DOI: 10.1515/ii-2017-0062

Original Article

The Prevalence and Levels of Anti-HEV IgG in the Population of Jiangsu Province, China

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Objective To investigate the prevalence and levels of anti-HEV IgG in the population of Jiangsu Province.

Methods Total of 2 656 samples from Qindong and 11 463 samples from Anfeng were colleted. The anti-HEV antibody was qualitatively and quantitatively detected using ELISA kits and the references had been established.

Results The positive rates of anti-HEV IgG in male and female were 55.6% and 40.1%, respectively. The positive rate of anti-HEV IgM in male and female were both 3.4%. In opposite to anti-HEV IgG, the positive rate of anti-HEV IgM in Anfeng was significant higher than that in Qindong. The mean anti-HEV IgG titers for 6 age groups were 0.94, 0.92, 1.07, 1.46, 1.27, 1.19 and 0.68, 1.31, 1.08, 1.14, 1.31, 1.68 IU/ml, in Qindong and Anfeng region, respectively. The positive rate of anti-HEV IgG tended to increase with age and the titer of anti-HEV IgG was associated with age ($R > 0.90$).

Conclusions The results in this study showed that HEV was widely prevalent in both Qindong and Anfeng of Jiansu Province and the prevalence and the anti-HEV IgG titer were associated with gender and age.

Key words: Hepatitis E virus; Quantitation of anti-HEV IgG; Immunoassay

Hepatitis E virus (HEV) is an important infectious agent, recognized globally as the major cause of enteric hepatitis both in epidemics and sporadic cases. Outbreaks caused by HEV have been observed in developing countries in Asia, and Africa.[1] However, in recent years, sporadic cases related to autochthonous transmission of hepatitis E in developed countries have been reported in the USA and European countries.[2,3] Although the disease is usually acute and self-limited, the fatality rate was up to 20% in infected pregnant woman, especially in their second and third trimesters.[4] In an ongoing hepatitis E epidemic in Uganda, which had caused illness in 10 196 persons and 160 deaths, a mortality rate of 13% was observed in children. [5]

The epidemiology of HEV is complex, and differs from that of other enteric pathogens such as hepatitis A virus (HAV). At least four major genotypes of HEV have been recognized: genotype 1 and 2 are restricted to humans and associated with epidemics in developping countries, whereas genotype 3 and 4 are zoonotic and infect humans and several other animals in both developping and industrialized countries. [6,7]

Some studies provided evidence that anti-HEV IgG protects against hepatitis E.[8,9] There are many data focused on the epidemiology of seropositive of anti-HEV IgG, however, few studies on the epidemiology of anti-HEV IgG levels were reported. In this study, the prevalence and levels of anti-HEV IgG in the population of Jiangsu Province, China were investigated.

MATERIALS AND METHODS

Subjects

The prevalence of hepatitis E virus infection in Jiangsu Province, which has many large scale swine farms, was higher than that of any other reported regions in China. [10-12] In this study, two counties in Jiangsu Province were randomly selected for the investigation. Healthy subjects were required to understand the study procedures and provide a self-written informed consent to participate. From Qindong and Anfeng, Jiangsu Province, China,

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2 656 samples and 11 463 samples were collected respectively in August, 2007. All the samples were stored in -20℃ until tested.

Establishment of a quantitative assay for anti-HEV IgG

To establish a quantitative reference, sera with high titer anti-HEV IgG from recovered hepatitis E patients were selected and mixed, the mixture named HEV-G01. According to instruction, WHO standard reference (NIBSC code: 95/584) was dissolved by sterile water. Both WHO standard reference and HEV-G01 were diluted 1∶1.5 by sera negative for anti-HEV IgG. Every diluted sample was detected in duplicate by EIA kit. Using parallel line assay, HEV-G01 was quantified with WHO anti-HEV IgG standard reference. The HEV-G01 then was diluted in the linear range of EIA kit and the anti-HEV IgG quantitative reference was established. Thirty independent tests were carried out and the mean concentration was calculated. The details of this assay were described previously.[13]

Detection of Anti-HEV IgG and anti-HEV IgM

The anti-HEV IgG and the anti-HEV IgM assays were conducted using EIA kits of Wantai Inc. (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China).[14-16] For determination of the anti-HEV IgG, microplate wells previously coated with the antigens were reacted with 10 μl serum samples followed by horse radish peroxidase conjugated anti-human IgG according to the manufacture's instruction. For determination of the anti-HEV IgM, microplate wells previously coated with anti-human IgM μ chain with 10 μl serum samples diluted in 100 μl buffer, followed by a horse radish peroxidase conjugated purified antigen. The results were determined according to the manufacturer's instruction.

