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Hepatitis E seroprevalence and related risk factors among seafood processing workers: a cross-sectional survey in Shandong Province, China

Weihong Cui [a], Yuan Sun [a], Aiqiang Xu [b], Rihong Gao [a], Lianfeng Gong [a], Li Zhang [b], Mei Jiang [a,*]

[a] Yantai Center for Disease Control and Prevention, 17 Fuhou Road, Yantai, Shandong 264003, PR China
[b] Shandong Center for Disease Control and Prevention, Jinan, Shandong, PR China

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SUMMARY

Objective: This study aimed to assess the seroprevalence of hepatitis E virus (HEV) infection and the potential risk factors for acquiring HEV infection in the seafood processing factories in Yantai City of Shandong Province, China.

Methods: A cross-sectional study was conducted in five randomly selected seafood processing factories in Yantai City. Subjects were 15–66 years of age and were raw seafood processing workers, semi-finished products processing workers, and administrative staff, etc. Each participant completed a structured questionnaire and agreed to blood drawing. Anti-HEV IgG antibody was detected in the blood samples by ELISA method.

Results: A total of 1028 of 1044 eligible workers were tested for HEV IgG antibody (response rate 98.5%). The prevalence of HEV IgG seropositivity was found to be 22.20%. Occupation was significantly associated with anti-HEV IgG antibody seropositivity ($p < 0.05$). Subjects who had direct contact with raw seafood had a higher anti-HEV IgG antibody prevalence (32.54%) than the semi-finished products processing workers (24.74%) and less exposed group (11.85%). HEV seroprevalence in the workers showed an increasing trend with the increase in working years, and this phenomenon was most obvious in raw seafood processing workers.

Conclusions: There is a higher risk of HEV infection in those who have direct contact with raw seafood. This study will help identify the risk factors for HEV infection and provide guidance on controlling HEV infection in the seafood processing occupations.

1. Introduction

Hepatitis E, an enterically transmitted acute hepatitis caused by hepatitis E virus (HEV), is widely endemic in many regions of the world,[1] especially in developing countries, where it causes large waterborne epidemics.[2] This disease is primarily transmitted by fecal–oral route.[3] Furthermore, HEV is now recognized as a zoonotic virus.[4] Hepatitis E is an infection that may often lead to an asymptomatic but self-limited acute hepatitis.[5] According to the China National Notifiable Disease Reporting System, the incidence of hepatitis E cases in Shandong has been increasing in the past decade; an increase from 0.13 per 100 000 in 1998 to 1.64 per 100 000 in 2013 was noted, and this phenomenon was most obvious in the coastal region.

Recent studies have suggested that coastal waters can be contaminated by HEV, leading to an accumulation of the virus in the digestive tissues of shellfish.[6,7] This increases the risk of human infection by ingestion.[8] Immune-compromised individuals and those in occupations requiring direct contact with the contaminated seafood might be at high risk of suffering from infectious diseases, including hepatitis E.

Research into HEV infection among seafood processing workers appears to be extremely limited. Thus, this cross-sectional study was conducted to estimate the seroprevalence of HEV infection and the potential risk factors for HEV infection in seafood

* Corresponding author. Tel.: +86 535 6700926.
E-mail address: meijiang8902@163.com (M. Jiang).

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processing workers in a coastal city, Yantai City of Shandong Province, eastern China.

2. Materials and methods

2.1. Population and sample collection

The research was carried out in Yantai City of Shandong Province, which is located in the eastern coast area of China (36°16′N–38°23′N, 119°34′E–121°57′E). This city has the biggest fishing harbor in Shandong Province and the seafood processing industry is currently an important economic activity.

In Yantai, there are an estimated 52 seafood processing factories registered with the Yantai Tax Bureau. Among these, five factories processing fish, crabs, and shellfish were selected at random and agreed to participate in the study. All persons employed by the factories during the period December 2014 to April 2015 were invited to participate in the study using the cluster sampling method. All participants signed written informed consent and agreed to provide study samples. This study was approved by the Ethics Committee of Shandong Provincial Center for Disease Control and Prevention.

To estimate the prevalence of anti-HEV IgG antibody in the seafood processing workers of Yantai, blood samples were collected from the participants at the five factories. The study population was categorized into three groups: raw seafood processing workers, semi-processed seafood workers, and low exposure workers including administrative staff, warehouse workers, and packers. In the factory, warehousemen transport raw seafood to the processing shop. There, raw seafood processing workers clean and fillet the seafood and cut them into slices. After this, some workers process the semi-finished products, e.g. sousing and barbecuing. In the final stage, packers pack the highly treated seafood. The employees who were in less contact with the raw seafood and worked in other premises (warehousemen, packers, and administrative staff) were included in the 'low exposure' group, and were considered as the reference group.

