Hepatitis E virus seroprevalence among employees in catering and public place industries in Nanjing, China

Jinlei Guo†, Juansheng Zhang‡, Lingling Li, Xingli Gao*

1. Qinhuai District Centre for Disease Control and Prevention, Nanjing, China.
2. Xi’an Centre for Disease Control and Prevention, Xi’an, China.

†Jinlei Guo and Juansheng Zhang contributed equally to this study.

* Corresponding author:

Xingli Gao, Qinhuai District Centre for Disease Control and Prevention, No.2, Baiguoshu Road, Nanjing, 210004, China.

E-mail: gaoxingli0130@163.com

Tel: 86-025-86531627

Fax: 86-025-86531627

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Abstract

This study aimed to assess the prevalence of anti-hepatitis E virus (HEV) immunoglobulin (Ig) M and elevated serum alanine aminotransferase (ALT) levels among employees in catering and public place industries. Blood samples were collected between January and December 2020 from 26790 employees working in Qinhui district of Nanjing, China. Anti-HEV IgM in the serum samples was tested by capture ELISA method and ALT was tested by IFCC method. Samples positive for anti-HEV IgM or with ALT levels over 200 U/L were subjected to PCR screening of HEV RNA. The overall seroprevalence of anti-HEV IgM was 0.41% and the seroprevalence was slightly higher in males (0.47%) than in females (0.37%), however did not present a substantial difference (P=0.177). Seroprevalence of anti-HEV IgM increased with age, reaching its peak level after 48 years of age. The prevalence of elevated ALT levels was 4.24% and males exhibited a higher prevalence than did females (6.78% vs 2.65%, P<0.001). Prevalence of elevated ALT levels differed in age groups and the 26-36-year-old group had the highest rate of elevated ALT levels. Employees with elevated ALT levels had a higher prevalence of positive anti-HEV IgM than that with normal ALT (0.57% vs 0.31%, P<0.001). Positive HEV RNA was detected in one anti-HEV IgM negative employee with ALT higher than 200 U/L. In our study, all the HEV RNA-positive and IgM-positive individuals are asymptomatic, the combination of ALT tests, serological and molecular methods are recommended to screen asymptomatic HEV carriers and reduce the risk of transmission.

Keywords: Seroprevalence; hepatitis E virus; immunoglobulin M; alanine aminotransferase; RNA
Introduction

Hepatitis E is a common infectious disease with a global distribution that is spread mainly through the fecal-oral transmission of hepatitis E virus (HEV). There are estimated to be 20 million HEV infections worldwide each year [1]. HEV is a non-enveloped, single-stranded, positive-sense RNA virus belonging to the Hepeviridae family. Currently, HEV are grouped to 8 genotypes (HEV1-8), of which HEV1-4 are the most frequent genotypes causing infections in humans. HEV1 and HEV2 are restricted to humans and transmitted via the fecal-oral route, mainly occur in developing regions and are associated with epidemic and sporadic hepatitis E infections. HEV3 and HEV4 infections mainly develop through zoonotic transmission that is caused by close contact with infected animals (such as pigs, goats, cats) or the consumption of contaminated food (most commonly raw or undercooked meat) and are common in industrialized countries [2]. In China, HEV1 was the most isolated genotype, however, HEV4 has overtaken HEV1 in frequency of isolation nationwide since the year 2000 probably due to improved sanitation and hygiene measures [3].

