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Hepatitis E outbreak in a mechanical factory in Qingdao City, China

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ABSTRACT

Objective: In July 2018, recurrent hepatitis E cases were reported from a factory in Qingdao City, China. The aim of this study was to identify additional cases, and help prevent future incidents by identifying possible risk factors for infection.

Methods: Participants were asked to provide blood samples for hepatitis E virus (HEV) IgM and IgG antibodies screening, as well as liver function test. A questionnaire that assessed demographics, potential risk factors, and clinical symptoms was completed by participants. HEV RNA genotyping was performed using a nested Reverse Transcriptional Polymerase Chain Reaction (RT-PCR) method. Adjusted Poisson regression model for participant characteristics and risk factors was constructed for multivariate analysis.

Results: Overall, 41(14.5%, 41/283) participants had recent acute infection (21 of these were symptomatic). The result of multivariate analysis demonstrated a significant association of acute HEV infection with consumption of pig liver within the past two months (Relative Risk 2.61, 95% confidence interval (CI) 1.10–6.17, p = 0.0294). Sequencing of HEV RNA from seventeen acute cases indicated three HEV isolates of genotype 4 induced this outbreak.

Conclusions: This was probably a common-source foodborne hepatitis E outbreak, related to the consumption of undercooked pig liver.

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Hepatitis E is a liver disease caused by the hepatitis E virus (HEV). HEV is a small virus, with a positive-sense, single-strand ribonucleic acid (RNA) genome. It is a member of the *Hepeviridae* family, from the genus *Orthohepevirus* (ICTV: Hepeviridae Study Group, 2016). The virus has at least eight genotypes (Lu et al., 2006; Meng, 2010; Takahashi et al., 2014; Woo et al., 2014; Lee et al., 2016; Woo et al., 2016). Genotypes 1- 4 have been detected in humans (Lu et al., 2006), genotypes 3 and 4 are zoonotic and can infect humans and several animals (Meng, 2010), genotypes 5 and 6 have been isolated from wild boar (Takahashi et al., 2014), genotype 7 has been isolated from dromedary camels (Woo et al., 2014) and humans (Lee et al., 2016) who regularly consume camel meat and milk, and genotype 8 has been reported in Bactrian camels (Woo et al., 2016). HEV is transmitted via the fecal-oral route, principally via contaminated water. However, HEV infections have been also linked to consumption of contaminated pig liver (Kamar et al., 2013; Pavio et al., 2014; Cossaboom et al., 2016) or shellfish (Crossan et al., 2012; Gao et al., 2015). Usually the infection is self-limiting, but occasionally causes a serious disease known as fulminant hepatitis, especially in pregnant women. Hepatitis E is found worldwide, but the prevalence is highest in East and South Asia. During 2014–2018, approximately 28,000 acute hepatitis E cases were reported in mainland China (Data from the official website of Chinese Center for Disease Control and Prevention (CCDC): http://www.chinacdc.cn/).

Abbreviations: HEV, hepatitis E virus; RNA, ribonucleic acid; ALT, alanine aminotransferase; RR, relative risk; CI, confidence interval.

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On July 4, 2018, the local CDC (Qingdao Municipal Center for Disease Control and Prevention of China) received a case report, which described an acute hepatitis E case in one mechanical factory. Two days later, two additional hepatitis E cases were identified in the factory. Recurrent cases in the same unit attracted the attention of the local CDC. The factory is located in a suburb of Qingdao City, comprised of three workshops and 330 individuals. Workers engaged in mechanical manufacturing shared one inner canteen, which provided breakfast and lunch. The local CDC initiated an investigation to identify additional cases and to implement epidemic control. Ethics was approved by National Institute for Viral Disease Control and Prevention (IVDC), and informed consent was obtained for experimentation.

Methods

All individuals working in the factory (workers, cooks, cleaners, security guards, and gardeners) were asked by the local CDC to provide blood samples. In all, 302 individuals provided the local CDC with blood samples for acute hepatitis E screening at the Qingdao Infectious Disease Hospital.