To ensure the accuracy of the test, each sample was tested before it was considered negative or positive for anti-HEV IgG. If the two results were different from each other, a third test was then performed with the same kit and the result from the third test was used. Anti-HEV IgM of these samples were tested with the same method. The anti-HEV IgG positive samples were further quantified with the established quantitative reference.

Detection of anti-HEV IgG titer

The anti-HEV IgG levels were determined by parallel line assay, each sample was serially diluted to 1∶4, 1∶16, 1∶64 and 1∶256. The anti-HEV IgG quantitative reference was used in duplicate on the same 96-well plate. Optical density (*A*) values and dilutions were log transformed to give linear dose-response plots. The anti-HEV IgG level was calculated by the diluted sample that was first in linear range of the standard curve. Samples tested at 1∶1 gave low values out of range, were thus reported as ≤ 0.077 IU/ml. Samples which gave high values out of range were diluted to 1∶512 and 1∶1 024 and was retest.

Control parameters and validation

Initial test runs showed that the outcomes of the quantification assays were strongly affected by the skill of the operator. Therefore, control parameters were required to ensure accuracy and consistency. Three quality control samples containing anti-HEV IgG ranging from low, medium to high were added to every plate and the anti-HEV IgG titers of the three samples were calculated according to the WHO standard reference. The result of each plate was accepted only if the anti-HEV IgG titer (IU/ml) of the quality control samples were in the range of mean ± 2 SD, and the CV of quantitative values were no more than 20%. Moreover, *K* values were in range from 0.8 to 1.2 and *R* values > 0.98 in the standard curve.

Statistical analysis

The Chi-square test (χ^2) was used to compare the prevalence between populations. All analyses were performed using SAS (version 9.1, SAS Institute Inc., Cary, NC, USA) and a significance level of $\alpha < 0.05$ was considered as statistically significant.

RESULTS

The prevalence of anti-HEV IgG in populations

The total positive rate of anti-HEV IgG was 46.9% in two regions. The positive rate of anti-HEV IgG for male and female were 55.6% and 40.1%, respectively. In both regions, the positive rates of anti-HEV IgG of male were significant higher than those of female ($P < 0.01$). The positive rate of anti-HEV IgG in Qindong was significant higher than that in Anfeng.

The prevalence of anti-HEV IgM in populations

The total positive rate of anti-HEV IgM was 3.4% in two regions. The positive rate of anti-HEV IgM for

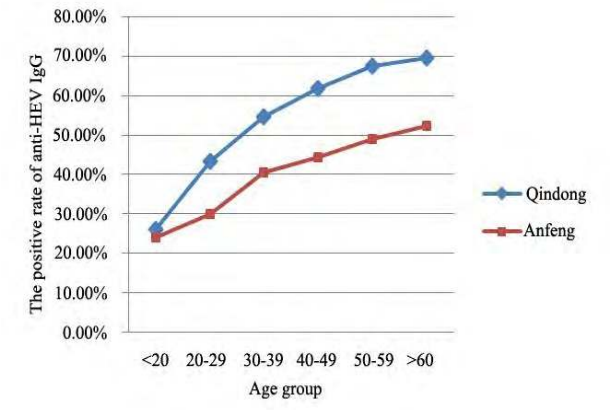

Figure 1. The trend graph of age related distribution of anti-HEV IgG positive rate in population in Qindong and Anfeng.

Notes: The positive rate of anti-HEV IgG tended to increase with age.