China used to be an endemic area for HEV, a massive outbreak of hepatitis E occurred in Xinjiang Uygur Autonomous Region of China from 1986 to 1988, which was one of the world’s largest epidemics of hepatitis E with 119280 cases and 707 deaths [4]. With the improved sanitation and the introduction of vaccines, China is now a low HEV endemic country with an incidence of 1.36 reported cases/100000 inhabitants/year according to the National Notifiable Infectious Disease Report of 2020 [5]. However, most people infected with hepatitis E are asymptomatic and they do not
go to hospital for a physical examination, the report may underestimate the true infection rate of the virus. A recent investigation revealed a high incidence of sporadic HEV infections in Shanghai, China [6]. Hepatitis E are foodborne diseases, food prepared by food handlers during their infectious period may put consumers at risk for HEV infection. In 2018, HEV caused an outbreak among mechanical factory workers in China because of eating contaminated pig livers [7]. Besides, person-to-person transmission of HEV was also confirmed in the HEV outbreak [8]. Public places are places where people gather to study, work, travel, rest, entertain, go shopping and so on. Employees working in the public places industries [9] such as the museum, shopping mall, hotel, cinema and barbershop who provide service to the public directly play an important role in the spread of the viruses. Since infections are mostly asymptomatic, asymptomatic HEV carriers can spread the viruses to susceptible populations during the acute infection period, leading to the emergency of HEV epidemics. Thus, the surveillance of HEV acute infections in those employees is important for HEV control.

Diagnosis of acute HEV infection usually depend on the detection of specific anti-HEV immunoglobulin (Ig) M antibodies in a person’s blood. However, the antibody test may leave out the suspected samples during the window period when the IgM is negative. Elevated serum alanine aminotransferase (ALT) levels which reflect the evidence of liver injury are helpful in the screening of the negative anti-HEV IgM samples [10], so ALT tests are performed to improve the HEV detection rates in our study.

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Currently, there is no routine HEV surveillance in the hospitals in China, but only monitoring of patients with liver disease. Recent investigations on HEV mainly focused on the IgG antibodies which indicated the previous infections [11], or focused on the seroprevalence of IgM among blood donors or pregnant women [12, 13]. Our study focused on the surveillance of acute HEV infections of catering and public place industries employees, trying to evaluate the risk of the HEV epidemics and propose prophylactic interventions for effective control of HEV.
Methods

Study subjects

Subject recruitment information of our programme was posted via internet, newspapers and television advertising. During the period of January to December 2020, subjects who were ≥16 years and employed in catering and public place industries in Qinhuaï district, the most densely populated district of Nanjing, eastern China, were included to detect the recent infections of HEV. The screening programme relied on subjects volunteering to come forward of their own volition. The demographic information of all subjects such as the age and sex were collected according to identity card number. For the subjects who are tested positive for anti-HEV IgM or with ALT levels over 200 U/L, the face to face interviews were carried out by trained personnel to collect further information on symptoms (such as jaundice, nausea, fever, anorexia, etc), travel histories (whether they have been to HEV epidemic areas in the past 15-75 days away from the sample collection day), and specific job types. Informed consent was obtained from each subject. Ethical approval from the Ethical Committee of our institution had been obtained. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Specimen collection, HEV IgM antibody and ALT test

Five milliliters of venous blood were drawn from each subject. Blood samples were left for 3-5 hours at room temperature to allow clotting and then centrifuged to separate the serum for the test of HEV IgM antibody and ALT on the sample collection day.
Commercially available ELISA kits (Wantai Biological Pharmacy Enterprise Co., Ltd, Beijing, China) were used to detect the anti-HEV IgM in serum samples. The positive criterion for anti-HEV IgM was based on the manufacturer’s instructions; optical density (OD) values were measured at 450 nm/630 nm. The cut-off value was determined based on the mean OD value of the negative control (NC) by the following formula: Cutoff value = 0.26+NCmean. Test samples with OD values equal to or greater than cutoff value indicated a positive sample. The serum biochemical parameter ALT was tested by IFCC method using the ALT detection kits (Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China). According to the Chinese Health Industry Standard (WS/T404.1-2012), males over 50 U/L or females over 40 U/L were classified as individuals who featured elevated ALT levels. All the kits are within their validity period and used according to the manufacturer’s instructions.