ELISAs for HEV antibodies test

Blood samples were first tested for alanine aminotransferase (ALT) and then HEV antibodies (IgM and IgG). The Enzyme-linked immunosorbent assays (ELISAs) reagents used in the screening were commercially kit produced by the Beijing Bioengineering Co., Ltd (http://www.beierbio.com/). Assays were performed according to the manufacturer's instructions. All IgM-seropositive samples were transported by cold chain to the IVDC.

RNA extraction and qualitative HEV real-time RT-PCR

Viral RNA was extracted from 140 μL of HEV IgM-positive serum by using the extraction Kit (QIAamp Viral RNA Mini Kit, Catalog no.:52906) according to the instructions of the manufacturer (QIAGEN, http://www.qiagen.com/). For the detection of HEV RNA, a realtime Reverse Transcriptional Polymerase Chain Reaction (RT-PCR) method was used, with forward primer (JVHEVF: 5′-GGTGGTTTCTGGGGTGAC-3′), reverse primer (JVHEVR: 5′-AGGGGTTGGTTGGATGAA-3′) and probe (JVHEVPmod: 5′-FAM-TGATTCTCAGCCCTTCGC-MGB-3′) (Garson et al., 2012). The One Step PrimeScript^{TM} RT-PCR Kit (Code no.:RR064A) produced by TAKARA BIO INC. (http://www.takarabiomed.com.cn/) was used, with RT-PCR condition as follows: at 42 °C for 30 min, at 95 °C for 10 s, 40 cycles of at 95 °C for 5 s and at 60 °C for 34 s. A Cycle threshold value less than 37 was judged as positive for HEV RNA.

HEV RNA genotyping

For all HEV IgM-seropositive samples, the IVDC conducted HEV RNA genotyping by a nested RT-PCR method (Ge et al., 2005). Primers used were targeted to the HEV open reading frame-2 (ORF-2). The second round of PCR primers were targeted to sites nt 6298 - nt 6486 (according to Burma strain D10330), and then the generated sequence was long for 189bps (Ge et al., 2005). Reverse transcription and the first round PCR were conducted using PrimeScriptTM One Step RT-PCR Kit Ver.2 (Code no.:RR055A, TAKARA BIO INC), with reaction condition: at 50 °C for 30 min, at 94 °C for 2 min, 35 cycles of at 94 °C for 30 s, at 53 °C for 30 s and at 72 °C for 40 s. The second round PCR was conducted using Premix Taq^{TM} Kit (Code no.:RR901A, TAKARA BIO INC), with PCR condition: at 94 °C for 5 min, 35 cycles of at 94 °C for 30 s, at 53 °C for 30 s and at 72 °C for 30 s.

For phylogenetic analysis, all generated sequences were compared to reference sequences of HEV genotype 1 – genotype 4, retrieved from GenBank. The ClustalW method of MEGA software (Version 5.0) was used for nucleotide sequences alignment. The maximum likelihood method with 1000 bootstrap replicates was used to construct a phylogenetic tree to determine the genotype of the samples. Estimated pairwise distances were also calculated using the bootstrap method with 1000 replicates. Further, sequences were blasted in GenBank to search for strains with the highest homology.

Epidemiological investigation

To assess the source of the hepatitis outbreak, the local CDC first performed an environmental hygiene survey of the inner canteen and the three workshops. The following were assessed in the canteen: sterilization of tableware, drinking water supply, and the menus for the past two months of the inner canteen. For the three workshops, the opportunity for workers to contact water during work and the mode for water drinking were investigated.

Based on the environmental hygiene survey, the local CDC designed a questionnaire that assessed three aspects for each individual, including demographics, potential risk factors, and clinical symptoms compatible with viral hepatitis. These demographics included gender, age, and occupation. Potential risk factors included history of ingestion of: shellfish, mussels, vongoles, pig liver, flounder, pork, salad (including peanut and celery), shrimp, cow tripe, unboiled water in the inner canteen, or having dined out within the last two months. Further risk factors included hand washing: before eating, after defecation, and the use of soap for hand washing. Symptoms compatible with HEV infection included fever, loss of appetite, weakness, dark urine, aversion to greasy food, jaundice, and abdominal pain. Questionnaires were self-completed by the individuals and collected later by the local CDC.

