook pork and pig offal thoroughly before consumption.

- When having hotpot, use separate chopsticks and utensils for handling raw and cooked foods to prevent cross-contamination.
- Wash hands thoroughly with running water and soap for 20 seconds before handling food and often during food preparation, after handling raw meat or offal and before eating.

OBJECTIVES

The objective of this study is to assess the prevalence of HEV in livers of locally slaughtered pigs and to determine the genetic relationship between HEV identified from pigs and human hepatitis E cases in our locality.

INTRODUCTION

2. Viral hepatitis is the inflammation of liver caused by virus. The disease is mainly caused by five different hepatitis viruses known as types A to E, of which types A and E are related to contaminated food or water.

3. Symptoms of hepatitis E are similar to those of hepatitis A such as fever, malaise, anorexia, nausea, abdominal pain, dark urine and jaundice. The incubation period for hepatitis E is longer and ranges from two to nine weeks. The disease usually is self-limiting and resolves in two weeks, leaving no sequelae, except for pregnant women where hepatitis E may result in serious complication such as death of the mother and fetus, abortion, premature delivery, or death of baby soon after birth.[1,2] In addition, the disease can cause adverse outcome in patients with preexisting chronic liver disease (CLD).[3-5]

4. Hepatitis E is caused by hepatitis E virus (HEV) and four main genotypes of mammalian HEV have been identified, which are characterised by their geographical distribution and host range.[2,6,7] Genotype I has been found throughout Asia, North Africa, and South America and suggested to be the major cause of water-borne epidemics and significant sporadic disease. Genotype II has been found from patients in Mexico, central Africa, and Nigeria. Genotype III has a wide prevalence in pig population worldwide and has been isolated from sporadic human cases in developed region such as United States and several European countries. Genotype IV is found mainly in Asian countries, including China, Japan, Taiwan and Vietnam and this genotype has been found from humans and domestic pigs. Both humans and pigs have been detected with genotypes III or IV HEV. In addition to these four main genotypes, possibly novel genotypes of HEV have also been reported in chicken, rex rabbit, and wild rats respectively.[8-10] HEV has also been detected in wild boar, deer, mongoose and shellfish.[11-13] Other animals may have contracted HEV, but the virus has not been identified in these animals.

5. Detection of antibodies against HEV (anti-HEV) has been reported in different kind of domestic and wild animals, such as dogs, cattle, goats, ducks, pigeons, horses, and rats, etc.[6,14-16] The anti-HEV are produced by immune system in response to contract with HEV, which means the animals may have contracted with HEV before. However, the virus has been rarely detected in these animals. For pigs, several studies in Mainland China reported higher prevalence of anti-HEV in comparison with other domestic animals.[14,15,17,18] Prevalence of anti-HEV in pigs has also been reported in other regions such as United States, United Kingdom, Canada, Australia, New Zealand, Japan, Thailand, and

Korea.[19-27] Contracting HEV is common in pigs and may occur more commonly in pigs than in other domestic animals.

6. As for detecting the HEV in pigs, a number of studies reported the detection of HEV in younger pigs. In an analysis of serum samples collected from commercial farms in 20 prefectures in Japan, HEV was detected in 6%, 10%, 6%, and 0.5% samples from two, three, four, and five months old pigs respectively, whereas none were found positive in one and six months old pigs.[25] In southern France, 65% of three months old pigs in a farm, sourced from different regions, and none of six months old pigs in a slaughterhouse were found HEV positive.[28] Around a quarter of samples from two months old pigs and around 6% of samples from three months old pigs, but none of the samples from pigs of other age group were positive for HEV in two principal swine farming areas in Thailand.[26] More than half of 97 pig farms in Netherlands were positive for HEV in the pooled faecal specimens of pigs from five to 27 weeks old in the farms.[29] Studies in different regions in Mainland have also found higher positive rate of HEV in younger pigs.[14,17] These imply that HEV is acquired by and disseminated among pigs during younger age.

7. Viral load and serological status of the pigs infected with HEV have also been studied. Pigs naturally infected with the swine HEV virtually present no symptom and appear clinically normal, but they do show anti-HEV, viremia and virus excretion. As demonstrated under experimental conditions, sero-conversion (antibodies production) occurs about 18 to 20 days post infection; virus is excreted in faeces for three to four weeks and is present in pigs for about one to three weeks.[19]

8. Some evidence suggests that the sporadic hepatitis E cases in

developed countries are zoonotic, in which compared with travel-related cases, the human cases showed the closest genetic homology to pig strains from the same region.[6] Live pigs in Hong Kong are mainly imported from Mainland China. Similar to overseas countries, there are also reports on detection of HEV in pig farms or abattoir in Mainland China.[6,14,15,17,18,30-36] The possibility of local population contracting the HEV from pigs may exist.