A total of 5 ml of venous blood was drawn from each participant. Blood samples were left for 3–5 h at room temperature to allow clotting and then centrifuged at 2500 rpm for 15 min. The serum samples were stored at −20 °C until testing. Testing was performed using ELISA kits (Wantai Biological Pharmacy Enterprise, Beijing, China) for the qualitative detection of anti-HEV IgG in serum samples, according to the manufacturer's instructions.

2.2. Data collection

A structured questionnaire was designed for data collection. The questionnaire contained questions on socio-demographic characteristics (name, age, sex, education level, duration of residence in the area, and occupation) and epidemiological characteristics (working years, the source of potable water, eating habits, and hygiene habits, such as hand-washing). The questionnaires were administered by well-trained interviewers who had no knowledge of the main hypotheses of the study.

2.3. Statistical analysis

Epidata 3.1 was used to build a database (Jens M. Lauritsen, Odense, Syddanmark, Denmark). Double-entry and logistic consistency checking was applied to ensure the accuracy of data. SPSS version 17.0 statistical software was used for the statistical analysis (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as the mean and standard deviation, and the Student *t*-test, one-way analysis of variance (ANOVA), and Mann–Whitney test were applied to compare the means between the groups. Categorical data were summarized as frequencies and proportions, and Pearson's Chi-square test or Fisher's exact test was applied to determine the statistical significance in prevalence between groups in the univariate analysis. To determine the correlation between the data obtained from the questionnaire and the laboratory results, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by binary logistic regression analysis. A two-sided p-value of <0.05 was accepted as statistically significant.

3. Results

In the survey, a total 1028 of 1044 eligible workers provided serum samples and were interviewed (response rate 98.5%), including 507 (49.32%) males and 521 (50.68%) females. They ranged in age from 15 to 66 years (mean 31.43 ± 10.25 years for males and 39.02 ± 9.51 years for females). Of the 1028 subjects, 228 (22.20%) were found to be positive for anti-HEV IgG antibody by ELISA. The mean age of raw seafood processing workers, semi-processed seafood workers, and low exposure workers including administrative staff, warehouse workers, and packers was 33.52 ± 10.55, 37.01 ± 10.68, and 35.49 ± 10.33 years, respectively; the difference was statistically significant ($p < 0.05$).

Table 1 shows the seroprevalence distribution of anti-HEV IgG antibody among the seafood processing workers in relation to the associated socio-demographic and epidemiological characteristics. Univariate analysis showed that there was no significant difference in sex, duration of residence in the area, frequency of eating seafood, frequency of washing hands before eating, and frequency of drinking unboiled water between the anti-HEV IgG-positive group and the negative group. However, significant differences were observed in age, working years, occupation, and education level between the two groups (Table 2).

The results showed that the individuals who were anti-HEV IgG antibody positive were older than those who were negative (Table 1) (37.03 years vs. 34.77 years, $p < 0.01$). The subjects processing raw seafood who had direct contact with raw seafood had a higher anti-HEV IgG antibody prevalence (32.54%) than the semi-finished products processing workers (24.74%) and less exposed group (11.85%). On close observation of working years associated with anti-HEV IgG antibody seroprevalence, it was found that HEV seroprevalence showed an increasing trend with the increase in working years. In addition, there was a significant association between education level and HEV seroprevalence ($p < 0.01$), and it was found that there was a higher risk of HEV infection for those with a lower level of education.

Multivariate unconditional logistic regression was applied to estimate the association between the risk of HEV infection and the factors above. The results showed the following factors to be significantly associated with HEV infection (Table 2): (1) age 40–49 years (OR 2.34, 95% CI 1.100–4.988), (2) working years 3–7 (OR 2.50, 95% CI 1.489–4.187) and working years ≥7 (OR 3.69, 95% CI 2.156–6.307), (3) raw seafood processing workers (OR 3.82, 95% CI 2.536–5.764) and semi-finished products processing workers (OR 2.62, 95% CI 1.696–4.043).

On trend test analysis, the probability of anti-HEV IgG antibody seropositivity increased significantly with the number of years working on raw seafood processing and semi-finished products processing (p for trend <0.05; Figure 1).

