Virus Research xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Review

Serological diagnostics of hepatitis E virus infection

Yury Khudyakov, Saleem Kamili*

Centers for Disease Control and Prevention, National Center for HIV/Hepatitis/STD/TB Prevention, Division of Viral Hepatitis, MS-A33/1600 Clifton Rd NE, Atlanta, GA 30333, United States

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Hepatitis E
Hepatitis E virus
IgM anti-HEV
IgG anti-HEV
Serology

ABSTRACT

Development of accurate diagnostic assays for the detection of serological markers of hepatitis E virus (HEV) infection remains challenging. In the course of nearly 20 years after the discovery of HEV, significant progress has been made in characterizing the antigenic structure of HEV proteins, engineering highly immunoreactive diagnostic antigens, and devising efficient serological assays. However, many outstanding issues related to sensitivity and specificity of these assays in clinical and epidemiological settings remain to be resolved. Complexity of antigenic composition, viral genetic heterogeneity and varying epidemiological patterns of hepatitis E in different parts of the world present challenges to the refinement of HEV serological diagnostic assays. Development of antigens specially designed for the identification of serological markers specific to acute infection and of IgG anti-HEV specific to the convalescent phase of infection would greatly facilitate accurate identification of active, recent and past HEV infections.

Published by Elsevier B.V.

Contents

1. Introduction

Until the discovery of its etiological agent, hepatitis E was described as enterically transmitted or water-borne non-A, non-B hepatitis and its diagnosis was based on the clinical and epidemiological features after exclusion of serological markers of hepatitis A and B virus infections (Khuroo, 2011). The causative agent of the disease, the hepatitis E virus (HEV) was first identified by immune electron microscopy in the feces of a human volunteer and experimentally infected cynomolgus monkeys (Balayan et al., 1983). Following the cloning and sequencing of the viral genome, serologic assays for the detection of antibodies against HEV were developed (Dawson et al., 1992; Tam et al., 1991) and soon it was found that almost all of the water-borne outbreaks from the Indian subcontinent, Central and Southeast Asia, the Middle East and North Africa were caused by HEV (Khuroo, 2011). Furthermore, the majority of the sporadic acute hepatitis cases in the endemic countries were etiologically linked to HEV (Khuroo et al., 1994; Khuroo, 2011). Current estimates indicate that one-third of the world's population living in the developing countries has been infected with HEV (Lancet editorial 2010).

HEV is the only member of the genus *Hepevirus* of the family *Hepeviridae* (Emerson et al., 2004). The virus has a single-stranded, positive-sense RNA genome of approximately 7.2 kb consisting of three open reading frames (Ahmad et al., 2011). Open reading frame (ORF) 1 codes for a non-structural protein with multiple functional domains; ORF2 codes for the capsid protein; and ORF3, the smallest of the ORFs, encodes a small protein which may have regulatory properties. Four major genotypes of HEV have been described: genotype 1 and 2 strains infect humans and are associated with epidemics; and genotype 3 and 4 strains sporadically infect humans as well as several other animal species (Purdy and Khudyakov, 2011).

* Corresponding author.
E-mail address: skamili@cdc.gov (S. Kamili).

0168-1702/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.virusres.2011.06.006

All four genotypes of HEV represent a single serotype, thus facilitating the development of diagnostic assays capable of detecting antibodies against any of the infecting genotypes.

The earliest assays used for laboratory diagnosis of hepatitis E included immune electron microscopy (Bradley et al., 1987), and fluorescent blocking antibody assay and HEV antigen detection in hepatocytes both of which depended on light microscopy (Krawczynski and Bradley, 1989). Although these assays played a critical role in the early identification of the virus and the immune response induced during HEV infection, they were laborious, technically difficult and not suitable for routine diagnosis of HEV infection. The cloning of the HEV genome facilitated the development of serologic and molecular assays which have unraveled the epidemiology of HEV infection showing a worldwide distribution of the disease prevalent in both the industrialized and non-industrialized countries albeit with varying proportions. A number of in-house conventional and real-time RT-PCR assays used for the detection of HEV RNA in blood and stool samples as well as contaminated water and sewage have been described (Mushahwar, 2008; Purdy and Khudyakov, 2011). These assays have been critical for delineating the molecular epidemiology of hepatitis E. This review focuses on the antigenic landscape of the HEV proteome and how it impacts the development of the most reliable and accurate serological assays for diagnosing acute and past HEV infections.

2. HEV antigenic composition

The antigenic composition of HEV proteins has been examined using synthetic peptides (Coursaget et al., 1993; Kaur et al., 1992; Khudyakov et al., 1993, 1999, 1994a,b) and recombinant proteins (Li et al., 1997, 1994; Purdy et al., 1992; Yarbough et al., 1991). Extensive studies on identification of antigenic epitopes in different HEV proteins were conducted almost immediately after the discovery of HEV. One of these early studies was exceptionally comprehensive. It was performed using a large set of overlapping synthetic 10-mer peptides completely spanning proteins encoded by all three ORFs (Kaur et al., 1992). This study showed that the largest ORF1-encoded protein contains at least 12 antigenic regions that could be modeled with short synthetic peptides. Only 3 antigenic regions at amino acid (aa) positions 25–38, 341–354, and 517–530 were identified within the ORF2-encoded protein, and a single antigenic region was mapped at the C-terminus of the ORF3-encoded protein. Although antigenic composition of the ORF1-protein received no further attention, the antigenic structure of the ORF2- and ORF3-encoded proteins was studied in great detail (Coursaget et al., 1993; Khudyakov et al., 1993, 1999, 1994a,b; Li et al., 1997, 1994; Qi et al., 1995; Yarbough et al., 1991; Zhao et al., 2009b).