**HEV RNA detection**

Serum samples positive for anti-HEV IgM or with ALT levels >200 U/L between January and December 2020 were stored at -20 °C for the test of HEV RNA by real-time reverse transcription polymerase chain reaction (RT-PCR) at the beginning of October 2022. Here we adopt the ALT>200 U/L as the cutoff point for HEV RNA test based on the previous studies [10, 14]. RNA was detected with commercially available HEV RNA detection kit (ACON, Hangzhou, China) with the lowest detection limit of 500 copies/mL. Viral nucleic acids were extracted from 100 µL of serum. The RT-PCR included 20 µL viral nucleic acids, 0.6 µL primers and fluorescence probe mixture, 1.4 µL enzyme and 18 µL RT-PCR mix. The RT-PCR amplification was carried on the
LightCycler 480II real-time PCR instrument (Roche, Basel, Switzerland) based on the following condition: 50 °C for 20 min and 70 °C for 15 min, followed by 45 cycles of pre-denaturation step at 94 °C for 15 s and extension at 60 °C for 30 s. Fluorescence signal was collected at 60 °C. Samples that had evidence of amplification were subjected to a conventional RT-PCR as previously described to determine the genotype [6].

Statistical analysis

IBM SPSS Statistics version 17.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Categorical data were tested using Pearson’s chi-square ($\chi^2$) test and a two-sided p value of less than 0.05 was considered statistically significant.
Results

Seroprevalence of anti-HEV IgM

A total of 26790 catering and public place industries employees working in Qinhuai district of Nanjing, were enrolled in our study for the screening of anti-HEV IgM, which aged between 16-79 years with a median age of 36 years, consisting of 16432 (61.3%) females and 10358 (38.7%) males.

In total, one hundred and nine employees were tested positive for anti-HEV IgM antibodies, which corresponding to a seroprevalence of 0.41% (95% confidence interval [CI], 0.34-0.49). The seroprevalence revealed a statistically significant increase with study-subject age by means of the Chi-square test for linear trend ($\chi^2=18.599$, P=0.000) (Table 1). The seroprevalence of anti-HEV IgM was slightly higher in males (0.47%, 49/10358) than that of females (0.37%, 60/16432), however did not present a substantial difference ($\chi^2=1.826$, P=0.177) (Table 1) (Figure 1).

All the 109 subjects with positive HEV IgM were asymptomatic and 11.0% (12/109) of them had elevated ALT levels. The age of the positive subjects ranged from 17 to 67 years with a median age of 42 years, consisting of 49 males and 60 females. 48.6% (53/109) of them were employed in catering industries. 35.8% (39/109) of the subjects were females in the childbearing age (18-49 years old). None of the females with positive HEV IgM were pregnant at the time of the blood collection. In our study, a total of 12591 subjects were females in the childbearing age (18-49 years old), among them, 39 females were HEV IgM positive; While the number of the rest of study population was 14199, of which 70 subjects were HEV IgM positive. Statistical
analysis showed that HEV positivity in females in the childbearing age (39/12591, 0.31%) is lower than that in the rest of study population (70/14199, 0.49%) ($\chi^2=5.53, P=0.019$).

**The correlation of elevated serum ALT level and the hepatitis E infection**

The ALT is abundant in liver cells and elevated serum ALT levels that reflect the evidence of liver injury can be used to improve the HEV detection rates. Our result showed 4.24% (1137/26790) of the employees were identified with elevated ALT levels (males higher than 50 U/L or females higher than 40 U/L) according to the reference intervals for common clinical biochemistry tests in China (WS/T 404.1-2012). Elevated ALT levels varied from 41 U/L to 424 U/L. The prevalence of elevated ALT levels was significantly higher in males than that of females (6.78% vs 2.65%, $\chi^2=266.662, P<0.001$). Statistics analysis showed that the prevalence of elevated ALT levels differed in different age groups, of which the 26-36-year-old group had the highest rate of elevated ALT level (Table 1). Positive HEV IgM was detected both in employees with elevated ALT levels and those with normal ALT. In the elevated ALT group, 12 samples were positive for anti-HEV IgM, corresponding to a prevalence of 1.05% (12/1137). While among the 25653 serum samples with normal ALT, 97 (0.38%) samples were positive for anti-HEV IgM. Employees with elevated ALT levels had a higher prevalence of anti-HEV IgM than those with normal ALT (1.05% vs 0.38%, $\chi^2=12.325, P<0.001$)

**PCR detection of HEV RNA**

The serum samples positive for anti-HEV IgM or with ALT levels over 200 U/L were
subjected to real-time RT-PCR screening of HEV RNA.