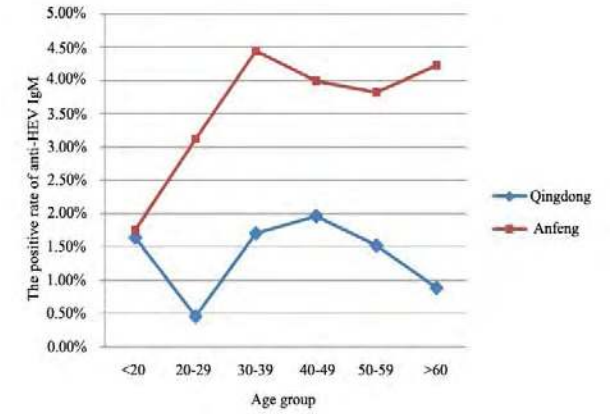

Figure 2. The trend graph of age related distribution of anti-HEV IgM positive rate in population in Qindong and Anfeng.

Notes: The positive rate of anti-HEV IgM were 1.6%, 0.5%, 1.7%, 2.0%, 1.5% and 0.9%, respectively in the 6 age groups in Qindong. The positive rate of anti-HEV IgM were 1.8%, 3.1%, 44%, 4.0%, 3.8% and 4.2%, respectively in the 6 age groups in Anfeng.

male and female were both 3.4%. The positive rate of anti-HEV IgM in Anfeng was significant higher than that of Qindong.

The distribution of anti-HEV IgG in different age groups in Anfeng and Qindong

Samples were grouped into 6 age groups, <19, 20-29, 30-39, 40-49, 50-59 and > 60 years old. The lowest positive rate of anti-HEV IgG was 26.2% in < 20 age group and the highest positive rate of anti-HEV IgG was 69.6% in > 60 age group in Qindong region. The lowest positive rate of anti-HEV IgG was 24.1% in < 20 age group and the highest positive rate of anti-HEV IgG was 52.2% in > 60 age group in Anfeng region. In both regions, the positive rate of anti-HEV IgG tended to increase with age (Figure 1) .

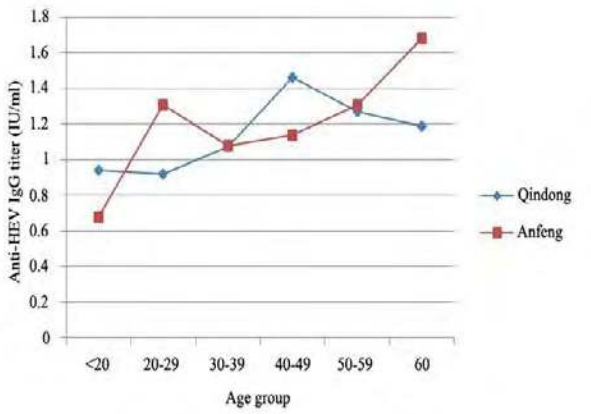

Figure 3. The trend graph of age related distribution of anti-HEV IgG titer in population in Qindong and Anfeng.

Notes: The means of anti-HEV IgG titer were 0.94 IU/ml, 0.92 IU/ml, 1.07 IU/ml, 1.46 IU/ml, 1.27 IU/ml and 1.19 IU/ml, respectively in the 6 age groups in Qindong. The means of anti-HEV IgG titer were 0.68 IU/ml, 1.31 IU/ml, 1.08 IU/ml, 1.14 IU/ml, 1.31 IU/ml and 1.68 IU/ml, respectively in the 6 age groups in Anfeng.

The distribution of anti-HEV IgM in different age groups in Anfeng and Qindong

The positive rate of anti-HEV IgM were 1.6%, 0.5%, 1.7%, 2.0%, 1.5% and 0.9%, respectively in the 6 age groups in Qindong. The positive rate of anti-HEV IgM were 1.8%, 3.1%, 44%, 4.0%, 3.8% and 4.2%, respectively in the 6 age groups in Anfeng. The highest positive rate of anti-HEV IgM was 2.0% in 40-49 age group in Qindong region while the highest positive rate of anti-HEV IgM was 4.4% in 30-39 age group in Anfeng region (Figure 2).

The distribution of anti-HEV IgG titer in different age groups in Anfeng and Qindong

The means of anti-HEV IgG titer were 0.94 IU/ml, 0.92 IU/ml, 1.07 IU/ml, 1.46 IU/ml, 1.27 IU/ml and 1.19 IU/ml, respectively in the 6 age groups in Qindong. The means of anti-HEV IgG titer were 0.68 IU/ml, 1.31 IU/ml, 1.08 IU/ml, 1.14 IU/ml, 1.31 IU/ml and 1.68 IU/ml, respectively in the 6 age groups in Anfeng.