Two additional antigenic regions were identified at aa positions 31–40 and 63–76 of the ORF3-protein (Khudyakov et al., 1993). It was found that variation in primary structure affects antigenic properties of its C-terminal region (Khudyakov et al., 1993; Yarbough et al., 1991). Although a strict strain specificity of immunoreactivity of recombinant proteins derived from this region originally reported by Yarbough et al. (1991) was not observed in later studies (Khudyakov et al., 1993), synthetic peptides derived from HEV genotypes 1 and 2 showed variation in the pattern of immunoreactivity with sera obtained from patients infected with these 2 genotypes (Khudyakov et al., 1994b). A more detailed analysis using overlapping 6-mer peptides showed that the C-terminal antigenic region contains several epitopes, which differentially immunoreact with serum specimens obtained from different patients (Khudyakov et al., 1994b). The most immunoreactive epitopes of genotype 1 and 2 strains were found to differ in their location within this region, with the genotype 1 epitope being mapped at aa positions 112–117 and genotype 2 at aa95–101. Additionally, it was suggested that variation in the HEV ORF3 primary structure affects not only specificity but also the extent to which epitope immunoreactivity is dependent on conformation (Khudyakov et al., 1994b).

Since ORF2 encodes the HEV capsid protein (Ahmad et al., 2011), understanding the antigenic composition of this protein has significant implications for diagnostic and vaccine development. Antigenic properties of the HEV capsid were extensively studied using fragments derived from different regions of this protein. Application of 3 sets of overlapping synthetic peptides of different sizes, with each set covering the entire genotype 1 capsid protein, allowed for the identification of 6 antigenic domains within this protein (Khudyakov et al., 1999). The most diagnostically relevant antigenic epitopes were found in domains 1 and 6 located at the N- and C-termini of the ORF2-encoded protein, respectively. Both domains were efficiently modeled with all tested peptides. However, the central domains showed inconsistent antibody binding patterns among the peptide sets, suggesting dependence of epitope immunoreactivity on protein conformation, which could be variably modeled with peptides of different sizes.

The complex capsid antigenic structure was further explored using recombinant proteins expressed in different hosts. Analysis of a large set of the HEV ORF2 proteins clearly showed that the antigenic properties of protein fragments and the whole protein significantly differ. For example, some fragments were found to bind HEV antibodies from convalescent serum specimens with a much greater efficacy than the full-length protein expressed in the same expression system (Li et al., 1997). Difference in immunoreactivity among recombinant proteins was also observed in an *in vitro* seroneutralization assay. It was shown that different HEV strains were more efficiently neutralized with antibodies against ORF2 fragments expressed in *Eschericia coli* (*E. coli*) than against the full-length protein expressed in the baculovirus genetic system (Meng et al., 1998). These findings indicate that many antigenic epitopes of the ORF2-encoded protein are conformation-dependent, at least to a degree, and may be differentially modeled with various protein fragments. Cross-inhibition among HEV monoclonal antibodies elicited against antigenic epitopes distinctly separated in the primary structure of the ORF2-encoded protein additionally supported the observation of a complex antigenic structure of this protein (Riddell et al., 2000). These observations have significant diagnostic relevance since they indicate the differential functional reproduction of the HEV epitopes by various protein fragments sharing the epitope sequences.

In general, viral neutralizing antigenic epitopes are diagnostically very important. The early experiments with recombinant proteins showed that the HEV neutralizing determinant was located within the C-terminal two-thirds of the ORF2 protein (Purdy et al., 1993). The exact location of this determinant was established in a series of experiments. The binding site for two neutralizing monoclonal antibodies against the ORF2 protein of the HEV SAR-55 strain was mapped in the capsid region at aa positions 578–607 (Schofield et al., 2000). An extensive analysis of the HEV neutralizing epitope(s) performed using an *in vitro* neutralization assay and antibodies against a large set of overlapping synthetic peptides and recombinant polypeptides derived from the HEV ORF2-encoded protein showed that the minimal region efficiently modeling the HEV neutralizing determinant was located at aa positions 452–617 and that this determinant was strongly conformation-dependent (Meng et al., 2001). This region forms domain P in the recently reconstructed three-dimensional structure of virus-like particles generated by expression of the ORF2-encoded protein in the baculovirus system (Xing et al., 2011). Further, it was shown that a recombinant protein comprising the ORF2 region at aa positions 394–606 formed homodimers after

Please cite this article in press as: Khudyakov, Y., Kamili, S., Serological diagnostics of hepatitis E virus infection. Virus Res. (2011), doi:10.1016/j.virusres.2011.06.006

expression in *E. coli* (Zhang et al., 2001a). Analysis of antigenic composition of this homodimer using neutralizing monoclonal antibodies revealed the presence of two conformation-dependent neutralizing sites (Zhang et al., 2005). This region of the ORF2-encoded protein was shown to contain at least one broadly immunoreactive conformation-dependent epitope responsible for antibody binding to all four known HEV genotypes (Liang et al., 2010; Zhang et al., 2009). Nevertheless, it was found that the variation in primary structure affects antigenic composition of this region, with a single aa substitution abrogating binding to a monoclonal antibody (Liang et al., 2010; Yamashita et al., 2009).

3. HEV diagnostic antigens

A number of different protein constructs have been used in the development of diagnostic assays for the efficient detection of HEV-specific antibodies. However, because of the complex antigenic composition of the HEV proteins, different protein constructs showed variation in diagnostically relevant antigenic properties resulting in variation in the performance of assays using these proteins. The major diagnostic target in all HEV assays is the ORF2-encoded protein. This protein or its fragments have been expressed in *E. coli* (Im et al., 2001; Li et al., 2000a, 1997; Zhang et al., 2001a), insect cells (Robinson et al., 1998; Tsarev et al., 1993; Zhang et al., 2001b), animal cells (Jameel et al., 1996), plant cells (Ma et al., 2003; Zhou et al., 2006) and *Trichoplusia ni* larvae (Jimenez de Oya et al., 2009). Another antigen that was applied to the assay development is the ORF3-encoded protein, which was expressed in *E. coli* (Ma et al., 2009; Purdy et al., 1994; Wang et al., 2001; Yarbough et al., 1991) and yeast (Lal et al., 1997). Synthetic peptides derived from the ORF2 and ORF3 proteins were also used for the development of HEV diagnostic assays (Favorov et al., 1994; Paul et al., 1994; Qi et al., 1995). However, the inferior performance of peptide-based assays for detection of anti-HEV in serum specimens (Ferguson et al., 2002; Mast et al., 1998) discouraged their broader application to HEV diagnostics.

