



Brief report

A hepatitis E outbreak by genotype 4 virus in Shandong province, China



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ABSTRACT

Hepatitis E vaccine was available in China in 2012, but the priority population for immunization is not clear. In 2013, a hepatitis E outbreak occurred in a company of Shandong province, China where most employees moved from other provinces and dined at the company's cafeteria. A total of fourteen (19%, 14/73) case-patients were identified, and three of them had symptomatic infection with one death. The proportion of symptomatic infection was much higher among those aged ≥ 50 years than those aged < 50 years (2/2 vs. 1/12, $P = 0.03$), and higher in males than females (3/8 vs. 0/6, $P = 0.21$). Food in the company's cafeteria might be the possible source of the outbreak. The findings from this outbreak investigation indicate that individuals aged ≥ 50 years, particularly males, might be the population of top priority for hepatitis E vaccination in China.

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1. Introduction

Hepatitis E virus (HEV) is an enterically transmitted virus, and outbreaks from HEV was first verified and confirmed in 1990s [1] and is responsible for about 50% of acute viral hepatitis cases in the endemic countries [2,3]. HEV is endemic in China and the seroprevalence among the general population was around 23% [4]. Hepatitis E cases are required to report to the National Notifiable Reporting System (NNDRS) in China since 1997, and the number of reported cases increased from 16,444 in 2004 to 19,202 in 2011 [5]. In 2012, hepatitis E vaccine became available in China, and offered an effective way to prevent HEV infection [6]; but the vaccination coverage is still very low.

Between December 2013 and January 2014, a hepatitis E outbreak occurred in a company in Donggang District, Rizhao Prefecture of Shandong province, China. We reported the HEV outbreak, and examined risk factors associated with HEV infection, and explored the implications for hepatitis E prevention.

2. Methods

2.1. General information of the company

Donggang District of Rizhao Prefecture was located in the east of China, having a population of 78,000. The company where the

outbreak occurred was established in Rizhao city in December 2011. There were 73 employees in the company, including 69 employees moved from other provinces of China during December 2011 and October 2012 and four local employees (two cooks and two janitors). All migrant employees lived in the apartments near the company. Free bottled water from dispensers was available in apartments and the offices. The company had a cafeteria which opened all day long for three meals.

2.2. Active case-finding

To identify all persons infected with HEV in the company, blood and stool samples were collected from all employees and were tested for HEV IgM and HEV IgG. The IgM-positive employees who developed signs and symptoms compatible with hepatitis were reported to NNDRS. Face-to-face interviews were conducted for all local employees and their family members on whether they had developed signs and symptoms indicative for hepatitis in the past two months. If any family had any sign/symptom for hepatitis, blood and stool samples would be collected and tested for HEV infection.

2.3. Case investigation

A face-to-face interview was conducted among HEV IgM-positive employees. Information on demographics information and the potential risk factors during the potential exposure period (from October 1, 2013 to November 15, 2013, 15–60 days prior to the disease onset of the index case) was collected, including the

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Table 1

The prevalence of HEV IgM and HEV IgG among the employees by age, gender and employee types.

	No. tested	HEV IgM positive n (%)	HEV IgG positive n (%)	Symptomatic hepatitis ^a n (% ^b)
Total	73	14 (19)	14 (19)	3 (21)
Age (years)				
20–49	67	12 (18)	12 (18)	1 (8)
≥50	6	2 (33)	2 (33)	2 (100)
Gender				
Male	45	8 (18)	8 (18)	3 (38)
Female	28	6 (21)	6 (21)	0 (0)
Employee types				
Local employee	4	1 (25)	1 (25)	0 (0)
No-local employee	69	13 (19)	13 (19)	3 (23)

^a Defined as HEV IgM+ with signs and symptoms for hepatitis.

^b The denominator was the number of the employees who were HEV IgM+.

histories of diet, drinking water, travel, contact with other persons infected with HEV, transplantation and transfusion and the clinical information including the date of disease onset, the symptoms and signs. The medical records were checked for those who were hospitalized to verify their clinical information. Verbal informed consents were obtained from all employees before interview and sample collection.

2.4. Laboratory testing

The blood samples were tested for HEV IgM and IgG using Wantai ELISA kit (Wantai Biological, Beijing, China) according to the manufactory's instructions. The sera and the stool samples from HEV IgM-positive employees were analyzed for HEV RNA by reverse transcription-nested PCR with primers designed within 644-bp region of open reading frame (ORF) 2 [7]. Phylogenetic analysis with 594 nt sequences of the ORF2 was performed to determine the HEV genotype using Mega 4.0 and the nucleotide similarity among the HEV isolations in the outbreak and other

