Expression of glutathione s-transferase-fusion hepatitis E virus ORF2, 3 antigens in *escherichia coli* and its application for diagnosis

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Context
Hepatitis E infection, caused by the hepatitis E virus (HEV), is a common cause of acute hepatitis in developing countries with poor sanitation and hygiene.
Aims
In this paper, we expressed HEV ORF2 C-terminal 802 bp and ORF3 C-terminal 107 bp fragments and its glutathione S-transferase (GST) fusion genes in *Escherichia coli* and tested the immunoreactivity of HEV antibody from sample serum with recombinant GST-HEV ORF2, 3 antigens by enzyme-linked immunosorbent assay (ELISA).
Settings and design
Eight primers designed for gene synthesis.
Materials and methods
HEV ORF2, 3 and its GST-fusion gene fragments were amplified by PCR. Expression of target genes in *E. coli* was induced by Isopropyl-β-D-Thio-alactopyranoside (IPTG) (0.1 mmol/l) and temperature (42°C). The detection of anti-HEV antibody with purified GST-HEV ORF2, 3 antigens was performed using the indirect ELISA method.
Results
When our ELISA was compared with the HEV-ELISA kit, the sensitivity of our assay is 97.9%, specificity is 100%, and consistency is 99.6%.
The positive rate of anti-HEV antibody from the serum in acute hepatitis patients with recombinant GST-HEV ORF2, 3 antigens by our ELISA was 29.5 and 26.1%, respectively, and this result corresponded with the HEV-ELISA kit.
Conclusion
The new HEV ELIA developed in the present study is a highly specific diagnostic assay for the detection of anti-HEV antibody in serum specimens obtained from acute hepatitis patients.

Keywords:
hepatitis E virus ORF2, 3 genes, expression vector, enzyme-linked immunosorbent assay

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Introduction

Hepatitis E presents as epidemic as well as sporadic disease.

Epidemic disease has been shown to be related to the consumption of fecally contaminated drinking water and this is a very important public health problem in many developing countries [1]. Sporadic disease not only occurs in developing countries but also uncommonly in industrialized countries. The source of this infection in industrialized countries suggested that persons with a history of travel to hepatitis E virus (HEV) epidemic regions and animals especially pig as a reservoir for HEV [2].

Other than hepatitis A, attack rates of HEV infection are higher among young adults and the illness may be particularly severe among pregnant women with mortality rates reaching as high as 25%.

Studies of pathogenic events in humans and experimental animals reveal that viral excretion begins approximately a week before the onset of illness and persists for nearly 2 weeks.

Viremia can be detected during the late phase of the incubation period.

Anti-HEV IgM appears early during clinical illness and disappears rapidly over a few months.

Anti-HEV IgG appears a few days later of illness and persists for at least a few years [3].

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Current laboratory tests for the diagnosis of human HEV infection include molecular and immuno-electron microscopy method for detection of the virus in the stool or serum and serological assay for the identification of anti-HEV IgM and/or IgG class [4–6].

For serological diagnosis of HEV infection, enzyme-linked immunosorbent assays (ELISA) is the most commonly used.

Target antigens in those assays are either recombinant HEV proteins or synthetic HEV peptides corresponding to immunodominant epitopes of structural HEV proteins (ORF2 and ORF3) of HEV strains [7].

Immunodominant epitopes locate on the different regions of HEV ORF2, 3 genes and according to the region of genes, the reaction of antigen with antibody have great difference [8].

Therefore, in preparation of a reagent for the detection of HEV antibody, it is important to select antigen regions having high immunoreactivity and specificity.

In this paper, HEV ORF2 C-terminal 802 bp and ORF3 C-terminal 107 bp fragments and its glutathione S-transferase (GST) fusion genes were expressed in *Escherichia coli* and the immunoreactivity of HEV antibody with GST-HEV ORF2, 3 antigens was tested using ELISA.