One of the first recombinant HEV antigens available for the development of diagnostic assays contained short regions of the C-terminal part from the ORF2- and ORF3-encoded proteins of HEV genotype 1 and 2 strains (Yarbough et al., 1991). These antigens were utilized in two solid-phase enzyme immunoassays (Dawson et al., 1992; Goldsmith et al., 1992). One assay was shown to detect anti-HEV in 8 of 386 (2.1%) randomly selected US blood donors (Dawson et al., 1992). The important finding in this study was that proteins derived from the genotype 1 or 2 strains were differently immunoreactive with sera from these 8 anti-HEV positive donors, thus indicating that variation in primary structure of the HEV antigens affects detection of anti-HEV activity in serum specimens. Difference in immunoreactivity of these antigens was also observed in another laboratory where the assay was applied to serum specimens obtained from Egyptian children with sporadic hepatitis E (Goldsmith et al., 1992). A surprising observation in this study was the detection of IgG anti-HEV in 25% of the controls in addition to 42% of the children with acute non-A, non-B hepatitis. Although detection of past HEV infections among patients without recorded history of hepatitis E was plausible in an endemic region such as Egypt, these data raised an issue of non-specific immunoreactivity of the HEV antigens.

All the 4 genotypes of HEV belong to a single serotype (Emerson and Purcell, 2003), thus suggesting that diagnostic antigens from a single HEV genotype should detect antibody against many HEV strains of different genotypes. Although broad cross-immunoreactivity among HEV genotypes was frequently observed (Arankalle et al., 2007; Herremans et al., 2007; Yarbough et al., 1991; Zhou et al., 2004), the aforementioned strain-specific variation of diagnostically relevant properties indicate that HEV sequence heterogeneity is a factor potentially affecting performance of diagnostic assays for detection of infections with various HEV strains. As discussed above, HEV proteins contain numerous antigenic epitopes, which may be differently affected by variations in the primary structure. Thus, the use of antigens comprising many epitopes increases probability of cross-immunoreactivity among genetically distant viral variants. This strategy for obtaining broadly immunoreactive antigens is most straightforward and was frequently implemented. Several groups used recombinant proteins comprising extended regions of the ORF2-encoded protein for the development of HEV assays (Favorov et al., 1992; Hu et al., 2008; Li et al., 1997, 1994; Purdy et al., 1992; Riddell et al., 2000).

The analysis of epitope composition of the ORF2- and ORF3-encoded proteins using synthetic peptides described above suggests an alternative strategy for constructing broadly immunoreactive HEV antigens. The strategy is based on identification of short regions of the HEV protein that can efficiently reproduce diagnostically relevant antigenic epitopes, and using these regions for construction of an artificial "mosaic" antigen (Fig. 1) designed for a specific detection of a broad range of antibodies against HEV (Khudyakov et al., 1994c; Ulanova et al., 2009). Artificial constructs offer a significant flexibility in using antigenic epitopes for diagnostics. It allows for increasing density of certain diagnostically relevant epitopes and presenting antigenic regions from different proteins of genetically distant HEV strains within a single diagnostic antigen (Ulanova et al., 2009). The selective use of epitopes with HEV-specific activity and exclusion of protein regions that showed marginal or non-specific antigenic reactivity (Khudyakov et al., 1994c) may substantially improve specificity of serological detection of HEV infections. These artificial antigens have successfully been used for the development of HEV diagnostic assays for the specific detection of anti-HEV activity in serum specimens obtained from patients infected with different HEV strains (Favorov et al., 1996; Obriadina et al., 2002).

Another successful strategy for obtaining broadly immunoreactive antigens involves expression of recombinant proteins that can efficiently model the HEV neutralizing epitope. A significant degree of cross-protection in animal challenge experiments (Huang et al., 2008, 2009; Sanford et al., 2011) and cross-neutralization in *in vitro* experiments (Meng et al., 2001, 1998) among HEV variants of different genotypes suggest conservation of the HEV neutralizing antigenic epitope. The conserved HEV neutralizing epitope should be broadly cross-immunoreactive and, as such, is a valuable component of diagnostic antigens for the efficient detection of antibody to genetically diverse HEV strains. Recombinant proteins reproducing the HEV neutralizing epitope were expressed in *E. coli* and baculovirus expression systems (Li et al., 2005; Meng et al., 2001; Tsarev et al., 1993). These antigens were used to develop a series of efficient HEV serological assays (Innis et al., 2002; Obriadina et al., 2002; Tsarev et al., 1993; Zhang et al., 2002). Application of the baculovirus-expressed ORF2-encoded protein (Tsarev et al., 1993) in various serological studies (Christensen et al., 2008; Kuniholm et al., 2009; Myint et al., 2006a) showed the significant efficacy of this antigen for detection of antibodies to different HEV strains.

4. Serological markers of HEV infection

Based on the analysis of serum specimens collected during various stages of human HEV infection, which include the incubation period, acute and convalescent phases, a classic serological pattern of IgM and IgG anti-HEV appearances has been observed (Dawson et al., 1992; Khuroo et al., 1994; Krawczynski et al., 2011). IgM anti-HEV appears during the early acute phase of illness and may be detected as early as 4 days after the onset of jaundice and lasts for

Please cite this article in press as: Khudyakov, Y., Kamili, S., Serological diagnostics of hepatitis E virus infection. Virus Res. (2011), doi:10.1016/j.virusres.2011.06.006