9. In recent years in Hong Kong, there was a rising trend of hepatitis E and a record high notification of 90 cases was noted in 2008 and a total of 73 cases were notified in 2009.[37,38] Apart from seasonal peak in March and April, there were also more cases recorded in January.[38,39] In an analysis of the 51 cases recorded by the Centre for Health Protection (CHP) in the first four months of 2008, some patients consumed raw or semi-cooked food such as shellfish (17 cases, 33%) or pig offal (13 cases, 26%) during the incubation period, but the sources of infection have not been identified from the food histories. HEV infection has been considered as a travel-related disease, where transmission in high endemic regions is generally via faecally contaminated water.[40] However, 65% of the 51 cases did not have history of travel to Mainland China or south Asian countries during the incubation period, they seemed to have contracted the virus locally.[39] Therefore attention is drawn to the potential of the presence of the causative agent, HEV, in food available in our locality.

10. Local data on HEV in food are scarce, while foodborne transmission through consumption of raw or undercooked meat or offal from deer and wild boar has been documented in Japan.[13,41] Prevalence of HEV in commercial pig liver has been found to range from 1.9% to

11% in some overseas studies.[42-44] Close genetic identity between the HEV isolates recovered from the patients and those from the pig liver samples has also been documented in Japan.[42] A local study comparing epidemiology and clinical features of sporadic hepatitis E and hepatitis A showed that significant higher proportion of hepatitis A cases had a recent history of taking shellfish, only travel to endemic area was identified as a risk factor for hepatitis E.[45] It seems that shellfish may not be a significant risk factor for hepatitis E as compared with that for hepatitis A. Previous examination of bivalve shellfish also did not show that they were the major vehicle locally.[46] However, HEV may survive in pig meat or offal, or shellfish if undercooked in some cases such as hot pot or congee cooking. Although contaminated water may be a potential source, local people generally drink boiled water and quality of water supply in Hong Kong is well monitored by Water Supply Department.[47] Two recent studies analysed HEV identified from local human cases, both suggested the exploration of potential of zoonotic transmission of HEV, in particular from pigs. One of the studies also reported that most local cases were related to isolates of HEV from pigs in Mainland China.[46,48]

11. The prevalence of HEV in locally slaughtered pigs is not known, and risk of exposure through pig offal or other pig products cannot be estimated. HEV is one of the emerging viruses identified as the viruses of primary concern in terms of foodborne transmission in recent microbiological risk assessment by the World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO).[41] As stated in the report, emerging viruses should be monitored, particularly when new problems arise, in an effort to assess the potential for foodborne transmission.[41] As such, this study was conducted to provide an overview of the prevalence of HEV in fresh pig livers

available in local market. In addition, in an attempt to see if pigs are potential sources of local human hepatitis E cases, partial sequences of HEV isolated from pig livers and that from human cases were compared.

SCOPE OF STUDY

12. Fresh liver from pigs slaughtered in local slaughterhouse was the target sample. All samples were collected from Sheung Shui Slaughterhouse, since around 80% of all pigs from different sources in Mainland China and local farms are processed by the slaughterhouse.

METHODOLOGY

Sampling

13. The sampling was conducted from mid-January to May 2009, because more human cases were recorded in the first few months of the year.[38]

14. Pig liver samples were collected from slaughterhouse by Slaughterhouse (Veterinary) Section under the CFS. The age and the origin of the pig, i.e. regions in Mainland China or Hong Kong, were recorded. Two types of live pigs of same species, i.e. roaster (around four months old) and porker (around six months old), are imported and slaughtered in Hong Kong and the porker is the majority. They are virtually different in age and the offal from both porker and roaster may be sold for consumption. Around ten porker and roaster liver samples were collected respectively in each month. Officers were at liberty to choose pigs for sampling; however, they should, as far as possible, take

samples from pigs imported from different farms in Mainland China in the same day and include one sample of porker from farms in Hong Kong in each month.

15. From mid-January to May, 49 porker liver samples were collected from six regions (Jiangxi, Henan, Hubei, Hunan, Guangdong, and Guangxi) in Mainland China and from local farms. On the other hand, 51 roaster samples were collected from pigs sourced from Shanghai and Guangdong, while no samples from other regions in Mainland China were available for collection during the sampling period. It seems that Shanghai and Guangdong are the main sources of roaster imported to Hong Kong. Breakdown statistics on farms that produce porkers or roasters are not available. The farms sampled and the number of samples taken are summarised in Table 1.