Materials and methods

E. coli DH5α, BL21(DE-3) (Novagen, USA), HEV cDNA Library (China), expression vector pGEX-3× (Pharmacia, Sweden), pBUG (Genetic Medicine Institute, Pyongyang Medical College), eight primer (Sangon, China), restriction enzyme *Bam*HI, *Eco*RI, *Kpn*I, *Pst*I (Takara), KOD Plus DNA polymerase (Toyobo, Japan), T4DNA ligase (Takara, Japan), dNTPs, DNA Glass-milk Rapid Purification Kit (BioDev, China), Sephacryl-200 (Sigma, USA), DEAE-sepharose (Sigma, USA), HRP-Mouse anti-human IgG (Sigma, USA), 137 acute hepatitis patients (Second specialized hospitals, Pyongyang City) and 239 healthy persons, HEV-ELISA kit (SABC, China).

Primer design for gene synthesis:

HEV ORF2: 5′-CAGGATCCAGCTGTTCTAC TCTCGCCC-3′
5′-GGAATTCTATAACTCCCGAGTTTTA-3′
HEV ORF3: 5′-CAGGATCCTCGTGTTCGC CAA-3′
5′-GGAATTCTTATAATTCGCGGCGC-3′
GST-HEV ORF2: 5′-GGAATTCATGTCCC CTATACTA-3′
5′-AACTGCAGCTATAACTCCCGAGT-3′
GST-HEV ORF2: 5′-GGAATTCATGTCCCC TATACTA-3′
5′-AACTGCAGTTATAATTCGCGGCGCGGC-3′

Method

Construction of recombinant plasmid-expressing HEV ORF2 802 bp, ORF3 107 bp and its GST-fusion gene fragments HEV ORF2, 3 and its GST-fusion gene fragments were amplified by PCR using restriction enzymes *Bam*HI, *Eco*RI, and *Pst*I. The gene fragments and plasmids were cut and throughout the ligation reaction and transformation, the *E. coli* strain expressing HEV ORF2 802 bp, ORF3 107 bp, and its GST-fusion gene fragments were made and they were identified by restriction cutting and finally sequencing [9].

Expression of target genes in *E. coli* was induced by Isopropyl-β-D-Thio-alactopyranoside (IPTG) (0.1 mmol/l) and temperature (42°C) and the expressed protein in its supernatant and pellet was identified by 12% SDS-polyacrylamide gel electrophoresis (PAGE) [9].

The purification of antigen proteins was performed throughout sonication, washing of inclusion body, solubilization of inclusion body, chromatography with sephacryl S-200, and DEAE-sepharose [9].

The detection of anti-HEV antibody with purified GST-HEV ORF2, 3 antigen was performed using indirect ELISA method [10].

To estimate the research results we determined the negative average OD_{492}+0.25 as a positive limited value. If the research value is high than the positive limited value, it was determined as positive and if the research value is low than the positive limited value, it was determined as negative.

Estimation of the sensitivity, specificity, and consistency of our ELISA is as follows [11]:

Our ELISA		HEV-ELISA kit (SABC)	
		ï¼	–
	ï¼	True positive	False positive
	–	False negative	True negative

$$\text{Sensitivity}(\%) = \left[\frac{\text{true positive}}{(\text{true positive} + \text{false negative})}\right] \times 100.$$

$$\text{Specificity}(\%) = \left[\frac{\text{true negative}}{(\text{true negative} + \text{false positive})}\right] \times 100.$$

$$\text{Consistency}(\%) = \left[\frac{(\text{true positive} + \text{true negative})}{\text{total number}}\right] \times 100.$$

Results

Construction of recombinant plasmid-expressing hepatitis E virus ORF2 802 bp, ORF3 107 bp gene fragments

HEV ORF2 802 bp, ORF3 107 bp gene fragments containing immunodominant epitopes were amplified by PCR and the PCR products were identified in 1% agarose gel (Fig. 1).

Following the cutting of gene fragments and the expression vector pGEX-3× with restriction enzymes *Eco*R?and *Bam*H?, ligation and transformation into the *E. coli*, the newly made recombinant plasmids were called pGE2, pGE3 (Fig. 2).

