Surveillance of biologic sources of hepatitis E viruses in community

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KEY MESSAGES

1. Food-borne route may play a role in hepatitis E virus (HEV) infection in Hong Kong.
2. HEV contamination is not uncommon in a variety of meat and seafood items for daily consumption.
3. HEV RNA was detected in pig liver, pig intestine, and oyster in local retail settings with a prevalence of 1.5%, 0.4%, and 0.2%, respectively.
4. Local co-circulating human and swine HEV strains were genetically indistinguishable from each other.
5. Local co-circulating human and swine HEV strains belonged to genotype 4 with highly comparable subtype distribution in which subtype 4b predominated over subtype 4d.

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Introduction

Hepatitis E virus (HEV) is a non-enveloped, positive-sense, single-stranded RNA virus in the family *Hepeviridae* and genus *Orthohepevirus*. HEV is transmitted primarily through the faecal-oral route. Non-travel-associated locally-acquired hepatitis E is re-emerging in developed countries. HEV is genetically classified into at least seven genotypes (HEV-1 to HEV-7). Because HEV-3 and HEV-4 can be detected in both humans and pigs, food-borne virus acquisition from consumption of contaminated food (such as pig liver sausage) and zoonotic transmission via close contact with infected pigs have been implicated.[1]

In Hong Kong, hepatitis E is a notifiable disease, and the number of cases has been on an upward trend since 2001.[2] Study to elucidate genetic relatedness of animal HEV detected in food and human HEV in clinical cases has been lacking. Thus, we reported the prevalence of HEV in different food items (lamb, oyster, pig blood curd, pig intestine, and pig liver) from retails stores over a 24-month period from 1 April 2014 to 31 March 2016 in Hong Kong. We also genotyped HEV from local clinical cases of the same period and provided molecular evidence suggesting that contaminated pig liver is one possible source of local human cases of autochthonous HEV infections.

Methods

From 1 April 2014 to 31 March 2016, five types of food items at risk of HEV contamination were purchased in two local market settings: supermarkets (lamb, oyster, and pig liver) and wet markets (oyster, pig blood curd, pig large intestine, and pig liver) at five districts (Hong Kong Island, Kowloon East, Kowloon West, New Territories East, and New Territories West) once every 2 weeks. Food tissues were homogenised and tested for HEV RNA by a quantitative RT-PCR assay. To monitor for viral RNA extraction efficiency and carryover of PCR inhibitors, each food sample was spiked with a known amount of TATAA Universal RNA Spike I. Archived human sera collected from hospitalised patients who tested positive for HEV IgM during the study period in our hospitals were retrieved. Viral RNA was extracted. To perform virus genotyping, HEV RNA from food samples and human sera was reverse-transcribed to cDNA, followed by a nested PCR targeting HEV open reading frame 1 (ORF1, 133 nucleotides) and ORF2/3 junction (97 nucleotides). PCR products were Sanger-sequenced. Phylogenetic inference was made using neighbor-joining clustering method. Sequences obtained in this study have been deposited into GenBank under accession numbers KX752737 to KX752775.

Results

A total of 240 lamb, 479 oyster, 240 pig blood curd, 240 pig intestine, and 479 pig liver samples were tested for HEV RNA by RT-qPCR. To monitor for the RNA extraction efficiency and PCR inhibition, 382 food samples were randomly selected for spike RNA detection, and 377 (98.7%) of them were tested positive, indicating satisfactory recovery and PCR efficiency at a 96% confidence level. HEV RNA was detected in seven pig liver, one pig intestine, and one oyster samples from four out of five districts (except

TABLE. Temporal distribution of food samples tested positive for hepatitis E virus RNA.

	No. of positive samples																							
	2014									2015									2016					
	Q2			Q3			Q4			Q1			Q2			Q3			Q4			Q1		
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Pig liver		1 (supermarket)		1 (wet market)				1 (supermarket) & 1 (wet market)					1 (wet market)			1 (wet market)		1 (wet market)						
Pig intestine			1 (wet market)																					
Pig blood curd																								
Oyster		1 (wet market)																						
Lamb																								

for New Territories West). The prevalence of HEV in pig liver, pig intestine, oyster, lamb, and pig blood curd samples was 1.5% (95% CI: 0.6%-3.0%), 0.4% (0.0%-2.3%), 0.2% (0.0%-1.2%), 0% (0.0% -1.5%), and 0% (0.0%-1.5%), respectively. In pig liver samples, HEV was detected in both supermarkets and wet markets. There was no significant difference in detection rate between retails settings. Food samples tested HEV RNA positive were collected all year-round with no observable seasonality (Table).

In our hospitals, 24 patients were tested positive for HEV IgM during the study period. The median age was 57 years and the male-to-female ratio was 5:1. A winter seasonality was observed in which 14 (58%) cases were admitted and diagnosed with hepatitis E during winter months from January to March. In contrast, there was only one (4%) case during summer months from July to September. Among these 24 patients, 22 archived HEV IgM positive sera had sufficient volume for further testing, and 18 (82%) of them tested HEV RNA positive. The median Ct value was 30.6 with an interquartile range of 27.6 to 32.8.