A symptomatic acute hepatitis E case was defined as HEV IgM-seropositive, ALT levels greater than 40 U/L, with signs and symptoms compatible with acute hepatitis (e.g., weakness, loss of appetite, abdominal pain, jaundice, and dark urine). Asymptomatic HEV infection was defined as those individuals who did not have the above clinical symptoms with either normal or abnormal liver function, but were seropositive for HEV IgM.

Statistical analysis

Statistical analysis was performed using SAS 9.1 (Statistical Analysis Software Institute Inc., USA). Adjusted Poisson regression models for participant characteristics and risk factors were constructed for univariate analysis (Wacholder, 1986). Demographic characteristics and risk factors with a global p value less than 0.05 by univariate analysis were included in the multivariate model.

Results

HEV IgM and IgG test

A total of 330 individuals worked in the factory. Of those, 302 individuals (91.5%) provided blood samples and completed the questionnaires. Of the 302 individuals, 41 (13.6%) were seropositive for HEV IgM. Of these 41, twenty individuals had both HEV IgM and IgG. Nineteen individuals (6.3%, 19/302) had HEV IgG only, indicative of a previous infection. The 19 individuals who had evidence of prior infection were excluded due to probable immunity to HEV infection. Of the remaining 283 individuals, 41 (14.5%) were seropositive for HEV IgM, consistent with a recent acute infection.

There were no acute hepatitis E case reports for residents near the factory, or for family members of the participants.

Risk factors screening

Demographic characteristics and possible risk factors were collected by questionnaire. Results of the univariate model indicated no statistical association between HEV IgM-sero-positivity and gender, age group, or occupation ($p > 0.05$). Acute infection rates for males and females were 16.0% (34/213) and 10.0% (7/70), respectively. For age groups: 20–39 years, 40–49 years, and 50 years or greater, the infection rates were 14.1% (18/128), 12.6% (12/95), and 18.3% (11/60), respectively. For office workers, workshop workers, and other persons, the infection rates were 13.9% (17/122), 17.3% (22/127), and 5.9% (2/34), respectively. By univariate analysis, ingestion of pig liver and hand washing with soap were significantly associated with HEV IgM-sero-positivity ($p < 0.05$). The adjusted Poisson regression models of ingestion of salad and shrimp were not constructed because of zero events, but the Fisher Exact tests were not significant ($p > 0.05$). Associations between HEV IgM-sero-positivity and other risk factors were not found by univariate analysis (Table 1).

Pig liver ingestion and hand washing with soap (both with a global p-value lower than 0.05 by univariate analysis) were used to construct the multivariate model (Table 2). The multivariate model demonstrated no associations among HEV IgM-sero-positivity and gender, age group, or occupation ($p > 0.05$). Adjusted by gender, age

Table 1
Univariate analysis of probability of having recent HEV infection, Qingdao factory workers, 2018, by participant characteristics and risk factors.