Construction of recombinant plasmid-expressing glutathione S-transferase-hepatitis E virus ORF2 802 bp, ORF3 107 bp gene fragments

To remove the nonspecification of antigens occurred during direct insertion of gene fragments into the temperature-inducible expression vector pBUG, HEV ORF2 802 bp, ORF3 107 bp gene fragments were fused with the GST gene [8].

GST-HEV ORF2, 3 gene fragments were amplified by PCR and these PCR products were identified in 1% agarose gel (Fig. 3). Following the cutting of fusion gene fragments and pBUG expression vector with restriction enzymes *EcoR* I and *Pst*?, ligation and transformation into the *E. coli*, the newly made recombinant plasmids were called pBGE2, pBGE3 (Fig. 4).

Expression of glutathione S-transferase-hepatitis E virus ORF2, 3 antigen proteins in *E. coli*

GST-HEV ORF2, 3 antigen proteins were introduced by IPTG and at 42°C in *E. coli* and the expressed fusion proteins were identified by 12% SDS-PAGE.

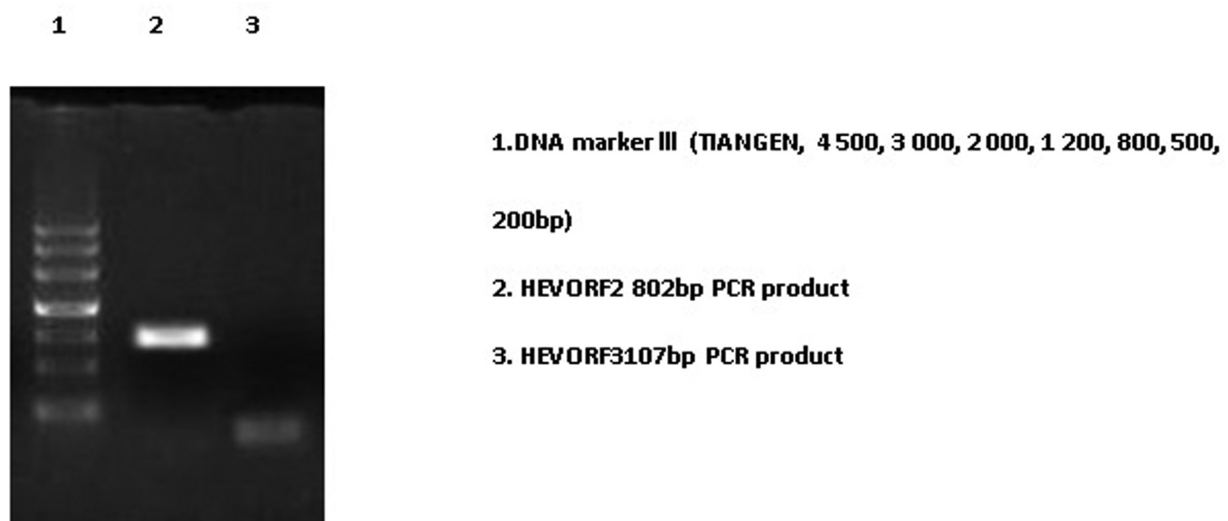
As shown in Fig. 5, GST-HEV ORF2 antigen protein expressed by IPTG and 42°C temperature induction are both in inclusion body and in same size on SDS-PAGE and the percentage of expression is 27% and the molecular weight is 55 kD.

As shown in Fig. 6, GST-HEV ORF3 antigen protein expressed by IPTG and 42°C temperature induction are both in inclusion body in the same size on SDS-PAGE and the percentage of expression is 27% and the molecular weight is 30 kD.