Table 1. Summary on samples collected in this study and live pigs import statistics in 2009

Regions	Porker samples		Roaster samples		Live pigs import /supply (2009)	
	No. of farms	No. of samples	No. of farms	No. of samples	No. of farms	No. of heads
Shanghai	N/A	N/A	4	27	6	18,461
Zhejiang	N/A	N/A	N/A	N/A	6	42,861
Fujian	N/A	N/A	N/A	N/A	2	4,238
Jiangxi	4	4	N/A	N/A	20	267,588
Henan	7	7	N/A	N/A	26	155,600
Hubei	4	4	N/A	N/A	13	74,929
Hunan	3	3	N/A	N/A	29	95,914
Guangdong	24	25	12	24	84	898,032
Guangxi	1	1	N/A	N/A	7	30,346
Hainan	N/A	N/A	N/A	N/A	3	6,411
Chongqing	N/A	N/A	N/A	N/A	1	2,993
Local	4	5	N/A	N/A	43	84,655
Total	47	49	16	51		

Note: N/A – Not applicable

Laboratory analysis

16. All samples, being kept at 4°C or below in viral transport medium, were submitted to the Public Health Laboratory Services Branch of the CHP, Department of Health for analysis. The samples were kept under -80°C until further processing. For positive samples, the partial Open Reading Frame 2 (ORF2), which was used previously by CHP to study human hepatitis E cases in Hong Kong, was sequenced and compared with the sequences of HEV isolated from human cases with onset date during January to July 2009 and sequences on Genbank database (http://www.ncbi.nlm.nih.gov/).[46] The determination of genotype was performed in accordance with the study of CHP.

Result analysis

17. Analysis on the genetic relationship between the HEV isolates from pig liver samples and human cases were performed by CHP. Results of the HEV detected in pig liver were analysed by the Risk Assessment Section of the CFS.

RESULTS

Prevalence of HEV in locally slaughtered pigs

18. Among the collected samples, 16 out of 51 (31%) roaster liver samples were found positive for HEV test, whilst none of the 49 porker liver samples were found positive. The positive rates were 22% (6/27)

and 42% (10/24) for pig samples sourced from Shanghai and Guangdong respectively. Two out of the four farms in Shanghai and seven out of the 12 farms in Guangdong sampled were found positive for HEV in one or more samples.

19. In addition, two porker samples which were taken from two farms (GD11 and GD19) in Guangdong with positive roaster samples were negative for the HEV.

Occurrence of HEV in roaster liver samples

20. During the sampling period, there were positive roaster liver samples found in each month with the highest positive rates in May, followed by January as shown in Table 2.

Table 2. HEV positive rate of roaster liver samples by month

Month	No. of Samples Positive/Tested (%)
January	3/8 (38%)
February	3/13 (23%)
March	2/10 (20%)
April	3/10 (30%)
May	5/10 (50%)

21. Consecutive samples from each farm in each month were not available due to limited sample size, however, repeated occurrence of HEV positive samples was observed in some farms collected with multiple samples. Nine farms were found with positive samples and seven of them were collected with more than one sample in different months (GD07, GD11, GD12, GD13, GD18, SH01, and SH03). Among

these seven farms, four (GD11, GD13, SH01, and SH03) of them were found with positive samples again within the sampling period.

Genetic relationship between HEV found in pig livers and identified from human cases

22. Partial ORF2 of HEV from all the 16 positive roaster samples was sequenced for determination of genotype. Sequence analysis of these samples showed that all the isolates are genotype IV.

23. In an attempt to see if the pig liver is a potential source of HEV of local cases, partial sequences of HEV isolated from human hepatitis E cases were compared with those of isolates found in the pig liver samples. According to the data of local human hepatitis E cases from CHP, there were 48 cases with onset date within January to July 2009 recorded. Seven (Samples 1 to 7 in Table 3) of them were found to have same partial sequences as those from pig samples in this study by CHP. Among the seven cases, three of them recalled that they had consumed pig offal, i.e. intestine or liver and onset dates of two out of the three cases fell within the sampling period plus expected incubation period of hepatitis E. For the other cases, one did not provide the food history and the other three had consumed pork and/or bivalve shellfish during incubation period. As shown in Table 3, four clusters of isolates from patients and pig samples, i.e. HEV isolates matched with same partial sequences, were identified. It is also noted that some HEV isolates within the same clusters were collected from pigs sourced from different regions.

24. In addition, partial ORF2 sequences of some HEV isolates in this

study were also found to be the same as that of isolates from human cases (Table 3) and animal specimens deposited on Genbank database. Sequences from HEV isolates of two previous humans cases (HK117-2006 and HEV/HK/2007/4308) in 2006 and 2007 respectively were found to be same as that of pig samples (V09-046 and V09-069) as well as four local human cases recorded with onset date within January to July 2009 (Sample 2 to Sample 5). Both pig samples were taken from pigs sourced from the same farm in Guangdong. Furthermore, isolate from another pig sample V09-061 was also found to be the same as that from a pig bile sample collected in northwest China (ZJKS56).