In both regions, anti-HEV IgG titers tended to increase with age. In Qindong, the anti-HEV IgG titer was highest in age group of 40-49, while the anti-HEV IgG titer was highest in the > 60 age group in Anfeng (Figure 3).

The relationship between anti-HEV IgG titer and gender in Anfeng and Qindong

Samples were grouped into 5 titer groups, >10 IU/ml, 5-10 IU/ml, 2-5 IU/ml, 0-2 IU/ml and negative. In

Table 1. Titer-related prevalence of anti-HEV IgG in male and female population from Anfeng and Qindong

Titer	Qindong			Anfeng		
	Male number (%)	Female number (%)	Total number (%)[f]	Male number (%)	Female number (%)	Total number (%)[f]
>10[a]	5 (0.46)	10 (0.64)	15 (0.56)	38 (0.80)	39 (0.58)	77 (0.67)
5-10[b]	25 (2.29)	27 (1.73)	52 (1.96)	65 (1.36)	84 (1.26)	149 (1.30)
2-5[c]	64 (5.87)	70 (4.47)	134 (5.05)	158 (3.31)	213 (3.18)	371 (3.24)
⩽2[d]	648 (59.40)	755 (48.42)	1 403 (52.82)	2 260 (47.34)	2 161 (32.31)	4 421 (38.57)
Negative[e]	349 (31.99)	703 (44.92)	1 052 (39.61)	2 253 (47.19)	4 192 (62.67)	6 445 (56.22)
Total	1 091	1 565	2 656	4 774	6 689	11 463

a Qindong, $\chi^2 = 0.37$ and $P > 0.05$; Anfeng, $\chi^2 = 1.89$ and $P > 0.05$
b Qindong, $\chi^2 = 1.07$ and $P > 0.05$; Anfeng, $\chi^2 = 0.24$ and $P > 0.05$
c Qindong, $\chi^2 = 2.61$ and $P > 0.05$; Anfeng, $\chi^2 = 0.13$ and $P > 0.05$
d Qindong, $\chi^2 = 32.08$ and $P < 0.01$; Anfeng, $\chi^2 = 116.03$ and $P < 0.01$
e Qindong, $\chi^2 = 44.94$ and $P < 0.01$; Anfeng, $\chi^2 = 77.44$ and $P < 0.01$
f Qindong, $R = 0.67$; Anfeng, $R = 0.94$

Table 2. The age related distribution of anti-HEV IgG levels in population in Qindong [sample, number(%)]

Anti-HEV IgG (IU/ml)	< 20 years old	20-29 years old	30-39 years old	40-49 years old	50-59 years old	> 60 years old	Total[f]
>10[a]	0 (0.00)	0 (0.00)	3 (0.51)	8 (1.12)	3 (0.35)	1 (0.44)	15
5-10[b]	0 (0.00)	3 (1.38)	8 (1.37)	13 (1.82)	21 (2.47)	7 (3.08)	52
2-5[c]	2 (3.28)	8 (3.69)	18 (3.08)	34 (4.76)	59 (6.93)	13 (5.73)	134
⩽2[d]	14 (22.95)	83 (38.25)	290 (49.57)	387 (54.13)	492 (57.81)	1 379 (60.35)	1 403
Negative[e]	45 (73.77)	123 (56.68)	266 (45.47)	273 (38.18)	276 (32.43)	69 (30.40)	1 052
Total	61	217	585	715	851	227	2 656

a $\chi^2 = 6.18$ and $P < 0.05$
b $\chi^2 = 5.18$ and $P < 0.05$
c $\chi^2 = 11.42$ and $P < 0.05$
d $\chi^2 = 19.69$ and $P < 0.01$
e $\chi^2 = 36.37$ and $P < 0.01$
f $R = 0.95$

Table 3. The age related distribution of anti-HEV IgG levels in population in Anfeng [sample, number(%)]