Purification of glutathione S-transferase-hepatitis E virus ORF2, 3 antigen proteins

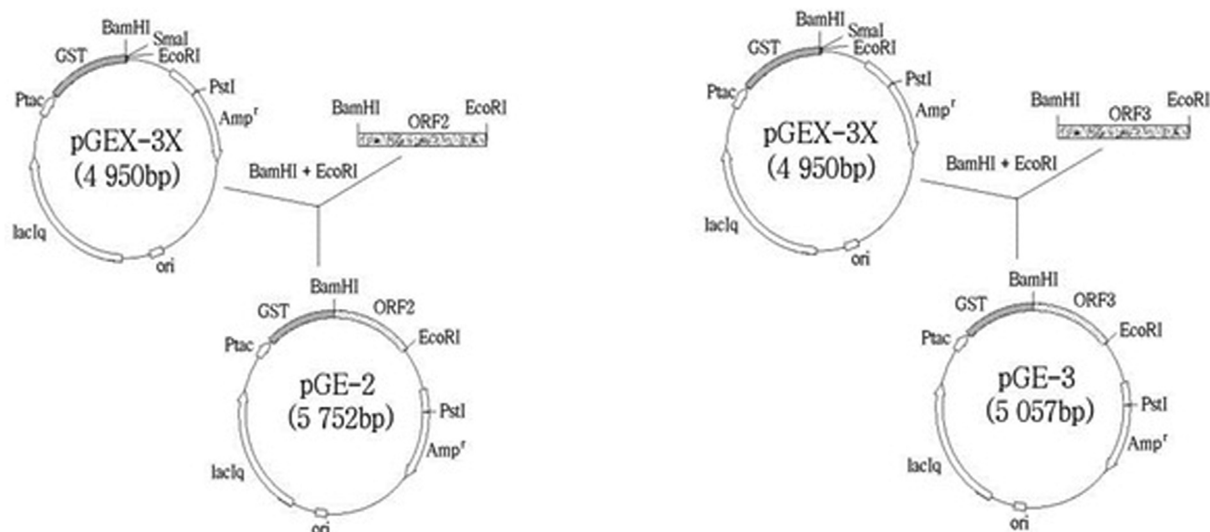
Purification of GST-HEV ORF2, 3 antigen proteins was performed through sonication of bacterium, washing of inclusion body, solubilization of inclusion body, sephacryl S-200, and DEAE-sepharose chromatography.

Figure 1



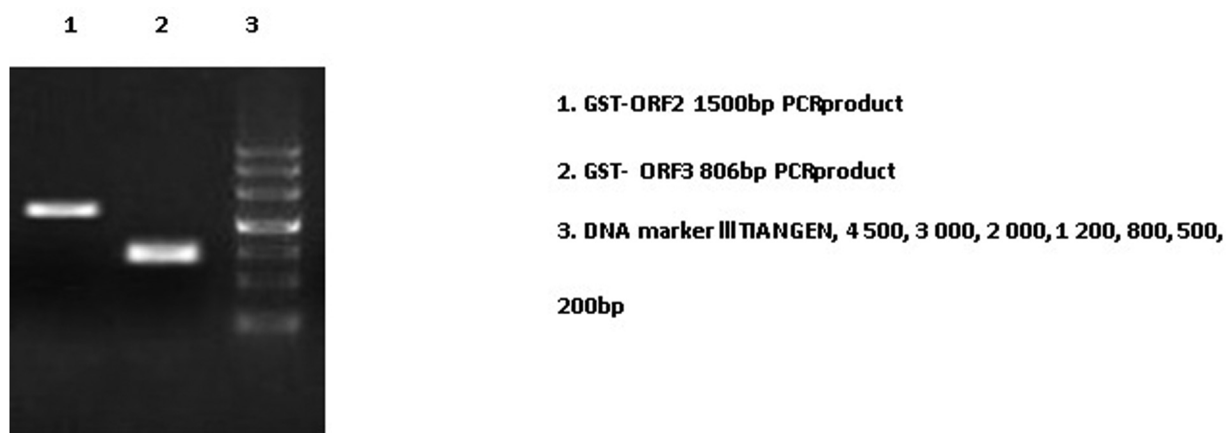
Identification of HEV ORF2, 3 PCR product. HEV, hepatitis E virus.