4. Discussion

HEV, the causative agent of hepatitis E, is an important public health problem in many industrialized countries and many developing countries, such as Africa and Asia.[9–11] The transmission of HEV infection is primarily by fecal–oral route through contaminated food or water.[4] Coastal waters can also be

Table 1
Characteristics of subjects who were positive and negative for the anti-HEV IgG antibody among the seafood processing factory workers

Subject characteristics	Anti-HEV IgG-positive (n = 228)				Anti-HEV IgG-negative (n = 800)			
	Mean	SD	n	%	Mean	SD	n	%
Sex								
Male			117	51.30			390	48.75
Female			111	48.70			410	51.25
Age, years	37.03	10.16			34.77	10.65		
Duration of residence in the area, years	11.74	13.64			10.38	12.74		
Working years	5.61	5.67			4.40	4.70		
Occupation								
Raw seafood processing			109	47.81			226	28.25
Semi-finished products processing			71	31.14			216	27.00
Less exposed jobs			48	21.05			357	44.63
Education								
Illiterate			22	9.65			32	4.02
Primary school			39	17.11			134	16.81
Junior middle school			128	56.14			436	54.71
Senior high school			27	11.84			132	16.56
Junior college			9	3.95			40	5.02
Bachelor degree or above			3	1.32			23	2.89
Frequency of eating seafood								
≥3 times a week			34	14.91			88	11.00
<3 times a week			194	85.09			712	89.00
Frequency of washing hands before eating								
≥2 times a day			222	97.37			776	97.24
<2 times a day			6	2.63			22	2.76
Frequency of drinking unboiled water								
≥3 times a week			17	7.46			62	7.76
<3 times a week			211	92.54			737	92.24

HEV, hepatitis E virus; SD, standard deviation.

contaminated by HEV, leading to the accumulation of the virus in the digestive tissues of shellfish, which increases the risk of human infection by ingestion.[8] HEV (genotype 3) has been reported to exist in bivalve mollusks in Japan, Thailand, and the UK since 2007.[6,12] In addition, as a result of the thermal stability of HEV, raw, rare-cooked, or slightly steamed contaminated seafood can transmit HEV to consumers.[13, 14] Jiang et al. have reported that the morbidity caused by HEV has been increasing over the recent decade and is centered on the coastal region.[15] All of this suggests that close contact with seafood may be a risk factor for HEV infection.

While many recent studies have investigated the prevalence of HEV infection in the general population and in animals serving as a reservoir for HEV, the prevalence of HEV infection in the related seafood processing occupations, in those who have close contact with seafood, has not been examined. Thus, this cross-sectional study was conducted in seafood processing factories to estimate the seroprevalence of HEV infection and the potential risk factors for acquiring HEV infection in this occupational population in Yantai City of Shandong Province, a coastal city of eastern China.

In this study, 1028 blood samples were collected from the seafood processing factories in Yantai City and the seroprevalence of HEV infection was found to be 22.20%. This is close to the seroprevalence of HEV infection in employees in the food trade in Yantai, but much higher than the 16.90% seroprevalence recently reported in blood donors in East China and 13.10% reported in Gansu Province of inland China.[16–18] Altogether, these results indicate that there is a higher HEV prevalence in the coastal region. In addition, this study showed a high anti-HEV seroprevalence in the age groups >20 years and that HEV prevalence increased with age. Furthermore, males were found to have a higher prevalence of HEV infection than females, which is consistent with the study by Jiang et al.[15] This study showed that those with a higher level of education had a lower seroprevalence of HEV infection, which might be related to preventive measures taken by them due to a high acceptance of health education for hepatitis E.

On multivariate analysis, it was shown that working with raw and semi-finished seafood was significantly associated with a positive serology, and the highest anti-HEV seroprevalence was found in the workers who had direct contact with raw seafood. Furthermore, a greater number of years working with raw seafood was associated with a higher seropositivity risk, suggesting that there is a greater probability of HEV exposure and higher risk of HEV infection for the long-term directly exposed worker. Moreover, the populations in jobs less exposed to the contaminated seafood products (including administrative staff, warehousemen, and packers) were less likely to develop a humoral response to HEV. Although prolonged exposure to raw seafood was associated with seropositivity in this study, it was not predictive. Many workers with almost identical exposure to raw seafood and a similar length of time working at the factory as those who were seropositive, showed a negligible antibody titer. This suggests that the antibody response varies from person to person, even within the exposure group. The current study applied trend test analysis to elaborate the seroprevalence of HEV infection with working years among the different types of occupation in order to estimate the association between HEV infection and contact with seafood. Of note, the results showed that the probability of anti-HEV IgG antibody seropositivity increased significantly with the number of years working on raw seafood processing and semi-finished products processing (p for trend <0.05).