Anti-HEV IgG (IU/ml)	< 20 years old	20-29 years old	30-39 years old	40-49 years old	50-59 years old	> 60 years old	Total[f]
>10[a]	0 (0.00)	4 (0.46)	11 (0.46)	20 (0.57)	26 (0.76)	16 (1.82)	77
5-10[b]	0 (0.00)	6 (0.69)	25 (1.04)	45 (1.28)	55 (1.61)	18 (2.05)	149
2-5[c]	6 (1.50)	15 (1.73)	65 (2.70)	107 (3.05)	145 (4.26)	33 (3.76)	371
⩽2[d]	90 (22.56)	235 (27.10)	876 (36.38)	1 385 (39.51)	1 444 (42.38)	391 (44.58)	4 421
Negative[e]	303 (75.94)	607 (70.01)	1 431 (59.43)	1 948 (55.58)	1 737 (50.98)	419 (47.78)	6 445
Total	399	867	2 408	3 505	3 407	877	11 463

a χ^2=22.88 and $P < 0.01$
b χ^2=15.19 and $P < 0.01$
c χ^2=23.28 and $P < 0.01$
d χ^2=62.38 and $P < 0.01$
e χ^2=54.40 and $P < 0.005$
f R=0.91

Qindong region, 648 male subjects (59.4%) were in the 0-2 IU/ml titer group, which had more subjects than the other 4 titer groups. Similarly, 755 female subjects (48.4%) were in the 0-2 IU/ml titer group, which had more subjects than the other 4 titer groups. There was a significant sex difference in the percentage of subjects in the 0-2 IU/ml titer group ($P < 0.01$). There was no significant sex difference in >10 IU/ml, 5-10 IU/ml, 2-5 IU/ml titer groups. Correlation coefficient (R=0.67) indicated that the titer of anti-HEV IgG was associated with gender to some degree in Qindong.

In Anfeng, 2 260 male subjects (47.3%) were in the 0-2 IU/ml titer group, which had more subjects than the other 4 titer groups. The results indicated that 2 161 female

subjects (32.3%) were in 0-2 IU/ml titer group, which had more subjects than the > 10 IU/ml, 5-10 IU/ml, 2-5 IU/ml titer groups. There were significantly more male than female subjects in the 0-2 IU/ml titer group ($P < 0.01$). In contrast to the 0-2 IU/ml, there was no significant difference between male and female in other titer groups. Correlation coefficient (R=0.94) indicated that the titer of anti-HEV IgG was associated with gender greatly in Anfeng (Table 1).

In both regions, there was no significant difference between male and female in the >10 IU/ml, 5-10 IU/ml and 2-5 IU/ml titer groups. The percentage of female in the 0-2 IU/ml titer group was significant lower than that of male. The two regions showed the same trend of gender related distribution of anti-HEV IgG levels in the population.

The relationship between anti-HEV IgG and age in Anfeng and Qindong

Samples were divided into 6 age groups, 0-19, 20-29, 30-39, 40-49, 50-59 and > 60 years old. Additionally samples were also grouped into 5 groups based on the titer of anti-HEV IgG. In Qindong, the 40-49 age group showed the highest percentage of distribution (1.1%) in the > 10 IU/ml titer group. The positive rate of anti-HEV IgG was increased with age in the 5-10 IU/ml titer group and was up to 3.1% in the > 60 age group. There was no significant difference between age groups in the > 10 IU/ml and 5-10 IU/ml titer groups. The positive rate of the 50-59 age group was highest (6.9%) in the 2-5 IU/ml titer group. The positive rate was increased with age in the 0-2 IU/ml titer group and was up to 60.4% in the > 60 age group. There was no significant difference in distribution of each age group in the > 10 IU/ml and 5-10 IU/ml titer groups. However, significant difference existed in other titer groups. Correlation coefficient (R = 0.95) indicated that the titer of anti-HEV IgG was closely associated with age (Table 2).

In Anfeng, the positive rate of anti-HEV IgG increased with age. For the > 60 age group, the positive rates were up to 1.8%, 2.1% and 44.6% in the > 10 IU/ml, 5-10 IU/ml and 0-2 IU/ml groups, respectively. The positive rate of the 50-59 age group was highest (4.3%) in the 2-5 IU/ml titer group. There was significant difference ($P < 0.01$) in age distribution in all titer groups, which was different from that of Qindong region. Correlation coefficient (R = 0.91) indicated that the titer of anti-HEV IgG was associated with age very closely (Table 3).

DISCUSSION

The result of anti-HEV IgG quantification using EIA assay is likely to be influenced by many factors, such as different operator and dilution method. In order to ensure the reliability of anti-HEV IgG quantification, a set of standard operating procedures (SOPs) to reduce operators' error was established and every operator was trained beforehand. Furthermore, three different quality control samples containing anti-HEV IgG from low to high were added to every plate as quality control for reliable results.