Figure 2



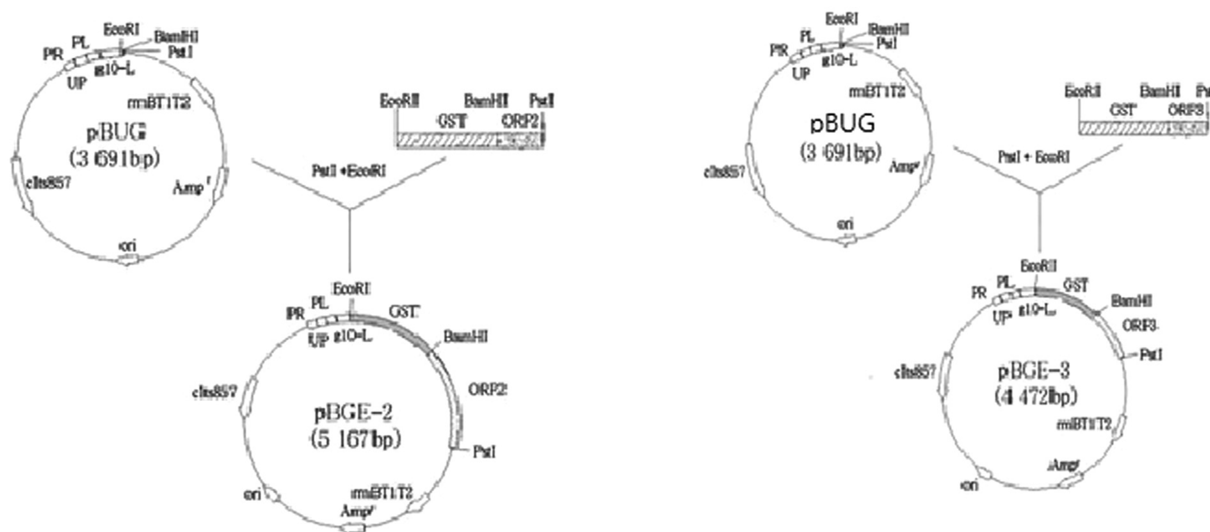
Insertion of HEV ORF2, 3 PCR products into pGEX-3× expression vector. HEV, hepatitis E virus.

Figure 3



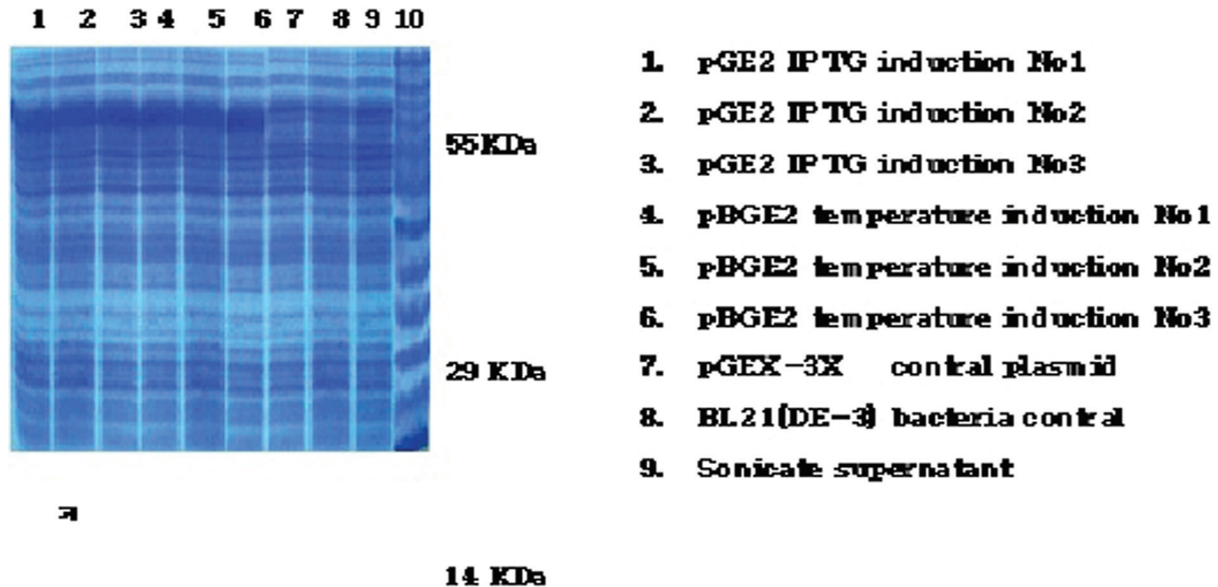
Identification of GST-HEV ORF2, 3 PCR product. GST, glutathione S-transferase; HEV, hepatitis E virus.