Variable name (n = 283)	HEV IgM		Univariate model		
	Negative	Positive	RR & 95% CI	p value	Global p value
Gender					
M	179	34	Ref.		
F	63	7	0.63 (0.29–1.35)	0.2323	0.1764
Age group					
20–39	110	18	Ref.		
40–49	83	12	0.90 (0.45–1.77)	0.7573	
≥50	49	11	1.30 (0.66–2.59)	0.4477	0.6408
Occupation					
Office worker	105	17	Ref.		
Workshop worker	105	22	1.24 (0.69–2.23)	0.4636	
Others	32	2	0.42 (0.10–1.74)	0.2323	0.1082
Ate shellfish					
No	145	24	Ref.		
Yes	97	17	1.05 (0.59–1.86)	0.8675	0.8681
Ate mussel					
No	237	39	Ref.		
Yes	5	2	2.02 (0.60–6.76)	0.2529	0.4226
Ate vongole					
No	169	29	Ref.		
Yes	73	12	0.96 (0.52–1.80)	0.9079	0.9072
Ate pig liver					
No	70	5	Ref.		
Yes	172	36	2.60 (1.06–6.37)	0.0372	0.0079
Ate flounder					
No	13	1	Ref.		
Yes	229	40	2.08 (0.31–14.06)	0.4519	0.3015
Ate pork					
No	29	2	Ref.		
Yes	213	39	2.40 (0.61–9.45)	0.211	0.081
Ate salad					
No	13	0			
Yes	229	41			0.2265[a]
Ate shrimp					
No	20	0			
Yes	222	41			0.0904[a]
Ate cow tripe					
No	36	4	Ref.		
Yes	206	37	1.52 (0.57–4.04)	0.3984	0.326
Drank unboiled water					
No	208	36	Ref.		
Yes	34	5	0.87 (0.36–2.08)	0.7523	0.7398
Dined out					
No	227	37	Ref.		
Yes	15	4	1.50 (0.60–3.77)	0.3863	0.4687
Wash hands before eating					
No	16	2	Ref.		
Yes	226	39	1.32 (0.35–5.05)	0.6806	0.6422
Wash hands after defecation					
No	21	2	Ref.		
Yes	221	39	1.72 (0.44–6.69)	0.4305	0.3241
Wash hands with soap					
No	50	2	Ref.		
Yes	192	39	4.39 (1.09–17.60)	0.0368	0.0009

[a] Fisher exact test.

Table 2
Multivariate analysis of probability of having recent HEV infection, Qing dao factory workers, 2018.

Variable name (n = 283)	Multivariate model	
	RR & 95% CI	p value
Gender		
M	Ref.	
F	0.85 (0.39–1.83)	0.6712
Age group		
20–39	Ref.	
40–49	0.97 (0.49–1.89)	0.9213
≥50	1.48 (0.72–3.01)	0.2828
Occupation		
Office worker	Ref.	
Workshop worker	1.32 (0.74–2.38)	0.3487
Others	0.40 (0.09–1.68)	0.2094
Ate pig liver		
No	Ref.	
Yes	2.61 (1.10–6.17)	0.0294
Wash hands with soap		
No	Ref.	
Yes	3.72 (0.92–15.09)	0.0658

group, and occupation, pig liver ingestion within the past two months was associated with HEV IgM-sero-positivity ($p < 0.05$). The participants who self-reported a history of pig liver ingestion in the past two months had a higher infection rate (17.3%, 36/208) than the other group (6.7%, 5/75) (RR = 2.61, 95% CI 1.10–6.17). The association between hand washing with soap and HEV IgM-sero-positivity disappeared with multivariate analysis ($p > 0.05$).

Acute HEV infection

Distributions of gender, age group, occupation, as well as the consumption of pig liver for the 41 participants who had acute HEV infection were analyzed. Of the 41 participants, 34 (82.9%) were male, with ages ranging from 22 to 57 years (mean 43.7 years), seven (17.1%) were female, with ages ranging from 31 to 47 years (mean 37.5 years). With respect to occupation, 23 (56.1%, 23/41) were workshop workers, 17 (41.5%, 17/41) were office workers, and one was a cook who worked in the inner canteen. Of the 41 participants who had acute HEV infection, 36 (87.8%, 36/41) reported a history of pig liver ingestion in the inner canteen of the factory within the past two months. Only five (12.2%, 5/41) denied a history of pig liver consumption.

As shown in Table 3, 21 (51.2%, 21/41) individuals had elevated ALT levels (> 40 U/L) and symptoms compatible with hepatitis. These were identified as symptomatic acute hepatitis E cases. Among the 21 symptomatic cases, 16 (76.2%) had ALT levels greater than 10000 U/L and 5 (23.8%) greater than 1000 U/L. Of the 21 symptomatic acute hepatitis E cases, 15 (71.4%, 15/21) complained of weakness, 13 (61.9%, 13/21) had dark urine, 12 (57.1%, 12/21) had a loss of appetite, eight (38.1%, 8/21) had aversion to greasy food, seven (33.3%, 7/21) had fever, and 15 (71.4%, 15/21) had jaundice or abdominal pain. Of the 41 participants, 16 (39.0%, 16/41) had normal ALT levels (<40 U/L) and no symptoms compatible with hepatitis. Only four (9.8%, 4/41) had slightly elevated ALT levels with no relevant symptoms.