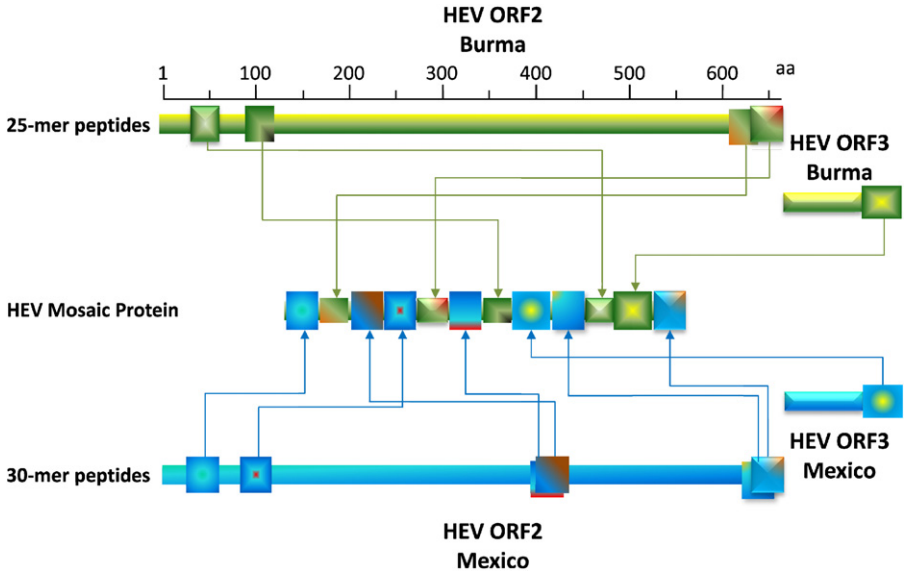

Fig. 1. Structure of the HEV "mosaic" antigen MA-II (Ulanova et al., 2009). The top and bottom bars represent the ORF2-encoded proteins. TheORF2 diagnostically relevant regions modeled with 25- or 30-mer peptides identified with boxes on the bars. The C-terminal regions of the ORF3-protein from the HEV Burma and Mexico strains contain broadly immunoreactive regions and as shown are included in the mosaic antigen. The arrows identify location of each antigenic region within the HEV MA-II (for details see Ulanova et al., 2009).

up to five months (Favorov et al., 1992). In outbreak settings, IgM anti-HEV was detectable in 40–100% of specimens collected from patients over varying periods ranging from less than a week up to one year after onset of illness. Overall, >90% of patients infected with HEV have detectable IgM anti-HEV in the first 2 weeks after the onset of illness (Favorov et al., 1992). IgM anti-HEV, therefore, is a marker eminently suitable for the diagnosis of acute infection. A number of in-house and commercially available assays (see Section 5) have been described for the detection of IgM anti-HEV. A recent evaluation of the performance characteristics of some of these assays revealed appreciable variability in their sensitivity and specificity (see Section 6). The serological appearance of IgM anti-HEV is succeeded shortly by IgG anti-HEV, so that both seem to appear almost simultaneously in the acute phase of infection. IgG anti-HEV persists for a longer time and may be detectable for 1–14 years (Bryan et al., 1994; Dawson et al., 1992; Khuroo et al., 1993). The decline of IgG anti-HEV over shorter durations also has been reported in several studies (Bryan et al., 1994; Myint et al., 2006b). However, the exact duration of the persistence of IgG anti-HEV remains to be determined. The studies that have addressed the issues of persistence of these antibodies have employed different assays. It is possible that different duration of persistence of IgG ant-HEV reported in these studies is due to the varying performance characteristics of the assays used. An evaluation of some of the in-house and commercially available IgG anti-HEV assays showed poor concordance ranging from as low as 40% to as high as 70% (Mast et al., 1998). The role of detection of IgA anti-HEV in conjunction with IgM anti-HEV in the diagnosis of acute infection also has been explored in several studies (Chau et al., 1993; Herremans et al., 2007; Takahashi et al., 2005b; Tokita et al., 2003). However, further studies are needed to establish the usefulness of IgA anti-HEV in aiding the diagnosis of acute HEV infection. The detection of HEV antigen in serum also has been reported recently (Zhang et al., 2006; Zhao et al., 2009a). HEV RNA can be detected in serum and stool of infected patients during the acute phase of infection by RT-PCR using conventional and real-time formats (Mushahwar, 2008). Although, the detection of HEV RNA has the advantage of providing additional information about the sequence and the genotype of the infecting viral isolate, the marker has a limited value in the diagnosis of acute infection due to brief periods of viremia (up to 2 weeks) and fecal shedding (up to 4 weeks) (Krawczynski et al., 2011). The laboratory diagnosis of acute hepatitis E is based on the presence of IgM anti-HEV in serum and/or detection of HEV RNA in serum or stool.

5. Diagnostic assay formats

HEV recombinant proteins have been used in different formats of diagnostic assays. A Western blot-based assay for the detection of HEV IgG and IgM antibodies was developed using a recombinant polypeptide containing the C-terminal half of the ORF2-encoded protein (Favorov et al., 1992). Although cumbersome to perform, this assay could detect IgG anti-HEV in 89–100% of non-A, non-C patients 1–24 months after onset of jaundice and IgM in 73% of patients within 26 days after onset of jaundice. Another Western blot assay based on a different set of fusion proteins containing sequences from ORF2 and ORF3 of the HEV genotype 1 strain was used to examine temporal appearance of HEV antibodies in experimentally infected rhesus monkeys (Li et al., 1994). A commercial immunoblot test recomBlot HEV IgG/IgM using 3 overlapping recombinant proteins covering the entire ORF2 and one recombinant protein covering ORF3 was recently developed and marketed by Mikrogen GmbH (Neuried, Germany) for the detection of IgG and IgM antibodies against HEV in human serum specimens (www.mikrogen.de). This test was adapted for detecting antibody in swine and used to show HEV-specific seroprevalence of 49.8% among domestic pigs in Germany (Baechlein et al., 2010).