Figure 4



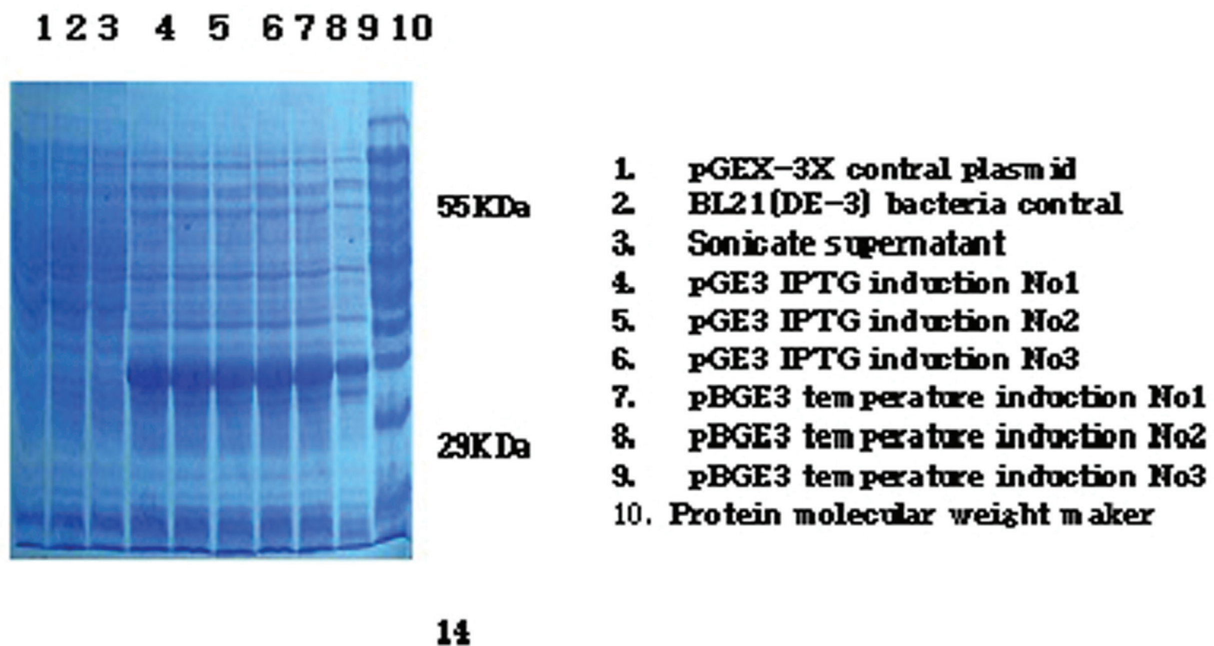
Insertion of GST-HEV ORF2, 3 PCR products into pBUG expression vector. GST, glutathione S-transferase; HEV, hepatitis E virus.

Figure 5



Expression of HEV ORF2 antigen (12% SDS-PAGE). HEV, hepatitis E virus; PAGE, polyacrylamide gel electrophoresis.

Figure 6



Expression of HEV ORF3 antigen (12% SDS-PAGE). HEV, hepatitis E virus; PAGE, polyacrylamide gel electrophoresis.

As shown in Table 1, the purification of HEV ORF2 antigen is 94.55% and the yield is 34.25%.

Detection of anti-hepatitis E virus antibody by enzyme-linked immunosorbent assay

To find the characteristic of ELISA that we established, the detection of anti-HEV antibody with the recombinant HEV ORF2, 3 antigens in 287 serum samples performed and estimated the sensitivity, the specificity, and the consistency of our assay compared with HEV-ELISA kit (Table 2).