HEV RNA genotyping results

Of the 41 HEV IgM-sero-positive samples, twenty-seven (65.9%, 27/41) were positive for HEV RNA judged by the real-time RT-PCR system (Table 3). For samples of symptomatic cases, the positive rate of HEV RNA was 100% (21/21), and it was 30% (6/20) for samples of asymptomatic infected individuals.

The partial fragment of ORF-2 was successfully amplified and sequenced for seventeen samples, with sixteen from symptomatic cases and one from an asymptomatic infected person. These genomic sequences are available in GenBank nucleotide databases under accession no.s **MN132863**-**MN132879**. Phylogenetic analysis of the 189 nt sequences indicated that all isolates belonged to genotype 4 (Figure 1). These sequences clustered into three clusters, with eight sequences (accession numbers: **MN132863**-**MN132865**, **MN132870**-**MN132874**) in Cluster 1, six sequences (**MN132866**, **MN132867**, **MN132876**-**MN132879**) in Cluster 2 and three sequences (**MN132868**, **MN132869**, **MN132875**) in Cluster 3. In Cluster 1, eight sequences were identical, with 100% homology. In Cluster 2, all the six sequences were identical, with 100% homology. And in Cluster 3, all the three sequences were identical, all with one nucleotide substitution of C6475 T (the site according to Burma strain D10330) contrast with sequences of Cluster 2. The pairwise distance between Cluster 1 and Cluster 2 is 0.069, with nucleotide divergence in twelve sites across the 189 nt sequence. The pairwise distance between Cluster 2 and Cluster 3 is 0.005, with nucleotide divergence in one site.

Results of the HEV sequences blasted in GenBank are given (Supplemental Material, Table S1). Sequences of Cluster 1 were similar to sequences isolated from Chinese swine and one Japanese patient who had travelled to China (Shao et al., 2009; Unpublished). Sequences of Cluster 2 and Cluster 3 were both similar to sequence isolated from Chinese swine (unpublished), with identity of 96.30% and 96.83%, separately.

Discussion

This hepatitis E outbreak among persons in a mechanical factory in Qingdao City was reported in July, 2018. All persons ate in an inner canteen. Office workers, workshop workers, cooks, cleaners, security guards, and gardeners were assessed for hepatitis E. More than 90% of these individuals provided blood samples for detection of HEV IgM and completed the

Table 3
Lab markers of acute HEV infected individuals.

Cases	HEV antibodies	ALT level (U/L)	Detected samples	
			Real-time RT-PCR	Nested RT-PCR
Symptomatic	IgM(+), IgG(−)	1000–10,000	4/4	0/4
		>10,000	11/11	11/11
	IgM(+), IgG(+)	1000-10,000	1/1	0/1
		>10,000	5/5	5/5
Asymptomatic	IgM(+), IgG(−)	<40	0/5	0/5
		40–80	1/1	0/1
	IgM(+), IgG(+)	<40	2/11	0/11
		40–160	3/3	1/3