"Indirect" ELISA is one of the most popular diagnostic formats. In this format, HEV-specific immunoglobulins captured from serum specimens by HEV antigens attached to the solid-phase surface (usually wells of microtiter plates) are detected using labeled species-specific antibody. The differential detection of IgG or IgM is achieved using, for example, antibody against gamma- or mu-chains of antibodies, correspondingly. There are numerous examples of application of this format to the detection of IgG anti-HEV in serum specimens using *E. coli*-expressed HEV antigens (Anderson et al., 1999; Dawson et al., 1992; Obriadina et al., 2002; Wang et al., 2001; Yarbough et al., 1991) and virus-like particles expressed in insect cells (Innis et al., 2002; Jimenez de Oya et al., 2009; Li et al., 2000b; Tsarev et al., 1993). With minor modifications,

Please cite this article in press as: Khudyakov, Y., Kamili, S., Serological diagnostics of hepatitis E virus infection. Virus Res. (2011), doi:10.1016/j.virusres.2011.06.006

this format was also used for the detection of IgA anti-HEV (Chau et al., 1993; Takahashi et al., 2005a) and IgM anti-HEV (Dawson et al., 1992; Li et al., 2000b; Lin et al., 2000; Takahashi et al., 2005a).

The robust detection of IgM anti-HEV has critical clinical implications, given the importance of IgM anti-HEV as a marker of recent or current HEV infection (Clayson et al., 1995; Seriwatana et al., 2002). However, the pathogen specificity of IgM detection using indirect ELISA is occasionally compromised by the presence of IgM-rheumatoid factor in test serum, and the sensitivity of IgM detection is frequently affected by the competition with specific IgG for interaction with antigens attached to solid phase (Champsaur et al., 1988). An alternative format for the detection of IgM is the class-capture ELISA, which takes advantage of immobilized antibodies against mu-chain of IgM to capture this class of antibodies for the subsequent pathogen-specific detection using labeled specific antigens. Such mu-capture assay was developed for the detection of IgM anti-HEV in serum specimens using the baculovirus-expressed virus-like particles. This assay was shown to detect an anamnestic response and reappearance of IgM anti-HEV in an experimentally infected chimpanzee. Importantly, it was found to be more sensitive than the indirect ELISA when applied to clinical samples from hepatitis E outbreaks. This improvement was most probably attributable to the reduction of competition between IgM and IgG anti-HEV (Yu et al., 2003). However, capture type assays are also affected by immunological interferences associated with rheumatoid factor binding, antinuclear antibodies (Naot and Remington, 1980), broadly immunoreactive IgG and IgM autoantibody (Avrameas and Ternynck, 1993) and human IgM binding to horseradish peroxidase conjugates (Tuuminen et al., 1999). Further improvement of the specificity and sensitivity of assays for detection of acute HEV infections remains a subject of research. In an effort to improve accuracy of serological diagnosis of hepatitis E, several laboratories developed technologies for controlling nonspecific IgM binding (Pan et al., 2010), supplementing IgM with IgA anti-HEV detection (Takahashi et al., 2005a), and identifying novel markers such as HEV antigen (Zhang et al., 2006). A commercially available assay based on mu capture has been developed and marketed by Beijing Wantai Biological Pharmacy (http://www.ystwt.com/wantai_english/IFU/HEV-IgM_CE_IFU.pdf).

All aforementioned diagnostic formats are based on the use of host-specific detection reagents, e.g. anti-human or anti-swine antibody. HEV genotypes 3 and 4 infect human and swine hosts (Meng, 2010). The detection of anti-HEV in humans or swine requires assays designed specifically for specimens from humans or animals. The host-independent detection of acute or past HEV infections has, however, significant advantages for epidemiological investigations in the field. The format that allows for such host-independent detection is the double-antigen sandwich ELISA. In this format, antigen attached to solid phase is used to capture specific antibody from serum specimens. Detection of this antibody is achieved using the same antigen labeled with, for example, horseradish peroxidase. An assay based on this format was recently developed for detection of anti-HEV in human and animal specimens (Hu et al., 2008). This assay showed the specificity of 98.8% with human samples. It could detect specific antibody in experimentally infected pigs 14 days after inoculation. Another very important feature of this assay is that it does not discriminate between classes of antibody, thus detecting total anti-HEV.

In addition to standard enzyme immunoassays, other assays formats like rapid tests have also been evaluated for HEV serology. The immunochromatographic methods for the detection of serological markers of infections are fast and simple and are frequently formatted into rapid diagnostic assays suitable for point-of-care testing. A rapid immunochromatographic assay ASSURE^{TM} has been developed by Genelabs Diagnostics, Singapore (http://www.genelabs.com.sg) and evaluated for the detection of IgM anti-HEV in serum specimens from patients with acute hepatitis E infection (Chen et al., 2005; Myint et al., 2005). This test is an IgM-capture lateral-flow immunochromatographic assay. In this format, anti-HEV IgM is captured by anti-human IgM monoclonal antibody immobilized onto the membrane and detected using a colloidal gold-labeled HEV antibody attached to HEV antigen. The HEV antigen ET2.1 used in this assay is a fusion protein containing the ORF2.1 fragment (aas 394–660) from the capsid protein of HEV genotype 1 strain (Riddell et al., 2000). This assay was evaluated using acute-phase serum specimens from Indonesia and Nepal, convalescent-phase specimens from Nepal and a large set of controls. It had a sensitivity of 93% and specificity of 99.7% (Myint et al., 2005). Rapidity and simplicity to perform are major advantages of the ASSURE^{TM} HEV IgM test. It was recently found to be highly sensitive (82%) and specific (100%) in detection of acute HEV genotype 3 infections (Legrand-Abravanel et al., 2009).