The detection of anti-HEV IgG in this study was based on the polymerized recombinant antigen in human serum and was performed using Wantai HEV IgG ELISA kits, which cover the main conformational epitope of HEV. Studies have confirmed that the sensitivity and specificity of this reagent are better than those of the traditional reagents (98% and 99.6%, respectively).[19, 20] Thus, the seroprevalence of HEV infection detected by the kits is close to the real seroprevalence in the population.

In conclusion, this is the first study to investigate HEV seroprevalence risk factors for HEV infection among the seafood processing occupations in eastern China. The results demonstrate

Table 2
Unadjusted (univariate analysis) and adjusted (multivariate analysis) odds ratios and 95% confidence intervals for anti-HEV IgG antibody seroprevalence by socio-demographic and epidemiological characteristics among seafood processing factory workers

Subject characteristics	No. of subjects	No. of positive antibody	Anti-HEV IgG antibody prevalence (%)	*p*-Value	OR (95% CI)	aOR[a] (95% CI)
Sex						
Male	507	117	23.10	0.550	1.10 (0.817–1.470)	1.44 (1.014–2.040)
Female	521	111	21.50		1	1
Age, years						
<20	86	11	12.79	0.042	1	1
20–29	287	58	20.21		1.73 (0.862–3.461)	1.88 (0.901–43.911)
30–39	254	55	21.65		1.88 (0.936–3.794)	1.78 (0.828–3.807)
40–49	331	90	27.19		2.51 (1.273–4.939)	2.34 (1.100–4.988)
≥50	67	14	20.90		1.80 (0.759–4.275)	1.71 (0.665–4.390)
Duration of residence in the area, years						
<3	406	84	20.69	0.338	1	
3–10	302	64	21.19		1.03 (0.715–1.486)	
≥10	320	80	25.00		1.28 (0.759–4.275)	
Working years						
<1	218	28	12.84	0.001	1	1
1–3	220	40	18.18		1.51 (0.893–2.547)	1.58 (0.909–2.738)
3–7	300	71	23.67		2.10 (1.305–3.393)	2.50 (1.489–4.187)
≥7	290	89	30.69		3.01 (1.880–4.801)	3.69 (2.156–6.307)
Occupation						
Raw seafood processing	335	109	32.54	0.001	3.59 (2.458–5.236)	3.82 (2.536–5.764)
Semi-finished products processing	287	71	24.74		2.45 (1.633–3.659)	2.62 (1.696–4.043)
Less exposed jobs	405	48	11.85		1	1
Education						
Illiterate	54	22	40.74	0.009	5.27 (1.408–19.729)	3.76 (0.874–16.199)
Primary school	173	39	22.54		2.23 (0.636–7.826)	1.76 (0.440–7.057)
Junior middle school	564	128	22.7		2.25 (0.665–7.617)	1.57 (0.414–5.910)
Senior high school	159	27	16.98		1.57 (0.439–5.597)	1.04 (0.265–4.068)
Junior college	49	9	18.37		1.73 (0.424–7.021)	1.61 (0.362–7.147)
Bachelor degree or above	26	3	11.54		1	1
Frequency of eating seafood						
≥3 times a week	122	34	27.87	0.131	1.42 (0.926–2.172)	
<3 times a week	906	194	21.41		1	
Frequency of washing hands before eating						
≥2 times a day	998	222	22.24	0.918	1.05 (0.420–2.619)	
<2 times a day	28	6	21.43		1	
Frequency of drinking unboiled water						
≥3 times a week	79	17	21.52	0.879	0.96 (0.548–1.673)	
<3 times a week	948	211	22.26		1	

HEV, hepatitis E virus; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval;
[a] Adjusted for confounding factors: sex, age, education, working years, occupation, duration of residence in the area, the source of potable water, eating and hygiene habits.

Figure 1. The changing trend of anti-HEV IgG antibody seropositivity with working years in the different occupations.

that there is a higher risk of HEV infection in those persons who are in direct contact with the contaminated seafood and water. This study contributes to knowledge on the immune response of persons exposed to raw seafood in an industrial setting and the associated risk factors. Exposure to repeated doses of HEV can cause an anti-HEV IgG response. Results suggest a dose–response association between exposure and antibody responses. Thus, protection measures are essential to prevent the disease, such as protective equipment and HEV vaccines. This study has some weaknesses; the data represent only a specific region of China. Further studies should be conducted to estimate the prevalence of HEV infection in the seafood processing occupations in other regions of China. In addition, further studies such as genetic characterization and phylogenetic analyses are required to assess the genomic homology of seafood and the involved occupational population and to identify the specific transmission route of HEV infection.

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