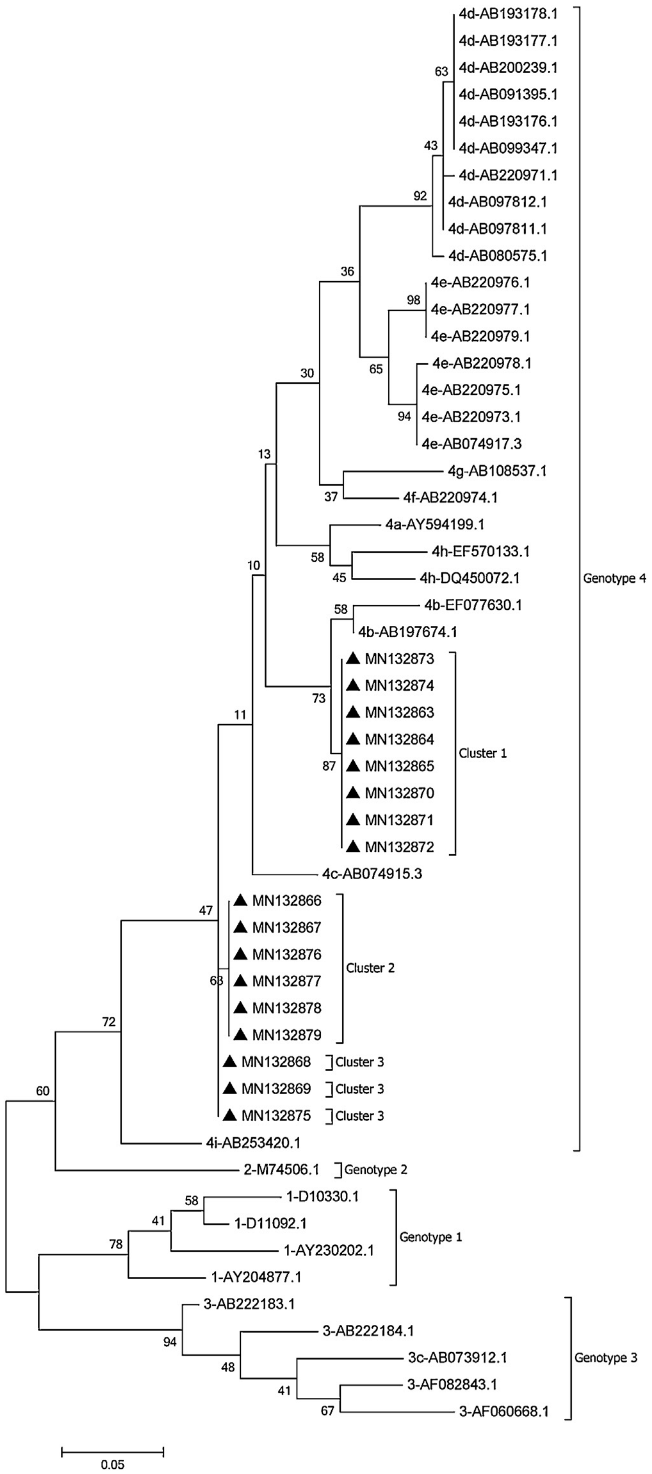

Figure 1. Phylogenetic tree showing the genotype of HEV strains from the outbreak, Qingdao City, China, 2018. The phylogenetic tree was calculated on the base of the 189 nt (nucleotides 6298 to 6486, according to Burma strain **D10330**) that code for structural protein, by using MEGA 5.0 software and the maximum likelihood method (1000 bootstraps). The percentage of trees in which the associated strains clustered together is shown next to the branches. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. ▲indicates our strains, **MN132863**-**MN132879**.

questionnaire. As such, we not only identified additional acute HEV cases in a timely manner but also had the opportunity to determine the probable cause of the outbreak.

Laboratory results indicated that there were 41(14.5%) individuals with recent acute infection. Twenty-one individuals reported illness compatible with acute hepatitis, with 20 (48.8%, 20/41) asymptomatic. This asymptomatic rate was much higher than previously reported (3–4%) (Panda et al., 2007; Mansuy et al., 2009), but lower than one other report (2/3) (Said et al., 2009).

Elevated liver function test results appeared to be associated with symptomatic cases. Our results indicated that all of the 21 symptomatic individuals had elevated ALT levels (>40 U/L), and among these, 16 had very high ALT levels (over 10,000 U/L). Most (80%, 16/20) of the asymptomatic cases had normal liver function. Hence, HEV infection can produce severe, little, or no effect on liver function, which may be due to differential interactions between virus and host.

The most often described symptoms of symptomatic hepatitis E included weakness, jaundice or abdominal pain, dark urine, and so on. These are typical symptoms related to viral hepatitis, but are not specific for hepatitis E. Therefore, diagnosis of HEV should rely on laboratory tests, including HEV antibody and RNA detection.

Associations among gender, age, occupation and acute HEV infection were not found. Of the 41 participants, the majority were male, shop workers, and office workers, with one individual a cook who worked in the inner canteen. Because the acute HEV infection was not specific for one particular occupation, the possibility of cases exposed to HEV in the course of work was excluded.