6. Performance of IgM and IgG anti-HEV assays

To date only a few studies have carried out a comprehensive evaluation of performance characteristics of IgG and IgM anti-HEV assays. In the first such study, a panel of 164 coded sera was tested for IgG anti-HEV by 12 different assays (10 EIAs and 2 Western blot assays) used in 10 laboratories. Seven of these assays used HEV recombinant proteins based on HEV ORF2 alone or in combination with HEV ORF3 expressed either in baculovirus or *E. coli* expression systems; four assays were based on synthetic peptides and one assay used an artificial mosaic protein consisting of short linear antigenic epitopes. A substantial variability in the performance of the assays was observed with sensitivities ranging from 17% to 100% and the overall concordance ranging from 49% to 94% (median 69%) (Mast et al., 1998). Although this study raised doubts about the seroprevalence rates reported from the countries where occurrence of hepatitis E was only considered to be travel-related, subsequent studies demonstrated that not only locally acquired HEV infections existed in these countries but were present in appreciable numbers (Miyamura, 2011; Teo, 2006). In a recent study we evaluated the performance of six IgM anti-HEV enzyme immunoassays using a serum panel that included specimens from all 4 genotypes of HEV (Drobeniuc et al., 2010). The assays included 2 in-house and 4 commercially available assays and the evaluation panel included a sensitivity panel of 50 serum samples and a specificity panel of 229 serum samples. All samples in the sensitivity panel were collected from acutely jaundiced patients negative for serological markers of hepatitis A, B and C virus infections and were HEV RNA positive with well characterized HEV genotypes. The samples in the specificity panel were obtained from household contacts of HEV-infected patients from outbreak settings, patients with acute hepatitis A, B and C virus infections and blood donors. The overall sensitivity of the 6 assays ranged from 72% to 98% and specificity ranged from 78% to 96% (Table 1) indicating an appreciable variability in the performance of these assays (Drobeniuc et al., 2010). It is imperative that assays well validated with proven performance characteristics be used for accurate diagnosis of HEV infections. Comparisons of commercially available or in-house assays for IgM and IgG anti-HEV tend to use a limited number of assays and the evaluations panels constituted by samples are restricted to only one or two genotypes (Baechlein et al., 2010; Bendall et al., 2008; Herremans et al., 2007; Seriwatana et al., 2002; Yu et al., 2003).

7. Unresolved issues of HEV serological assays

Diagnostic assays are only as good as their sensitivity and specificity. Unfortunately, assessing these fundamental diagnostic

Please cite this article in press as: Khudyakov, Y., Kamili, S., Serological diagnostics of hepatitis E virus infection. Virus Res. (2011), doi:10.1016/j.virusres.2011.06.006

Table 1
Anti-HEV-IgM reactivities among 6 immunoassays in the sensitivity and specificity panels.

Assay	Sensitivity panel						Specificity panel	
	Genotype (gt) and number of samples reactive					Sensitivity (%)	No. of samples reactive (n = 229)	Specificity (%)
	gt 1 (n = 15)	gt 2 (n = 4)	gt 3 (n = 15)	gt 4 (n = 17)	Total (n = 51)			
I	15	4	14	17	50	98	49	78.5
II	15	3	15	17	50	98	15	93.4
III	15	2	13	12	42	82.4	19	91.7
IV	13	1	10	13	37	72.5	16	93
V	15	4	14	17	50	98	10	95.6
VI	15	3	13	16	47	92.2	9	96.1

Assay I (NIH): ORF2 aa112–606, Pakistan, genotype (gt) 1, expressed in baculovirus; Assay II (CDC): ORF2 aa452–617, gt 1 (Morocco), gt 2 (Mexico), gt3 (US) and gt4 (China) expressed in *E. coli*; Assay III: International Immuno-Diagnostics (Foster City, CA); Assay IV: MP Biomedicals (Singapore); Assay V: Diagnostics Systems (Nizhnii Novgorod, Russia); Assay VI: Mikrogen GmbH (Neuried, Germany).

parameters is not always straightforward. Although crucial for all diagnostic assays, the problem of sensitivity and specificity seems to be especially relevant to HEV diagnostics. Since the inception in early 1990s, serological testing occasionally produced results that did not fit comfortably with the available body of hepatitis E knowledge, resulting in reservations regarding the actual accuracy of the HEV diagnostic assays. Detection of HEV immunoreactivity among "negative" controls (Goldsmith et al., 1992) or high HEV seroprevalence in nonendemic countries (Thomas et al., 1997) suggested a substantial number of subclinical HEV infections in endemic and nonendemic regions of the world. However, these findings could be also interpreted as indicating poor specificity of the existing HEV assays for IgG anti-HEV. Surprisingly, both suppositions found support in further investigations.

Indeed, in endemic regions of the world, the number of subclinical human infections is more than two times greater than symptomatic infections among sporadic cases and during outbreaks (Clayson et al., 1997; Teshale et al., 2010), with one estimate projecting that only 1–2% of HEV infections in China are accompanied with clinical symptoms in adults (Wedemeyer and Pischke, 2011). The number of subclinical infections in developed countries is not clearly established. The HEV seroprevalence in these countries was found ranging from 7% to 21% (Buti et al., 2006; Dalton et al., 2007; Ijaz et al., 2009; Kuniholm et al., 2009; Mansuy et al., 2008; Tanaka et al., 2005). Since autochthonous hepatitis E cases are rarely reported from developed countries, such high seroprevalence rates detected using a variety of diagnostic assays may reflect the existence of frequent asymptomatic infections.

The use of different diagnostic assays in these studies, however, still leaves room for attribution of significant differences in the antibody detection rate to inconsistent performance of these assays. In fact, the comparative evaluation of several HEV serological assays showed highly discrepant results in the detection of anti-HEV among U.S. blood donors, indicating significant differences among these assays in sensitivity and specificity, serving as a caution that seroprevalence data obtained using different HEV assays should be carefully interpreted (Mast et al., 1998). Taking into consideration that (1) non-specific IgG anti-HEV activity is not frequently observed (Elkady et al., 2007; Khudyakov et al., 1994a), and (2) only a small fraction of serological reactions can be potentially attributed to cross-immunoreactivity with non-viral proteins (Srinivasappa et al., 1986), the observed poor concordance among assays for detection of IgG anti-HEV in convalescent-phase specimens (Mast et al., 1998) may be explained by variation in sensitivity rather than specificity of these assays.