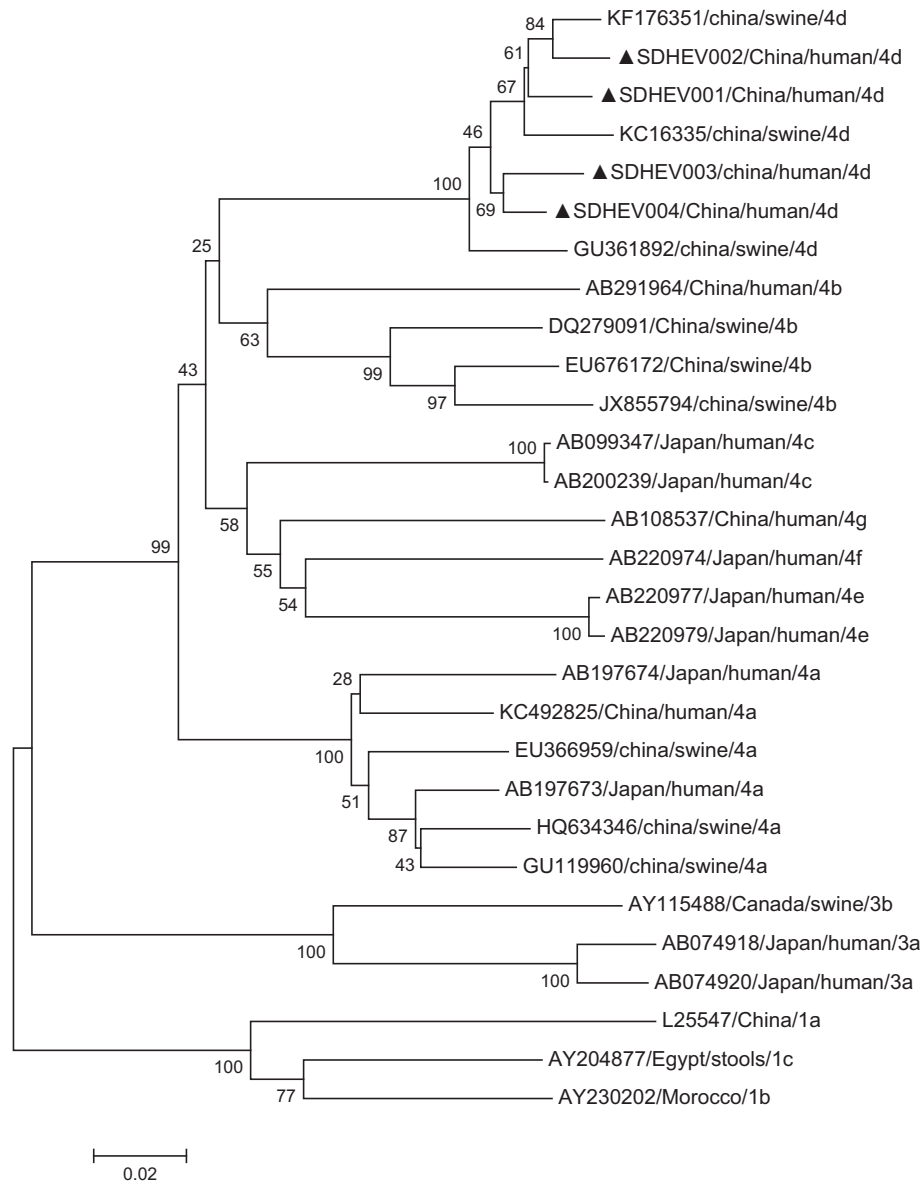


Fig. 1. Phylogenetic tree based on partial open reading frame (ORF) sequences of the hepatitis E virus monophyletic strain in Rizhao city of Shandong province, China. Using the neighbor joining method. Genetic distances were calculated using the Kimura two-parameter method.

HEV sequences downloaded from Genbank. No food samples were collected because the cafeteria had been thoroughly sanitized before investigation.

2.5. Data analysis

HEV recent acute infection was defined as positive HEV IgM, and symptomatic hepatitis E was defined as recent acute HEV infection with signs and symptoms compatible with hepatitis including acute jaundice, dark urine, anorexia, malaise, extreme fatigue and right upper quadrant tenderness. The distributions of HEV recent acute infection were examined by age and gender using Fisher's exact test and $P < 0.05$ was regarded as statistically significant.

3. Results

Fourteen (19%, 14/73) employees were positive for HEV IgM as defined as recent acute infection. There was no difference in the attack rates between those aged < 50 and ≥ 50 years (18% vs. 33%, $P = 0.32$), between males and females (18% vs. 21%, $P = 0.76$), or between the local and migrant employees (19% vs. 25%, $P = 1.00$) (Table 1).

Three employees were diagnosed with symptomatic hepatitis. The index case became ill on December 1, 2013 and died on January 9, 2014. The other two had disease onset on December 30, 2013 and January 9, 2014, respectively, and fully recovered soon. The clinical characteristics of these cases were shown in Table 2. The proportion of symptomatic infection was much higher among those aged ≥ 50 years than those aged < 50 years (2/2 vs. 1/12, $P = 0.03$), and higher in males than females (3/8 vs. 0/6, $P = 0.21$). Moreover, a cook of the company was positive for HEV IgM and her husband developed symptoms of hepatitis on January 5, 2014 and was diagnosed as symptomatic hepatitis E.