Table 3: Genetic relationship between the HEV isolated from pig liver samples, HEV isolated from human cases, and HEV sequences from Genbank database

	Sample no.#	Month◊	Source	Farm*	Remark/ Accession no.
Samples that were found to have same partial ORF2 sequence					
Cluster 1	Sample 2	Feb	Human	/	This study
	Sample 3	Mar	Human	/	This study
	Sample 4	Apr	Human	/	This study
	Sample 5	May	Human	/	This study
	HEV/HK/2007/4308	/	Human	/	FJ217978[46]
	HK117-2006	/	Human	/	FJ438454[48]
	V09-046	Feb	Pig	GD13	This study
	V09-069	Mar	Pig	GD13	This study
Cluster 2	Sample 7	Apr	Human	/	This study
	V09-058	Feb	Pig	GD11	This study
Cluster 3	Sample 6	Apr	Human	/	This study
	V09-035	Jan	Pig	SH03	This study
	V09-082	Apr	Pig	SH03	This study
	V09-116	May	Pig	SH03	This study
	V09-117	May	Pig	GD18	This study
Cluster 4	Sample 1	Jan	Human	/	This study
	V09-003	Jan	Pig	SH01	This study
	V09-079	Apr	Pig	SH01	This study
	V09-110	May	Pig	GD07	This study
Other samples that are not in the clusters above					
	V09-005	Jan	Pig	GD08	This study
	V09-055	Feb	Pig	SH01	This study
	V09-061	Mar	Pig	GD19	This study
	V09-097	Apr	Pig	GD13	This study
	V09-099	May	Pig	GD11	This study
	V09-108	May	Pig	GD12	This study

Notes:
#: "V09" – coding for pig liver samples
"Sample" – coding for human isolates
Please refer to the text for the others
◊ : Sample collection
*: "SH" – coding for farms in Shanghai
"GD" – coding for farms in Guangdong

DISCUSSION

25. In this study, genotype IV HEV was found in fresh pig liver samples collected from roaster pigs, i.e. around four months old. On the other hand, some isolates from pigs and human cases were found to have same partial ORF2 sequence. Sources of pigs of the said isolates were not limited to same farm as well as same region.

Prevalence in Pigs

26. HEV is widespread in pig population in different regions, which is shown by the high prevalence of anti-HEV.[19-27] Studies in northeast China, Japan, Thailand, and Midwestern United States also found increase in prevalence of anti-HEV in pigs after two to three months of age.[17,24-26,49] Several studies in Mainland China also reported anti-HEV positive rate in pigs with a range from 54% to more than 80% depending on age groups tested.[14,15,17,18] As shown in Table 4, HEV has been detected in different regions in Mainland China as well as in overseas countries. Although detection of anti-HEV in pigs is not included in this study, it is believed that for live pigs imported to Hong Kong it may not be uncommon for them to have contracted HEV during young age. The virus may be present in pigs for some period of time, i.e. one to three weeks as demonstrated under experimental condition.[19] Production of anti-HEV following the infection may provide protection to the pigs against further infection by HEV and the infected pigs recover without showing symptoms. As can be seen in this study, HEV was only detected in liver of roaster pigs, but none were detected from liver of porker which is around six months old.

27. The roaster samples collected in this study were sourced from two regions in Mainland China, namely Shanghai and Guangdong. No roaster samples were taken from other regions in Mainland China, it appears that these two regions are the major supplier of live roaster to Hong Kong. Although positive samples were only found in samples from these two regions, presence of HEV in pigs is not limited in these two regions. As shown in Table 4, detection of HEV has also been reported elsewhere in Mainland China as well as in overseas countries.

28. The positive rate (31%) was higher than that in previous studies in Shanghai and Guangdong as well as some other regions as shown in Table 4. One of the studies reported a positive rate of around 26% among farms located in Shanghai suburban districts and the prevalence of HEV varied from 0 to 41.7% within different districts. However, the age of pigs sampled was not known.[33] The positive rate may be quite variable among different farms.

29. Furthermore, different target age groups of the pigs and the types of samples collected, i.e. liver samples were collected in this study while faecal or serum samples were collected in previous studies in Shanghai and Guangdong, may result in different positive rates. It has been suggested that, in comparison with serum or faeces, higher detection sensitivity was achieved using samples such as liver, mesenteric lymph node and bile, possibly due to the fact that these organs were the site where the virus replicates and accumulates.[50]

Table 4: Some recent studies on HEV prevalence in samples collected from pigs in Mainland China and overseas