There are two major factors that potentially affect sensitivity of detection of anti-HEV in human serum specimens. The first factor is varying diagnostic properties of antigens. As noted above, HEV proteins have a very complex antigenic composition, with many diagnostically relevant epitopes being conformation-dependent. As a result, even recombinant antigens sharing HEV sequences may significantly differ in their antigenic structure (Li et al., 1994; Meng et al., 1998), owing to the different presentation of epitopes. Additionally, it is known that HEV is genetically heterogeneous (Ahmad et al., 2011) and the variation in sequence affects activity of the HEV antigenic epitopes (Khudyakov et al., 1994b; Yarbough et al., 1991), thereby affecting sensitivity of detection of antibodies to various HEV strains (Herremans et al., 2007).

The second factor is the variable nature of the HEV-specific antibody responses. HEV infections are characterized with a complex kinetics of anti-HEV immune response. The duration of antibody response to different HEV epitopes was shown to vary widely (Li et al., 1994; Ma et al., 2009). Moreover, the antibody response may be poorly developed, especially during subclinical infection (Aggarwal et al., 2001; Clayson et al., 1995; Husain et al., 2010; Nicand et al., 2001), or may rapidly decline after acute infection (Goldsmith et al., 1992; Khuroo, 2010; Myint et al., 2006b), so ensuing a broad range of temporal antibody patterns in patients with current or past HEV infection.

These patterns should be differentially detected by diagnostic antigens differing in their epitopic composition. Given the geographically distinct distribution of HEV genotypes and variation in frequency and types of exposure to HEV infections in different parts of the world (Aggarwal and Naik, 2009), it is conceivable that these temporal patterns of immune response to HEV differ among patients residing in endemic and non-endemic regions. This consideration suggests that discrepant performance of diagnostic assays for the detection of past infections (Ferguson et al., 2002; Mast et al., 1998) is rather associated with variation in sensitivity than specificity. Thus, the development of accurate assays suitable for seroprevalence studies, producing comparable results from different parts of the world, should be based on using carefully assembled serum panels containing specimens from patients with different durations of past symptomatic and asymptomatic infection, who were infected with a variety of HEV strains representing all 4 genotypes through different modes of transmission.

Both factors described above are relevant to the development of assays for diagnosis of hepatitis E in clinical settings. The major serological marker of acute infection is IgM anti-HEV. However, the detection of IgM is prone to many non-specific reactions, some of which are described above (see Section 5). There are several reports describing the detection of IgM anti-HEV in patients without any other serological markers and clinical signs of acute hepatitis E (Elkady et al., 2007; Goto et al., 2006; Fogeda et al., 2009; Meky et al., 2006). Detection of IgM anti-HEV in HEV PCR-negative specimens or viremia in the absence of IgM response in patients with acute HEV infection (Zhang et al., 2002; Echevarría et al., 2011; Beale et al., 2011) further suggests problems with sensitivity in addition to specificity of IgM detection. To improve accuracy of detection of acute HEV infections, several research groups have investigated

Please cite this article in press as: Khudyakov, Y., Kamili, S., Serological diagnostics of hepatitis E virus infection. Virus Res. (2011), doi:10.1016/j.virusres.2011.06.006

the use of IgG avidity (Bigaillon et al., 2010), IgA (Takahashi et al., 2005a) and a combination of serological markers (Huang et al., 2010). Although the estimation of IgG avidity and detection of IgA anti-HEV may significantly improve the identification of recent or current HEV infections, the final assay format for the reliable diagnosis has yet to be established.

8. Conclusion

Over the last 2 decades of HEV research, substantial progress has been achieved in understanding the HEV-specific immune responses, antigenic composition of HEV proteins and development of serological assays. Nonetheless, the problem of sensitivity and specificity of these assays is yet to be satisfactorily resolved. A significant difference in serological markers of acute, recent or past HEV infections in conjunction with the variable antibody kinetics exhibited by the infected host shows the complexity of immune responses during HEV infections. This complexity is compounded by the heterogeneity of the HEV genome and the diverse antigenic structure of the HEV proteins. The further improvement of HEV diagnostics is contingent on better understanding of the epidemiology of hepatitis E and HEV infections in different parts of the world and the availability of specific and broadly immunoreactive antigens. It is conceivable that accurate detection of HEV infections will be achieved through the development of antigens specifically designed for the identification of serological markers specific to acute infection or of IgG anti-HEV specific to the convalescent phase of infection.