Of the 109 anti-HEV IgM positive subjects, 105 serum samples tested for HEV RNA were RNA negative, while HEV RNA tests of the remaining 4 subjects were not performed because of limited serum samples.

There are 14 samples with elevated ALT levels higher than 200 U/L in our study, of which 11 samples were subjected to HEV RNA screening (the remaining 3 samples did not have enough serum to do the test), among the tested, one sample was RNA positive. The RNA positive employee was anti-HEV IgM negative and asymptomatic with an elevated ALT level of 312 U/L, indicating that she was in the window period of HEV acute infection. With no history of recent travel in HEV endemic areas or eating raw meat, she worked in a fast food restaurant and prepared raw meat in the daily work, besides she kept a pet cat in her house. Unfortunately, due to the low viral load in the serum sample, we were unable to obtain the sequences and determine the genotype.
Discussion

Located in the Yangtze River Delta region of China, Nanjing is the provincial capital of Jiangsu, of which Qinhuai district is the most densely populated with prosperous business and tourism attracting large numbers of migrant workers and tourists each year. Here we investigate the catering and public place industries employees working in the Qinhuai district who provide service to the public directly, and try to provide a view of the recent infections of HEV in the employees and help evaluate the risk of the HEV epidemics in the local residents.

Anti-HEV IgM is the first antibody that appears after HEV infection, the positive IgM is an important marker of recent HEV infection and is increasingly used in routine diagnosis of hepatitis E since its reliability has been dramatically increased by using antigens with immunodominant epitopes of HEV [15]. The overall prevalence of HEV IgM for the local catering and public place industries employees in Nanjing was 0.41%.

In order to gain a better analysis of the prevalence of anti-HEV IgM in our area, we reviewed recent published representative reports from domestic and international regions, as listed in Table 2. The prevalence of anti-HEV IgM in different studies has a high heterogeneity, ranging from 0.41% to 3.8%. This discrepancy in prevalence of anti-HEV IgM may be influenced by different test systems with varying sensitivities, effects of subjects selection such as exposure to contaminated food sources (raw meat) or contact to certain animal species (pigs, wild boars and so on), study areas with varying levels of hygiene and local sanitation infrastructure. Additionally, the discrepancy in seroprevalence results across locations could be attributed to the actual
intensity of HEV infection and circulation in each geographic area. Our investigation indicated that employees in catering and public place industries in Nanjing had a low seroprevalence of anti-HEV IgM, which is comparable to study populations in Italy and Portugal [16, 17], and lower than the swine workers of Guangzhou [18] or healthy individuals of Nigeria [19], or the general populations (1.8%) in mainland China according to a meta-analysis [20]. Nanjing is a developed city with high socioeconomic status and good sanitary conditions, which may explain the low HEV infection rate of the local catering and public place industries employees. Although our results showed the HEV seroprevalence was higher in men than in women, it was not considered statistically significant, similar to some previous studies [17, 19, 21], whereas other report provided a contrasting result [13]. Thus, whether HEV infection exhibits gender bias or not needs further verification. None of the positive anti-HEV IgM samples tested in our study had detectable RNA, and similar observations have been described previously in some studies [13, 22, 23]. We speculate there are several possibilities: firstly, the subjects in our study are apparently healthy individuals without symptoms and the blood samples might be taken in the convalescent period when the viremia subsides as the period of viremia lasts very short while IgM which is produced early in acute phase can persist for a relatively longer time until and after the infection resolves [24]. Secondly, HEV RNA was detected retrospectively in our study and RNA in stored serum may degrade over time. Besides, some subjects might be reactive to anti-HEV IgM non-specifically due to unknown reasons. Although the RNA is the direct evidence of acute infection, there are evidences show HEV of 20%-30% of the infected persons
are cleared when symptoms develop [25], thus the negative RNA results cannot exclude
the acute infection. Our results show the likelihood of contracting HEV increases with
age, reaching peak level after 48 years of age, which are in accord with the previous
investigations [19, 26]. In China, the aging of the population is becoming more and
more serious, the elderly tend to have a variety of chronic diseases such as diabetes,
hypertension and heart diseases, and have a higher chance of developing severe
hepatitis E. It is worth noting that 35.8% of the anti-HEV IgM positive study subjects
are females in the childbearing age in our study, while infections in pregnant women
usually have more severe consequences [2]. This underscores the importance of
vaccination and surveillance of HEV in the elderly and the childbearing age women
who are at high risk.