Four samples, three from symptomatic cases including the cook's husband and one from an asymptomatic case, showed positive for HEV RNA. The sequence data had been submitted to Genbank (Accession No. KT873295, KT873296, KT873297 and KT873298). All of them were classified into genotype 4, subgenotype d and the nucleotide similarity were 96–98% among them. The strains were also closely related to the strains KF176351

(Nucleotide similarity: 96.6–98.6%), KC16335 (Nucleotide similarity: 96.0–97.3%) and GU361892 (Nucleotide similarity: 94.6–96.0%), all of which were isolated from the swine of China (Fig. 1).

All HEV IgM-positive employees drank the bottled water provided by the company and usually dined in the cafeteria of the company, but most of them could not provide the details of the food. Thirty-two dinners outside the cafeteria were reported, but no dinner even included all IgM-positive employees. The IgM-positive cook frequently brought the food leftovers from the cafeteria and shared with her husband. No common travel history was found and nobody reported the histories of transfusion or transplantation.

4. Discussion

The determination of immunity to HEV usually depends on the detection of HEV IgG and antibody concentrations of 20 Walter Reed Units/ml are protective [8]. In this outbreak, all HEV IgG-positive employees had positive results for HEV IgM, which suggested that all employees in the company were negative for HEV IgG or only had low concentration of HEV IgG before the outbreak. This might be an important reason for the outbreak.

In this outbreak, field investigation indicated that the HEV parenteral transmission might not be the cause for this outbreak because no histories of transfusion or transplantation were reported. The transmission via contamination of drinking water might be impossible as the same water company supplied lots of local residents of Donggang District, and the number of hepatitis E cases reported to NNDRS during December 2013 and January 2014 was similar to what reported in the same period of past year in Donggang District. Moreover, the waterborne HEV usually is genotype 1 or 2 [9] while HEV in this outbreak is genotype 4. The contaminated food in the cafeteria might be the possible source of outbreak as all employees infected with HEV dined in the cafeteria during the potential exposure period and there was no meals outside of the cafeteria involved all IgM-positive persons. Moreover, the appearance of the case for the cook's husband who often ate the foods from the cafeteria during the same period further provided evidence that the foods in the cafeteria might have been contaminated with HEV.

Table 2

Clinical and demographic characteristics of the 4 symptomatic hepatitis E outbreak cases in Rizhao, Shandong Province, China.

Characteristic	Case 1 ^a	Case 2	Case 3 ^b	Case 4
Age, year/gender	51/Male	51/Male	42/Male	25/Male
Date of symptom onset	Dec 1, 2013	Dec 30, 2013	Jan 5, 2014	Jan 9, 2014
Symptoms	General malaise, fatigue, inappetence, dark urine, jaundice	Fever, inappetence, jaundice, dark urine	Fever, inappetence, nauseating, dark urine	Diarrhea, dark urine
ALT on admission, IU/L	9	580	839	104
AST on admission, IU/L	1649	40	356	43
TBil on admission, umol/L	303.66	30	132.39	25.8
Duration of hospitalization (days)	9	9	33	10
Clinical outcome	Death	Recovery rapidly	Recovery after artificial liver treatment	Recovery rapidly
Anti-HEV IgM	Positive ^c	Positive	Positive	Positive
Anti-HEV IgG	Positive ^c	Positive	Positive	Positive
HEV RNA	NA	Positive	Positive	Positive
HEV isolate no.	NA	SDHEV001	SDHEV002	SDHEV003
HBsAg ^c	Negative	Negative	Negative	Negative
IgM anti-HAV ^c	Negative	Negative	Negative	Negative
Anti-HCV ^c	Negative	Negative	Negative	Negative

NA: not applicable.

^a The death.

^b The husband of the cook.

^c The results were obtained from medical records in the hospital.

The four HEV strains isolated in this outbreak were closely related with the other strains obtaining from Shandong province of China, indicating that the individuals were infected by local strains. Although epidemiological data showed the outbreak was common-source, the molecular data suggested the cases were infected with closely related but different HEV strains [10,11]. It was difficult for the cases to recall the details of the food because of the long incubation period of the disease. According to the cafeteria's menu, seafood, pork and pig liver were provided before the outbreak, all of them had been reported as sources of HEV outbreak [12–14].

HEV infection can be asymptomatic or symptomatic, which could be severe or even life-threatening. In this outbreak, the proportion of symptomatic infection among all cases identified was similar to previous reports in China [9,10]. Moreover, the higher proportion of symptomatic infection among those aged above 50 years, including the death case, indicates elder population might be more likely to develop severe outcomes after HEV infection. All symptomatic HEV-infected individuals were males in the outbreak, which was in accordance with the previous study [15].

Therefore, those aged ≥ 50 years, particularly males, might be the population of top priority for vaccination in order to mitigate severe outcomes, in addition to reduce HEV infection.

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