Regions	Pig ages and sample locations	Sampling time	Type of Samples	Positive rate	Genotype
Shanghai and Guangdong					
Eastern China(Deqing in Zhejiang Province, another one near Shanghai)[30]	Swine farms	2002 and 2004	Faecal samples	9.6% (27/282)	Among selected isolates, all were gIV
Eastern China(Deqing)[30]	Abattoirs	2004	Bile samples	3.1% (5/160)	Among selected isolates, all were gIV
Shanghai city and Jiangsu province[14]	39 swine farms	2004 to 2006	Serum samples	Sow (8 to 30 months): 0%(0/135) Swine (slaughterhouse): 6.7% (2/30) Swine (4 to 6 months): 5.2% (5/96) Swine (1 to 3 months): 8.3% (11/133)	Among the isolates, 1 was gIII and the rest were gIV.
Shanghai area [35]	2 to 4 months old from 23 farms	Sep to Nov 2007	Faecal samples	5% (24/480)	All were gIV
Shanghai suburban districts[33]	37 pig farms in 10 Shanghai suburban districts	-	Faecal samples	26.1% (111/426)	Among 32 sequenced isolates, 22 were gIII and 10 were gIV
Shaoguan region, Guangdong province[36]	1 to 5 months old, 3 pig farms	-	Faecal samples	1 to 5 months old + sow samples: 6.1% (6/99) 3-5 months old: 8.6% (6/70)	All were gIV
Other regions in Mainland China					
Beijing, south suburbs[51]	Younger swine (< 3 months old)	-	Faecal samples	19/83 (22.9%)	All were gIV
Central China (Fuyang, Huaibei, and Suzhou)[52]	4 to 26 weeks old, 11 swine farms	Mar to Aug 2008	Faecal samples	39/554 (7.0%)	All were gIV
Northwest China (Xi'an, Kashi, and Datong)[34]	4 to 6 months old	Apr to May 2007	Bile Samples	1.8% (11/603)	All were gIV
Overseas countries					
Southern France[28]	3 months old pigs: farm in Vaucluse Department, sourced from different region 6 months old pigs: Drôme slaughterhouse	Jan to Jul 2007	Faecal samples	3 months old pigs: 65% (65/100) 6 months old pigs: 0% (0/107)	All were gIII
Northern Italy[53]	From 6 farms: 3 to 4 months old (weaners) 8 to 9 months old (flatteners) Gilts (0 partities) Young sows (1-2 partities) Old sows (>2 parities)	Jan to Jun 2006	Faecal samples	Gilts: 43.1% (25/58) Young sows: 38.6% (22/57) Old sows: 53.4% (31/58) Weaners: 42.2% (27/64) Flatteners: 27.0% (10/37)	gIII: 16 isolates
Japan[25]	1 to 6 months old in 92 commercial farms in 20 prefectures	-	Serum samples	Overall: 4 % (55/1425) 1 months old pigs: 0% (0/218) 2 months old pigs: 6% (11/198) 3 months old pigs: 10% (32/310) 4 months old pigs: 6% (10/180) 5 months old pigs: 0.5% (2/383) 6 months old pigs:0% (0/136)	gIII: 52 isolates gIV: 3 isolates
Japan (Hokkaido, Aomori and Akita on mainland	25 commercial farms 2500 serum samples For each farms,	2000 and 2002	Serum sample	2 months old pigs: 0% (0/180) 3 months old pigs: 15% (113/750) 4 months old pigs: 13% (24/180)	gIII (128/137 isolates) gIV (9/137

Honshu and Miyazaki and Kagoshima on Kyushu Island)[24]	2 months of age: 20 pigs 3 months: 30 pigs 4 months: 20 pigs 5 months: 20 pigs 6 months: 10 pigs			6 months old pigs: 0% (0/250)	isolates)
Korea[27]	1 to 7 months old pigs and sows from 13 swine farms	-	Serum samples	1 months old pigs: 0% (0/21) 2 months: 1.6% (1/62) 3 months: 6.7% (2/30) 4 months: 0% (0/15) Total 2.3% (3/128)	gIII
Netherlands[29]	Pooled specimens of pigs from 5 to 27 weeks (mean 20 weeks)	2005	Faecal samples	Farms positive rate: 55% (53/97)	All were gIII
Thailand (Nakhon Pathom and Ratchaburi provinces)[26]	5 pig farms	Sep 2006 to Jan 2007	Serum and faecal samples	Serum samples: Overall: 7.75% (20/258) 1 months old pigs: 0% (0/41) 2 months old pigs: 27.5% (11/40) 3 months old pigs: 5.7% (4/70) 4 months old pigs: 0% (0/42) 6.5 months old pigs: 0% (0/19) Sows:10.8% (5/46)	gIII (20 selected samples)

30. All the identified HEV in this study were genotype IV and this genotype is also commonly reported in pigs in other studies in Mainland China as can be seen in Table 4. Although it was reported that higher prevalence of genotype III instead of genotype IV in Shanghai suburban regions, genotype III HEV was not detected in samples sourced from the four farms in Shanghai in this study and was rarely found in local human cases.[33,39,46,48] Another study in Shanghai also detected only genotype IV HEV in farms in Shanghai.[35]

31. One of the local HEV isolates from pig sourced from Guangdong in this study was found to have the same sequence as a pig bile samples taken in northwest China in previous study.[34] In addition, some samples from pigs sourced from Shanghai and Guangdong in this study were also found to have same partial ORF2 sequence. The travelling of humans or trading of pigs, which are infected with or carried the virus, may facilitate the transmission of virus to wider areas. Different variants of genotype IV HEV and genotype III HEV were identified in Shanghai and some subgroups were found to be closely related to strains isolated in different

regions, even countries.[33,35] It has been estimated that 60% of pigs consumed in Shanghai were imported from other regions of Mainland China to Shanghai.[35] On the other hand, contaminated water may also contribute to the transmission of HEV, in which people lived in downstream area of swine farms were found to have a 29% higher risk of infection by HEV than people lived in the upstream area.[54]

Transmission of HEV to humans

32. The genetic relationship between the HEV isolates from pigs and local human cases may provide clues on the potential source of HEV. Majority of HEV isolates in local human cases have been found to belong to genotype IV and only some isolates were found to belong to genotypes I or III, while all the identified HEV in pig livers in this study were genotype IV.[46,48] Study in central China reported that, despite HEV prevalence in the region were all genotype IV, there was no evidence of cross-species transmission between human and swine.[52] Yet, some local HEV isolates from humans and pigs were found to be closely related, i.e. having the same partial ORF2 sequence in this study.