When our assay was compared with the HEV-ELISA kit, the sensitivity of our assay is 97.9%, specificity is 100%, and consistency is 99.6%.

We detected the anti-HEV antibody with recombinant HEV ORF2 among healthy persons using our ELISA method.

Table 1 Purification and yield of glutathione S-transferase-hepatitis E virus ORF2 antigen in each purification step

Purification step	Total protein (mg/ml)	Purification (%)	Yield (%)	Total yield (%)
Before	802.1±12.5	27.5±2.4		
Sonication	518.3±7.5	38.5±1.5	90.5±2.8	90.5±3.2
Washing of inclusion body	389.5±3.4	45.8±1.8	89.4±0.9	80.9±1.9
Solubilization	201.4±2.7	65.4±2.3	73.7±1.5	59.7±2.0
Sephacryl S-200	101.57±5.1	88.2±2.1	68.0±1.4	40.6±1.6
DEAE-sepharose	78.90±1.2	94.5±1.5	84.3±2.2	34.2±2.8

Data are presented as mean±SD.

Table 2 Comparison of our assay with hepatitis E virus-enzyme-linked immunosorbent assay kit

Detection method of anti-HEV antibody	HEV-ELISA kit		
	Positive (*n*)	Negative (*n*)	Total (*n*)
Our ELISA assay			
Positive			
ORF2	48	0	48
ORF3	48	0	48
Negative			
ORF2	1	238	239
ORF3	1	238	239
Total	49	238	287

ELISA, enzyme-linked immunosorbent assay; HEV, hepatitis E virus.

Table 3 Detection rate of anti-hepatitis E virus antibody in healthy persons

Sample	Positive [*n* (%)]
168	1 (0.59)

Table 4 Detection rate of anti-hepatitis E virus antibody in acute hepatitis patients

	Antigens	Our assay	ELISA kit
Sample	ORF2	88	88
	ORF3	88	88
Positive [*n* (%)]	ORF2	26 (29.5)	26 (29.5)
	ORF3	23 (26.1)	23 (26.1)

ELISA, enzyme-linked immunosorbent assay.

As shown in Table 3, the positive rate of anti-HEV antibody by our ELISA in healthy persons is 0.59%.

We detected the anti-HEV antibody in 88 acute hepatitis patients by our ELISA.

As shown in Table 4, the positive rate of anti-HEV antibody from the serum in acute hepatitis patients with recombinant HEV ORF2, 3 antigens by our ELISA was 29.5 and 26.1%, respectively, and this result corresponded with HEV-ELISA kit.

Discussion

Immunodominant epitopes determining the antigenicity of this virus in the HEV genome mainly locates on ORF2, 3 regions. Therefore, the ideal reagent for the diagnosis of anti-HEV antibody must contain HEV ORF2, 3 regions that have high immunoreactivity and specificity.By the literature, the C-terminal region of HEV ORF2 has several epitopes and the N-terminal region of HEV ORF2 has a little epitope and the middle region of HEV ORF has not epitope at all.

Therefore, the C-terminal of HEV ORF2 is used for the diagnosis of acute and convalescent phase of hepatitis E and total length and the N-terminal of HEV ORF2 for the diagnosis of acute phase of hepatitis E [10,12,13].

By the literature, during the preparation of reagent for the diagnosis of anti-HEV antibody using recombinant HEV ORF2, 3 antigens the rate of detection of antibodies was higher in the combination of HEV ORF2, 3 than HEV ORF2, 3 alone [10,14].

In this research paper, we first amplified HEV ORF2 802 bp, ORF3 107 bp gene fragments containing immunodominant epitopes by PCR and inserted them into the IPTG-inducible expression plasmid pGEX-3×. GST-HEV ORF2 802 bp, ORF3 107 bp gene fragments also amplified by PCR and inserted them into the temperature-inducible expression plasmid pBUG.

GST-HEV ORF2, 3 antigens were expressed in *E. coli* and purified. Purified antigens used in immunoreactions with anti-HEV antibody in the serum of hepatitis E patients by ELISA.