In general, the HEV seroprevalence is higher in developing countries than in industrialized countries. Now, more and more studies on epidemiology of HEV in industrialized countries were reported. One previous study showed that 11.3% of females and 15.2% of males in England were anti-HEV IgG seropositive. [17] Another study showed that 3% cases in North American population were anti-HEV IgG seropositive. [18] However, the seroprevalence of antibodies to HEV in epidemic regions is much higher. In rural Bangladesh, 255 (22.5%) out of 1 134 specimens tested from a representative random population were anti-HEV IgG seropositive. In general, seroprevalence of anti-HEV IgG was lower among women (19.7%) than among men (25.8%).[19] The seropositive rate of anti-HEV IgG in male (56.1%) is higher than that of female (49.1%) in Jiangsu Province, China. [12] In this study, consistent with previous reports, the seropositive rate of anti-HEV IgG in male (68.0%, 52.8%) is higher than that in female (55.1%, 37.3%) in both Qindong and Anfeng. The higher seropositive rate of male might be due to the living habits. Persons engaged in occupations related to swine farming were found to have a 74% higher risk of infection than others. [20] There are many large-scale swine farms in the 2 counties. Many local people are occupied with swine farming. As fecal-oral transmission is the predominant mode of HEV infection, the higher seropositive rate in the 2 counties might be explained by swine farming.

The presence of anti-HEV IgG antibodies is believed to be the evidence of prior exposure to HEV. The level of anti-HEV IgG in population was influenced by some factors: 1) the speed of anti-HEV IgG declining during convalescence. The duration of persistence of circulating anti-HEV IgG antibodies

remains unclear. In one study, anti-HEV IgG were detected in nearly half (47%) the patients with epidemic HEV infection after 14 years. [21] In another study of serial sera from patients with acute hepatitis E, antibodies remained detectable 14 months later, though total anti-HEV IgG titers showed a rapid decline after first 3 months.[22] 2) Higher levels of anti-HEV IgG associated with recent infection. Generally, people infected with HEV within 3 months have higher anti-HEV IgG level than those infected more than 3 months. In one study, 6 serial specimens from 6 women obtained before and after they sustained acute hepatitis E were examined. Among five women whose serum specimens were collected several months before illness, the mean level of pre-illness antibody was quite low (1.6 WR U/ml) and rose several hundred-fold to more than 1000 WR U/ml during acute illness.[23] 3) Higher levels of anti-HEV IgG associated with re-infection of HEV. Anti-HEV IgG raises significantly in infected people when they re-contacted HEV. The chance of re-contact HEV may be influenced by many factors, such as water, personal sanitation and occupation, ect. In developed countries, veterinarians and swine farm workers who come in close contact with pigs have higher anti-HEV seroprevalence rates than general population.[24]

It is interesting that IgM positivity is the reverse of IgG in the two counties (Figure 1 and Figure 2). It may indicate peoples in Anfeng were at greater risk for high anti-HEV IgM positive rate. Further studies need to be carried out to investigate these problems.

Many studies have been conducted to explore the epidemiology of HEV. However, there are only few studies on whether the anti-HEV IgG titer is associated with different age and gender. In this study, 2 656 samples from Qindong and 11 463 samples from Anfeng were collected and the anti-HEV IgG levels were quantified. The results indicated that there was an association between an increase of anti-HEV IgG titer with an increase in age. In both Qindong and Anfeng regions, there was no significant sex difference in the groups with anti-HEV IgG titers of >10 IU/ml, 5-10 IU/ml and 2-5 IU/ml, respectively. In contrast, there was significant sex difference in the 0-2 IU/ml and negative titer group, suggesting that the rate of male with past HEV infection were higher than that of female. Higher levels of anti-HEV IgG were associated with new infection or re-infection of HEV. The fact that there's no sex difference in the three high titer groups might suggest that male and female were at equal risk of HEV new infection or re-infection.

The results from Anfeng indicated that the titer of anti-HEV IgG was associated with age closely. There was a clear association between an increase of anti-HEV prevalence with an increase in age. The results of this study are concordant with the previous study.[17]

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