Qingdao is a seaside city in the east of China, located in the southeast of Shandong Peninsula and near the Yellow Sea. Seafood is common on the tables of residents. Because consumption of HEV-contaminated seafood can lead to HEV infection (Crossan et al., 2012; Gao et al., 2015), we doubt that the outbreak was caused by consuming seafood. Based on menus provided by the inner canteen, we investigated the seafood consumption history of the participants. We found no association between seafood consumption and acute HEV infection, including shellfish, flounder, and shrimp. Thus, seafood was excluded as a cause of the outbreak.

With respect to drinking water, we investigated the water source. Most participants drank bottled water supplied by one water treatment plant. Residents near the factory who consumed the same brand of water had no acute HEV cases reported. There was no statistical association between drinking unboiled water and acute HEV infection by univariate analysis. Hence, a waterborne outbreak was excluded.

By multivariate analysis using the Poisson regression model, ingestion of pig liver was found to be associated with acute HEV infection. The participants who self-reported a history of eating pig liver within the past two months had a higher infection rate than the other group. Although acute HEV infection was found in various occupations, most of the cases reported the consumption of pig liver in the inner canteen. Therefore, we infer that the hepatitis E outbreak in the factory was foodborne and related to the consumption of pig liver. Similar zoonotic HEV transmission linked to consumption of contaminated pig liver has been previously reported (Kamar et al., 2013; Pavio et al., 2014; Cossaboom et al., 2016).

Pigs are reservoirs of HEV, especially genotypes 3 and 4 (Meng, 2010). One study found that HEV antibodies positivity was 83.6% in Chinese swine (Wang et al., 2002). If pigs carry HEV, humans can be infected by eating undercooked pig liver. Fried pig liver is a common food found on Chinese tables. Medium cooked pig liver is often considered to have a good taste and is preferred by people. The fresh pig liver used in the canteen was supplied by local farm product markets, and the pig liver was probably not adequately fried during the cooking process. The local CDC advised the canteen to sufficiently cook pig liver and to ensure that raw and cooked foods are kept separate.

The potential source of this acute HEV infection was native instead of imported. HEV RNA genotyping was successful for seventeen samples. Phylogenetic analysis indicated that all isolates belonged to genotype 4, which is the main type of HEV prevalent in China (Liu et al., 2012). When blasted in GenBank, all the sequences were similar to HEV sequences isolated from Chinese swine (except for one Japanese traveler). We infer that the potential source of this acute HEV infection was native instead of imported. The genotyping results supported the results of epidemiological investigation, which indicated pig liver supplied by local farm product markets was the potential source of infection.

It was possible that three HEV isolates induced this hepatitis E outbreak. The phylogenetic tree indicated that these sequences clustered into three clusters, with eight sequences in Cluster 1, six sequences in Cluster 2, and three sequences in Cluster 3. And in each cluster, the sequences had 100% homology. So we infer that this was a possible common source of infection. In combination with the results of epidemiological investigation, it is therefore possible that this outbreak is an example of an HEV zoonotic transmission from swine to human. The first animal strain of HEV to be obtained from a pig was in the United States (Meng et al., 1997), with evidence that infection was transmitted zoonotically to humans (Meng, 2000).

Results of the multivariate model and RNA genotyping indicated that pigs were a potential source for HEV infection in this outbreak and that HEV may be transmitted zoonotically to humans by eating undercooked pig liver. This was probably a common-source foodborne hepatitis E outbreak.

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Ethical approval

Ethics was approved by National Institute for Viral Disease Control and Prevention (IVDC), Chinese Center for Disease Control and Prevention (CCDC), and informed consent was obtained for experimentation.

Conflict of interest statement

None declared.

Author contributions

Wenjiao Yin wrote the main manuscript and fully participated in all experiments. Yalin Han, Hualei Xin participated in data collection. Weilin Liu, Qing Song, Zhizhi Li and Shuli Gao participated in the specimen collection. Fachun Jiang designed the study. Jingyuan Cao, Shengli Bi and Hongtu Liu participated in the analysis and interpretation of data. All authors made substantial contributions to writing and revising the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.07.006.

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