The ALT and HEV RNA testing are combined to screen HEV in the window period
of negative IgM in our study. We adopt the ALT>200 U/L as the cutoff point for HEV
RNA test based on the previous studies. Positive HEV RNA was detected in one anti-
HEV IgM negative employee with elevated ALT of 312 U/L among the tested 11
employees with ALT levels higher than 200 U/L, however we cannot determine the
genotype due to the low viral load of the serum sample. The routes of HEV transmission
of the RNA-positive employee are not clear as she had no history of recent travel in
HEV endemic areas or eating raw meat, and we speculate that the occupational contact
with raw pork meat might result in her infection as the HEV is a zoonotic virus.

Consistent with the results of the previous study [27], our results reveal that male
gender or a younger age group (26-36-year-old) is associated with a high likelihood of
elevated serum ALT levels. It has been shown that high estradiol level and estrogen receptors in the liver can protect females from the acute liver injury [28]. Besides, alcohol and staying up late which may cause liver injury are the general lifestyles of the modern young people in urban China, and men are more likely to engage in heavy drinking [29, 30]. Taken together, this may partly help explain why males and the younger age group had a higher prevalence of elevated ALT levels. However, other associated risk factors that cause the liver injury deserve further investigation. The two groups should receive screening for liver function more regularly in order to avoid serious liver injury.

There were some limitations in our study. First, the epidemiological data of the study subjects were scarce. Therefore, we could not comprehensively evaluate the risk factors. Second, the job types of the majority of the subjects were not distinguished between catering industries and public place industries when they were enrolled, the lack of the information disabled us to separate out the seroprevalence by type of industry and assess the effects of job types on the HEV infection, which we will improve in the future investigations. Besides, we could not determine the HEV genotypes circulating in the local area as we could not obtain the virus sequences. However, it is possible that most prevalent strains circulating in the local residents are genotype 4 according to a previous report of Nanjing [31].

In China, the hepatitis E vaccine was licensed in 2012. In 2020, only about 100000 HEV vaccines were issued by National Institutes for Food and Drug Control in China (https://bio.nifdc.org.cn/pqf/search.do), suggesting a very low HEV vaccination rate of
Chinese people. A recent meta-analysis show that overall prevalence of HEV IgG seropositivity was only 27.3% among the general population in mainland China [20]. The anti-HEV IgM seroprevalence (0.41%) of the subjects indicates a low HEV endemicity state of Nanjing, China. However, possible epidemics may break out once there are imported HEV cases since most of the local individuals are antibody-naïve due to the lack of naturally acquired immunity and the vaccination of HEV. In our study, all the HEV RNA-positive and IgM-positive individuals are asymptomatic who may act as a major source of transmission, thus a comprehensive surveillance of HEV that combines the ALT tests, serological and molecular method is recommended to screen asymptomatic virus carriers and help propose prophylactic interventions for effective control of HEV.
Conflict of interest

The authors declare that they have no competing interests.