Among the nine HEV-positive food samples, three samples with the highest viral load (lowest Ct value) were pig livers. They were successfully genotyped by both nested ORF1 and ORF2/3 PCR. In addition, partial ORF1 sequence was determined from a pig liver sample that had the fourth highest viral load. Among the 18 HEV RNA positive human sera samples, 14 (78%) were successfully genotyped by both nested ORF1 and ORF2/3 PCR. The remaining four human sera samples with lowest HEV viral load were genotyped by nested ORF2/3 PCR only. Genotyping failure was associated with lower HEV viral load ($P<0.05$). Phylogenetic neighbour-joining trees were constructed using concatenated and individual partial ORF1 and ORF2/3 sequences (Fig). All HEV from pig liver samples and human sera clustered into genotype 4. All but one swine HEV strains belonged to subtype 4b and the remaining strain grouped into subtype 4d based on partial ORF1 sequence. Pairwise nucleotide identity in concatenated ORF1 and ORF2/3 of swine HEV in subtype 4b ranged from 97.4% to 98.7%. Similarly, in human sera, all but one HEV were assigned to subtype 4b and another strain was assigned to subtype 4d. Pairwise nucleotide identity in concatenated ORF1 and ORF2/3 of human HEV in subtype 4b ranged from 96.5% to 100.0%. The mean pairwise nucleotide identity between human and swine HEV strains was 97.8%. Both human and swine HEV strains interspersed in the subtype 4b lineage, and there was no species-specific sub-lineage observed from the phylogenetic tree.

Discussion

We detected HEV RNA in pig liver, pig intestine, and oyster samples. We provided compelling molecular evidence supporting close genetic relatedness between human and swine HEV genotype 4 strains to the subtype level circulating during the study period.

An increasing number of hepatitis E cases has been reported in non-endemic developed countries.[3] Most cases had no recent travel history to hepatitis E endemic area; this implicates a local source of infection and food-borne and zoonotic transmission from pigs. The prevalence of HEV RNA in pig liver samples was 1.5%. There was no observable seasonality or temporal association with human hepatitis E cases after taking into account the long incubation period. Among the seven swine HEV strains, we were able to sequence four of them with the highest viral loads and all belonged to genotype

4. The local HEV RNA prevalence in pig liver samples is comparable to those reported by others in Asia. In China, where HEV genotype 4 strains are predominant,[4] HEV RNA prevalence in pig herds is around 5%. In Japan, where HEV genotypes 3 and 4 co-circulate, HEV RNA prevalence in retail pig liver products ranges from 2% to 5%. In contrast, the detection rate of HEV in pig livers outside Asia, where HEV genotype 3 predominates, is much higher. In North America, the prevalence of HEV contamination in retail pig livers typically ranges from 5% to 10%. In Europe, the prevalence is even higher and can reach up to over 40% in very high-risk pig liver-derived food products such as figatellu. It is of interest to know whether or not HEV prevalence in pig livers is genotype-dependent.

To better understand association between human and swine HEV strains, an additional 18 human HEV strains from clinical hepatitis cases during the same period were genotyped. Phylogenetic analysis showed that all sequenced human and swine HEV strains were genotype 4. Among both human and swine HEV strains, subtype 4b is predominant whereas subtype 4d is the minority. This concordant HEV genotype distribution indicates possible association between human and swine HEV strains. Interestingly, HEV subtype distribution seems to vary in different cities in China.[4] For instance, subtype 4b is the predominant type in both humans and pigs in southern China, but subtypes 4a and 4i are predominant in eastern China and subtype 4g is predominant in northeast China. Such a highly matched HEV genotype and subtype distribution between human and swine HEV strains strongly suggest one common HEV reservoir and probable cross-species transmission in our populations.

This is the first report of HEV RNA detection in pig intestine, which is a popular food in Chinese cuisine. It is not surprising to detect HEV in pig intestine as extrahepatic HEV dissemination is common in naturally infected pigs. Our findings extend the range of food items at risk of HEV contamination. We also detected HEV RNA in oysters but at a very low frequency of 0.2%. This is consistent with studies from Croatia, France, and Japan that detected no HEV in oysters. However, a higher HEV prevalence of 8.7% in oysters was reported in South Korea coastal region. Considering that oysters are common vehicles of other food-borne viruses such as norovirus and hepatitis A virus and are consumed raw, the risk of food-borne HEV transmission from oysters and other shellfish cannot be ignored.