33. Among the HEV from pig liver samples that were found to have same sequence as those from human cases, certain isolates (V09-046 and V09-069) collected from the same farm but from different months, were found to be closely related with isolates in several human cases in this study as well as those recorded in the past.[46,48] This suggests that these strains may have circulated among pigs import to Hong Kong and local people for quite a while. In Shanghai, genotype IV HEV has also been found co-circulating among swine and humans and swine was suggested to be the principal reservoir of HEV. This is because higher amount of

virus was found in swine than that in humans.[30] Furthermore, humans may not be an efficient vector for spreading the virus in comparison with swine, as a silent outbreak occurred in rural community was not spread to neighbouring communities in a seroepidemiological study.[30,55] In these regards, it is more probable that pigs rather than humans spread the infection to humans.

34. For these seven human cases, only three persons had reported pig offal consumption, i.e. liver or intestine, during incubation period, while the other three had consumed pork and/or shellfish during incubation period and food history of one case was not available. Although other tissues of pigs may harbour HEV, the risk of contracting HEV infection through consuming uncooked virus-contaminated liver may be higher than that of skeletal muscle.[56] In local culinary settings, it is not uncommon to find pig livers that are not thoroughly cooked, while well-done pork is usually consumed. In addition, pork meat is the meat from porker but only roaster pigs were found to have HEV in this study. On the other hand, other risk factors for local hepatitis E cases also exist. Contaminated water, consumption of raw or undercooked shellfish, for example, are also well documented risk factors of HEV. According to investigation of CHP, so far the human hepatitis E infections were all sporadic without obvious epidemiological linkage. Only a proportion of the cases could recall consumption of pig offal. It is difficult to determine the exact source of infection of individual cases.

35. Shellfish has been reported to be a potential source of HEV. The virus has been detected from two out of 32 packages of a type of bivalve shellfish from Japanese river.[11] Consumption of shellfish has also been associated with hepatitis E cases.[57,58] Bivalve shellfish are

filter feeders; they absorb food particles and nutrients by filtering out the seawater. They can concentrate virus, including HEV, present in the polluted water. Hence, consuming undercooked shellfish harvested from or maintained in polluted water may also be a potential source of local cases, despite previous studies did not indicate that shellfish is a major vehicle.[45,46]

36. The epidemiological importance of the foodborne transmission is still unclear. Documented proof of HEV illness as a result of food consumption was only available from Japan, but not from other countries until recently where figatellu, a traditional pig liver sausage widely eaten in France and commonly consumed raw (although their manufacture includes smoking for a few days), was reported as a source of HEV infection in France.[40,59] In addition, there is less definitive evidence for "foodhandler" transmission of emerging viruses, such as HEV, in comparison to norovirus and HAV, as stated in microbiological risk assessment by WHO/FAO.[40] Although the exact transmission patterns remain to be established, foodborne transmission is plausible based on available information.[40] There may be other potential transmission routes not related to food. Transmission of HEV through blood transfusion has been reported in some regions.[6] Some seroepidemiological studies also suggest the possibility of occupational exposure to HEV in veterinarians and swine farmers.[54,60,61] The contribution from other potential sources, i.e. food related or not, for local hepatitis E cases is difficult to determine due to the long incubation period of hepatitis E.

Sources of HEV in farms

37. Repeated occurrence of HEV positive samples was observed in some farms collected with multiple samples; the farm environment may be the source of the HEV for pigs. In an experimental setting, it was observed that the faecal-oral route, but not tonsil and nasal secretions and contaminated needles, was the only route where HEV transmission was achieved. Yet, this was only demonstrated in one of the three pigs. The author suggested that higher dose of HEV or repeated exposure are required to initiate infection via this route.[62] This may occur in the field condition where larger number of pigs is held in a confined facility and faeces from multiple pigs accumulate and expose to pigs in the pool.[62] HEV has been detected in the farm environment, such as in manure slurry.[63,64] Furthermore, sow can harbour HEV which may be transmitted to piglets, one study suggested that early weaning and segregation of the pigs from their dams may reduce the contract of HEV by pigs.[65-67] Currently, specific risk factors for on-farm and between farm transmission of HEV are not clear.[19]

38. Sanitary condition of farms may have effect on HEV prevalence in which pigs in farms with comparatively poor sanitation were reported to have higher chance of contracting or harbouring HEV.[31,33] On the contrary, it was demonstrated that implementation of stricter sanitary measures did not have much effect on prevalence of genotype IV HEV.[32] At present, recommendation on farm control for HEV is not available from World Organisation for Animal Health (OIE).