Acknowledgments

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Author contribution

X.G. conceived and designed the study; J.G., L.L., X.G. performed the experiments; J.G., J.Z. performed data acquisition, statistical analysis, data interpretation and the original draft writing. All authors have approved the final article version submitted.

Data availability statement

The data that support the findings of this study are available upon request from the corresponding author.
References


Table 1. Prevalence of hepatitis E virus and elevated ALT levels by gender and age among 26790 employees in Nanjing, 2020.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Sample Size</th>
<th>Elevated ALT level</th>
<th>Anti-HEV IgM positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Prevalence (%)</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10358</td>
<td>702</td>
<td>6.78</td>
</tr>
<tr>
<td>Female</td>
<td>16432</td>
<td>435</td>
<td>2.65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sample Size</th>
<th>Elevated ALT level</th>
<th>Anti-HEV IgM positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Prevalence (%)</td>
<td>P-value</td>
</tr>
<tr>
<td>16-25</td>
<td>6906</td>
<td>282</td>
<td>4.08</td>
</tr>
<tr>
<td>26-36</td>
<td>6783</td>
<td>340</td>
<td>5.01</td>
</tr>
<tr>
<td>37-47</td>
<td>6760</td>
<td>270</td>
<td>3.99</td>
</tr>
<tr>
<td>≥48</td>
<td>6341</td>
<td>245</td>
<td>3.86</td>
</tr>
<tr>
<td>Total</td>
<td>26790</td>
<td>1137</td>
<td>4.24</td>
</tr>
</tbody>
</table>

ALT= alanine aminotransferase; Anti-HEV IgM= Hepatitis E virus immunoglobulin M antibody; Elevated ALT level= males higher than 50 U/L or females higher than 40U/L.

The percentages (%) was calculated by dividing the number of subjects with elevated ALT level or seropositive for IgM by the total number of subjects tested in each gender or age group.
<table>
<thead>
<tr>
<th>Stud</th>
<th>Region</th>
<th>Year</th>
<th>Subjects</th>
<th>Nu</th>
<th>Preva</th>
</tr>
</thead>
<tbody>
<tr>
<td>[12]</td>
<td>Nanjing,</td>
<td>2020</td>
<td>Catering or public place industry employees who</td>
<td>267</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>InnerMong</td>
<td>2018</td>
<td>Pregnant women who visited the Inner Mongolia</td>
<td>327</td>
<td>0.6%</td>
</tr>
<tr>
<td>[18]</td>
<td>Guangzhou, China</td>
<td>2015</td>
<td>Swine workers who are ≥16 years and exposed to pigs over 5 h/week for over 10 weeks are included and those who are immunocompromised or pregnant</td>
<td>332</td>
<td>2.71%</td>
</tr>
<tr>
<td>[23]</td>
<td>Southern Brazil</td>
<td>2013</td>
<td>Risk populations (crack users, low-income area residents, cirrhotic and liver transplant patients) who</td>
<td>400</td>
<td>1.5%</td>
</tr>
<tr>
<td>[21]</td>
<td>Shiraz, Iran</td>
<td>2011</td>
<td>Healthy populations without ongoing infectious</td>
<td>103</td>
<td>0.9%</td>
</tr>
<tr>
<td>[16]</td>
<td>Italy</td>
<td>2017</td>
<td>Blood donors</td>
<td>717</td>
<td>0.5%</td>
</tr>
<tr>
<td>[17]</td>
<td>Portugal</td>
<td>2015</td>
<td>General populations</td>
<td>165</td>
<td>0.48</td>
</tr>
<tr>
<td>[19]</td>
<td>Osun State,</td>
<td>2015</td>
<td>Apparently healthy individuals from 6 communities</td>
<td>653</td>
<td>3.8%</td>
</tr>
</tbody>
</table>

*: the year of sampling
Figure legends

Figure 1. Age and gender distribution of hepatitis E prevalence among males (open block) and females (shaded block).