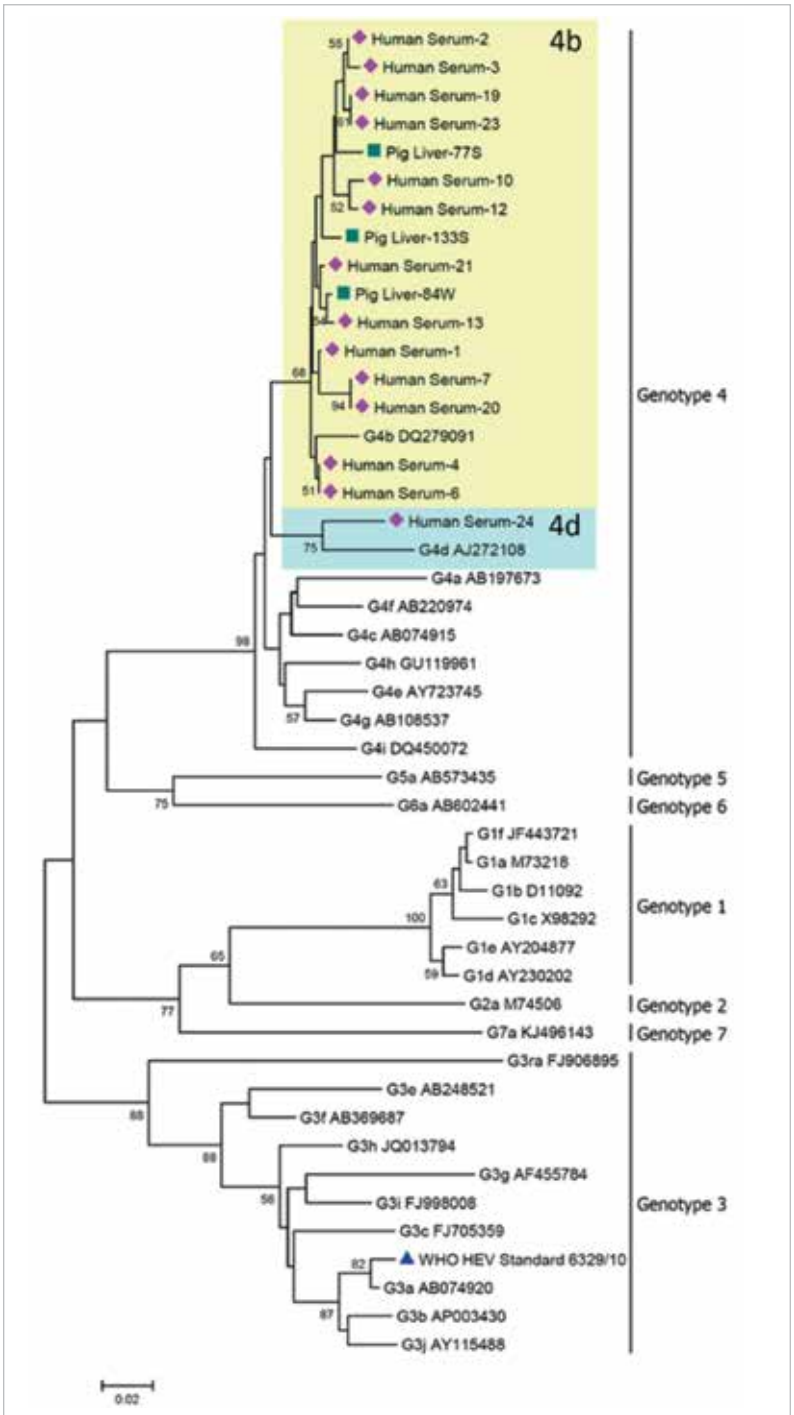

FIG. Neighbour-joining phylogenetic tree of human and swine hepatitis E virus (HEV) obtained from 2014 to 2016 in Hong Kong. The tree was constructed using Kimura 2-parameter distance method with 1000 bootstrap replicates. Sequences used were concatenated partial open reading frame (ORF) 1 and ORF2/3 junction sequences of 230 nucleotides in length. Green squares denote pig liver samples and those collected in supermarket and wet market are labelled with a suffix S and W, respectively. Pink diamonds indicate human serum samples and the indigo-blue triangle refers to the World Health Organization HEV RNA standard 6329/10. Reference sequences of 29 known HEV subtypes are labelled in the format of genotype and subtype, followed by GenBank accession number. Bootstrap values with a cut-off value of 50% are shown at nodes on the phylogenetic tree. The tree is mid-point rooted. Scale bar indicates the number of nucleotide substitutions per site. (Reproduced with permission from: Chan MCW, Kwok K, Hung TN, Chan PKS. Molecular Epidemiology and Strain Comparison between Hepatitis E Viruses in Human Sera and Pig Livers during 2014 to 2016 in Hong Kong. J Clin Microbiol 2017;55:1408-15).

Conclusion

HEV contamination is not uncommon in a variety of meat and seafood items for daily consumption. The HEV genotype 4 strains isolated from human

samples during the study period are genetically indistinguishable from those isolated from swine samples, suggesting that contaminated pig liver is one possible source of local human cases of autochthonous HEV infections. Food-borne transmission may play a role in HEV infections in Hong Kong.[5]

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