Anti-HEV antibody in 287 clinical samples was detected using the ELISA method that we established and the results were compared with HEV-ELISA kit. When our assay method compared with the HEV-ELISA kit, the sensitivity of our assay is 97.9%, specificity is 100%, and consistency is 99.6%.

We detected anti-HEV antibody among healthy persons and acute hepatitis patients by our ELISA.

The detection rate of anti-HEV antibody with recombinant HEV ORF2 in healthy persons was 0.59% and the detection rate of anti-HEV antibody with recombinant HEV ORF2, 3 antigens in acute hepatitis patients was 29.5, 26.1%, respectively, and this result corresponded with the HEV-ELISA kit.

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Nil.

Conflicts of interest

There are no conflicts of interesting.

References

1 Kumar S, Subhadra S, Singh B, Panda BK. Hepatitis E virus: the current scenario. Int J Infect Dis 2013; 17:228–233.

2 Chang Y, Wang L, Geng J, Zhu Y, Fu H, Ren F. Zoonotic risk of hepatitis E virus (HEV): a study of HEV infection in animals and humans in suburbs of Beijing. Hepatol Res 2009; 9:1153–1158.

3 Chandra NS, Ojha D, Chatterjee S, Chattopadhyay D. Prevalence of hepatitis E virus infection in West Bengal, India: a hospital-based study. J Med Microbiol 2014; 63:975–980.

4 La Rosa G, Muscillo M, Vennarucci VS, Garbuglia AR, La Scala PL, Capobianchi MR. Hepatitis E virus in Italy: molecular analysis of travel-related and autochthonous cases. J of Gen Virol 2011; 92:1617–1626.

5 Zhang Y, Mcatee P, Yarbough PO, Tam AW, Fuerst T. Expression, characterization, and immunoreactivities of a soluble hepatitis E virus putative capsid protein species expressed in insect cells. Clin Diagn Lab Immunol. 1997; 4:423–428.

6 Obriadina A, Meng JH, Ulanova T, Trinta K, Burkov A, Fields HA, Khudyakov Y. A new enzyme immunoassay for the detection of antibody to hepatitis E virus. J Gastroenterol Hepatol 2002; 17:360–S364.

7 Zhang H, Dai X, Shan X, Meng J. Characterization of antigenic epitopes of the ORF2 protein from hepatitis E virus genotype 4. Virus Res 2009; 142:140–143.

8 Ma H, Song X, Li Z, Harrison TJ, Zhang H, Huang W, *et al.* Varing abilities of recombinant polypeptids from different regions of hepatitis E virus ORF2 and ORF3 to detect anti-HEV IgM. J Med Virol 2009; 81:1052–1061.

9 Green MR, Sambrook J. Molecular cloning. Vol. 1. 3rd ed. New York, NY: CSHL Press; 2001.

10 Anderson DA, Li F, Riddellb M, Howard T, Seow H-F., Torresi J, *et al.* ELISA for IgG-class antibody to hepatitis E virus based on a highly conserved conformational epitope expressed in *Escherichia coli*. J Virolog Methods 1999; 81:131–142.

11 Shengli B, Yongzhen J, Honglan Z, Jingyuan L, Jian L, Jingyuan C, Chongbai L. Expression of the hepatitis E virus structural proteins in E. coli and application of the recombinant products for diagnosis. Chin J Virol 1996; 12:118–122.

12 Ponterio E, Di Bartolo I, Orrù G, Liciardi M, Ostanello F, Ruggeri FM. Detection of serum antibodies to hepatitis E virus in domestic pigs in Italy using a recombinant swine HEV capsid protein. BMC Vet Res 2014; 10:133.

13 Verghese VP, Robinson JL. A systematic review of hepatitis E virus infection in children. Clin Infect Dis 2014; 59:689–697.

14 Yang E-C., Chi B-R., Li X, Liu Y, Gao P, Jia P, *et al.* Expression and characterization of a recombinant truncated capsid protein of hepatitis E virus in *Pichia pastori*. Chem Res Chinese Uni 2010; 26:235–239.