39. Inspecting every pig from farm positive for HEV may not be useful as infection by HEV is not uncommon and adult pigs have usually recovered from HEV infection. Regarding the potential on transmission

from younger pigs, further information is required on the transmission routes and the contribution from this source compared with other potential risk factors for hepatitis E, to determine appropriate risk management option.

Food safety advice

40. The transmission from pigs could be one possible source for local hepatitis E cases, but there may be other sources. In addition, there are still uncertainties on the routes of infection, source, and incidence of hepatitis E in developed countries.[6,19,40] In a recent review on transmission routes and risk factors for locally acquired HEV infection in Europe, it was also concluded that there was no evidence for one main transmission route of HEV infection or risk factor for hepatitis E, however zoonotic transmission seemed likely.[68] While there is evidence that point to the transmission from pigs, recommendation on food safety may help to prevent hepatitis E.

41. Liver samples were tested in this study, but the presence of HEV is not limited to pig livers. It was found that extrahepatic replication of HEV occurs in lymph nodes, colons, small intestines, and spleens for pigs infected with swine HEV and additionally in stomach, kidneys, tonsils, and salivary glands of pigs infected with human HEV.[69] In addition, bivalve shellfish may be a potential source of HEV as discussed. These foods should be handled or prepared properly to minimise the risk of HEV as well as other foodborne pathogens.

42. HEV can be eliminated by cooking food thoroughly. It was demonstrated that pigs inoculated with the homogenates of HEV-positive

livers that were stir-fried at 191°C for five minutes or boiled for five minutes (with a minimal internal temperature of 71°C) did not develop HEV infection.[70] In addition, immersion in boiling water to raise the internal temperature to 90°C for no less than 90 seconds is a mandatory treatment for bivalve molluscs harvested from waters a fairly high degree of pollution in European countries and this treatment can also inactivate hepatitis A virus, which is more resistance than HEV.[71-73] Some people may prefer the pig liver or shellfish just done and consider this delicious and may not cook pig livers and other pig products as well as shellfish thoroughly in some cases, e.g. hotpot or congee cooking. These may pose risk of contracting HEV and other foodborne pathogens, especially for those at-risk populations.

43. Hepatitis E can be fatal to pregnant women, in particular those in third trimester pregnancy. Apart from pregnant women, patient with CLD should particularly aware of foodborne HEV, since it has been reported that superinfection with HEV in patients with underlying CLD can cause severe hepatic decompensation leading to increased morbidity and mortality in developed country.[4] In addition, it was noted that the median age of local hepatitis E cases were 48.5 years old in a review of hepatitis E cases from 1998 to 2007;[74] this means that half of patients were older than this age. Hence, elderly should also aware of food with higher risk of HEV.

44. Food trade and consumers are advised to observe good personal and hygiene practices; cook meat and offal thoroughly; prevent post cooking contamination. The CFS has developed guidelines on safe preparation and handling of meat and meat products including offal as well as food hygienic practice to prevent hepatitis A and hepatitis E.

Limitations and future study

45. The emergence of HEV requires closer monitoring. As can be seen from this study, some strains of HEV with same partial sequence were found in both pigs sourced from Mainland China and patients in Hong Kong. Surveillance of this virus in animals and humans is required to follow the trend of infection.

46. In this study, it was found that pigs can be one of the sources of local hepatitis E cases. Only partial ORF2 of HEV was sequenced. Additional gene sequence data, i.e. other genes, can help to confirm the results and determine the relatedness of other strains. In addition, partial sequences of HEV isolates from human cases that are not same as that from pigs were not analysed in this study. Analysis of HEV sequence data from other human cases may help to determine the prevalence of different strains of HEV and if the strains found in pigs are more prevalent in humans.

47. It should be noted that the sample size was limited and only pig livers were collected for testing. Other sources of HEV may also exist. The testing of other types of foods such as non-ready-to-eat bivalve shellfish may provide further information on other potential source of local hepatitis E cases. In addition, the possibility of acquiring the HEV through other sources, such as contaminated water, is not included in this study.

CONCLUSION AND RECOMMENDATIONS

48. This study showed that genotype IV HEV can be found from locally available fresh pig livers of roaster pigs but none were found from those of porker pigs which are the major live pigs imported from Mainland China. Partial sequences of HEV isolates from some pigs were found to be the same as that from local human cases during the sampling period as well as some local cases recorded in the past. These suggested that consumption of pigs is a possible source of HEV in local human hepatitis E cases. On the other hand, it is well documented from literature that HEV infection can be transmitted through other sources such as contaminated water or undercooked shellfish. In fact, some of the human cases with same partial sequence as pigs did not give a food history of consuming pig offal.

49. Current recommendations focus on food safety advice based on evidence showing pigs as a possible source. Food trade and consumers are advised to observe good personal and hygiene practices; cook meat and offal thoroughly; prevent post-cooking contamination. These are particularly important for at-risk populations such as pregnant women, elderly, and patient with CLD. The CFS has developed guidelines on safe preparation and handling of meat and meat products including offal as well as food hygiene practices to prevent hepatitis A and hepatitis E.

50. Last but not least, to prepare safe and wholesome food, it is recommended to practise the Five Keys to Food Safety in the daily operation:

1. Choose (Choose safe raw materials)
2. Clean (Keep hands and utensils clean)
3. Separate (Separate raw and cooked food)
4. Cook (Cook thoroughly)
5. Safe Temperature (Keep food at safe temperature)

Below are some practical tips for trade and public to apply five keys in daily life to minimise the risk of contracting HEV as well as other foodborne pathogens that may be present in raw meat and offal.

Advice to Trade

Purchase and Receiving

- Obtain food and food ingredients from approved and reliable sources.
- Use fresh and wholesome food ingredients and check the quality of the ingredients upon receipt.

Storage

- Ideally, use two separate refrigerators for storing raw food and cooked food or ready-to-eat food.
- If cooked food or ready-to-eat food and raw food such as raw meat and offal have to be stored in the same refrigerator, do the following :
  - Store food in containers with lids to avoid contact between raw food and ready-to-eat food or cooked food.
  - Store raw food below ready-to-eat food or cooked food in the refrigerator to prevent juices from dripping onto ready-to-eat food or cooked food.

Preparation

- Food contact surfaces of equipment and utensils should be maintained in a clean and sanitary condition.
- Wash utensils and worktops with hot water and detergent after each use.
- Slice raw meat and offal into thin strips to allow thorough cooking, especially those for hotpot or congee cooking.
- Use separate utensils to handle raw food (including raw meat and offal) and cooked food or ready-to-eat food e.g., use different colours codes for different utensils (including cutting boards and knives):
  Red- Raw food
  Blue- Cooked food
  Green- Ready-to-eat food

Cooking

- For sliced pig liver, depending on thickness and quantity, boil at 100°C or stir-fry in hot skillet/wok for at least three to five minutes.
- Food handlers should be made aware that heating to an internal temperature of 90°C for 1.5 minutes is required for inactivation of hepatitis A virus in molluscan shellfish. Hence, for cooking shellfish, boil at 100°C until their shells open; boil for additional three to five minutes afterwards.
- For meat and offal, make sure that juices are clear, not red, blood is not visible when you cut the cooked meat and offal.

Personal Hygiene

- Always follow good personal hygiene practices, including:
  - Wash hands thoroughly with running water and soap for 20 seconds before handling food and often during food preparation, after handling raw meat or offal and after handling soiled equipment or utensils;
  - Open wound should be covered by bright-coloured waterproof bandages or gloves;
  - Suspend from engaging in any food handling work when suffering or suspected to be suffering from an infectious disease or symptoms of illness such as flu, diarrhoea, vomiting, fever, sore throat and abdominal pain.

Advice to Public

Purchase and Receiving

- Obtain food and food ingredients from approved and reliable shops.
- Select fresh and wholesome food ingredients and check the quality of the ingredients upon receipt.

Storage

- Keep raw food including raw meat and offal separate from other food items in your grocery cart and shopping bags to prevent their juices from contaminating other food items.
- In the refrigerator:
  - Store food in containers with lids to avoid contact between raw food and ready-to-eat food or cooked food.
  - Store raw food below ready-to-eat food or cooked food in the refrigerator to prevent juices from dripping onto ready-to-eat food or cooked food.

Preparation

- Food preparation areas and food contact utensils should be maintained in a clean and sanitary condition.
- Wash utensils and worktops with hot water and detergent after each use.
- Slice raw meat and offal into thin strips to allow thorough cooking, especially during hotpot or congee cooking.
- Use separate utensils to handle raw food (including raw meat and offal) and cooked food or ready-to-eat food e.g., use different colours codes for different utensils (including cutting boards and knives):
  Red- Raw food
  Blue- Cooked food
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Cooking

- For sliced pig liver, depending on thickness and quantity, boil at 100°C or stir-fry in hot skillet/wok for at least three to five minutes.
- Consumers should be made aware that heating to an internal temperature of 90°C for 1.5 minutes is required for inactivation of hepatitis A virus in molluscan shellfish. Hence, for cooking shellfish, boil at 100°C until their shells open; boil for additional three to five minutes afterwards.
- For meat and offal, make sure that juices are clear, not red, blood is not visible when you cut the cooked meat and offal.
- When having hotpot, use separate chopsticks and utensils for handling raw and cooked foods to prevent cross-contamination.

Personal Hygiene

- Always follow good personal hygiene practices, including:
  - Wash hands thoroughly with running water and soap for 20 seconds before handling food and often during food preparation, after handling raw meat or offal and before eating.

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