References

Aggarwal, R., Kamili, S., Spelbring, J., Krawczynski, K., 2001. Experimental studies on subclinical hepatitis E virus infection in cynomolgus macaques. J. Infect. Dis. 184 (11), 1380–1385.
Aggarwal, R., Naik, S., 2009. Epidemiology of hepatitis E: current status. J. Gastroenterol. Hepatol. 24 (9), 1484–1493.
Ahmad, I., Holla, R.P., Jameel, S., 2011. Molecular virology of hepatitis E virus. Virus Res..
Anderson, D.A., Li, F., Riddell, M., Howard, T., Seow, H.F., Torresi, J., Perry, G., Sumarsidi, D., Shrestha, S.M., Shrestha, I.L., 1999. ELISA for IgG-class antibody to hepatitis E virus based on a highly conserved, conformational epitope expressed in *Escherichia coli*. J. Virol. Methods 81 (1–2), 131–142.
Arankalle, V.A., Lole, K.S., Deshmukh, T.M., Chobe, L.P., Gandhe, S.S., 2007. Evaluation of human (genotype 1) and swine (genotype 4)-ORF2-based ELISAs for anti-HEV IgM and IgG detection in an endemic country and search for type 4 human HEV infections. J. Viral Hepat. 14 (6), 435–445.
Avrameas, S., Ternynck, T., 1993. The natural autoantibodies system: between hypotheses and facts. Mol. Immunol. 30 (12), 1133–1142.
Baechlein, C., Schielke, A., Johne, R., Ulrich, R.G., Baumgaertner, W., Grummer, B., 2010. Prevalence of hepatitis E virus-specific antibodies in sera of German domestic pigs estimated by using different assays. Vet. Microbiol. 144 (1–2), 187–191.
Balayan, M.S., Andjaparidze, A.G., Savinskaya, S.S., Ketiladze, E.S., Braginsky, D.M., Savinov, A.P., Poleschuk, V.F., 1983. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. Intervirology 20 (1), 23–31.
Beale, M.A., Tettmar, K., Szypulska, R., Tedder, R.S., Ijaz, S., 2011. Is there evidence of recent hepatitis E virus infection in English and North Welsh blood donors? Vox Sang 100, 340–342.
Bendall, R., Ellis, V., Ijaz, S., Thurairajah, P., Dalton, H.R., 2008. Serological response to hepatitis E virus genotype 3 infection: IgG quantitation, avidity, and IgM response. J. Med. Virol. 80 (1), 95–101.
Bigaillon, C., Tessé, S., Lagathu, G., Nicand, E., 2010. Use of hepatitis E IgG avidity for diagnosis of hepatitis E infection. J. Virol. Methods. 164, 127–130.
Bradley, D.W., Krawczynski, K., Cook Jr., E.H., McCaustland, K.A., Humphrey, C.D., Spelbring, J.E., Myint, H., Maynard, J.E., 1987. Enterically transmitted non-A, non-B hepatitis: serial passage of disease in cynomolgus macaques and tamarins and recovery of disease-associated 27- to 34-nm viruslike particles. Proc. Natl. Acad. Sci. U.S.A. 84 (17), 6277–6281.
Bryan, J.P., Tsarev, S.A., Iqbal, M., Ticehurst, J., Emerson, S., Ahmed, A., Duncan, J., Rafiqui, A.R., Malik, I.A., Purcell, R.H., et al., 1994. Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. J. Infect. Dis. 170 (3), 517–521.
Buti, M., Dominguez, A., Plans, P., Jardi, R., Schaper, M., Espunes, J., Cardenosa, N., Rodriguez-Frias, F., Esteban, R., Plasencia, A., Salleras, L., 2006. Community-based seroepidemiological survey of hepatitis E virus infection in Catalonia, Spain. Clin. Vaccine Immunol. 13 (12), 1328–1332.
Champsaur, H., Fattal-German, M., Arranhado, R., 1988. Sensitivity and specificity of viral immunoglobulin M determination by indirect enzyme-linked immunosorbent assay. J. Clin. Microbiol. 26 (2), 328–332.
Chau, K.H., Dawson, G.J., Bile, K.M., Magnius, L.O., Sjogren, M.H., Mushahwar, I.K., 1993. Detection of IgA class antibody to hepatitis E virus in serum samples from patients with hepatitis E virus infection. J. Med. Virol. 40 (4), 334–338.
Chen, H.Y., Lu, Y., Howard, T., Anderson, D., Fong, P.Y., Hu, W.P., Chia, C.P., Guan, M., 2005. Comparison of a new immunochromatographic test to enzyme-linked immunosorbent assay for rapid detection of immunoglobulin m antibodies to hepatitis e virus in human sera. Clin. Diagn. Lab. Immunol. 12 (5), 593–598.
Christensen, P.B., Engle, R.E., Hjort, C., Homburg, K.M., Vach, W., Georgsen, J., Purcell, R.H., 2008. Time trend of the prevalence of hepatitis E antibodies among farmers and blood donors: a potential zoonosis in Denmark. Clin. Infect. Dis. 47 (8), 1026–1031.
Clayson, E.T., Myint, K.S., Snitbhan, R., Vaughn, D.W., Innis, B.L., Chan, L., Cheung, P., Shrestha, M.P., 1995. Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E. J. Infect. Dis. 172 (4), 927–933.
Clayson, E.T., Shrestha, M.P., Vaughn, D.W., Snitbhan, R., Shrestha, K.B., Longer, C.F., Innis, B.L., 1997. Rates of hepatitis E virus infection and disease among adolescents and adults in Kathmandu, Nepal. J. Infect. Dis. 176 (3), 763–766.
Coursaget, P., Buisson, Y., Depril, N., le Cann, P., Chabaud, M., Molinie, C., Roue, R., 1993. Mapping of linear B cell epitopes on open reading frames 2- and 3-encoded proteins of hepatitis E virus using synthetic peptides. FEMS Microbiol. Lett. 109 (2–3), 251–255.
Dalton, H.R., Thurairajah, P.H., Fellows, H.J., Hussaini, H.S., Mitchell, J., Bendall, R., Banks, M., Ijaz, S., Teo, C.G., Levine, D.F., 2007. Autochthonous hepatitis E in southwest England. J. Viral Hepat. 14 (5), 304–309.
Dawson, G.J., Chau, K.H., Cabal, C.M., Yarbough, P.O., Reyes, G.R., Mushahwar, I.K., 1992. Solid-phase enzyme-linked immunosorbent assay for hepatitis E virus IgG and IgM antibodies utilizing recombinant antigens and synthetic peptides. J. Virol. Methods 38 (1), 175–186.
Drobeniuc, J., Meng, J., Reuter, G., Greene-Montfort, T., Khudyakova, N., Dimitrova, Z., Kamili, S., Teo, C.G., 2010. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. Clin. Infect. Dis. 51 (3), e24–e27.
Echevarría, J.M., Fogeda, M., Avellón, A., 2011. Diagnosis of acute hepatitis E by antibody and molecular testing: a study on 277 suspected cases. J Clin. Virol. 59, 69–71.
Elkady, A., Tanaka, Y., Kurbanov, F., Hirashima, N., Sugiyama, M., Khan, A., Kato, H., Okumura, A., Mizokami, M., 2007. Evaluation of anti-hepatitis E virus (HEV) immunoglobulin A in a serological screening for HEV infection. J. Gastroenterol. 42 (11), 911–917.
Emerson, S.U., Purcell, R.H., 2003. Hepatitis E virus. Rev. Med. Virol. 13 (3), 145–154.
Emerson, S.U., Anderson, D., Arankalle, V.A., Meng, X.J., Purdy, M.A., Schlauder, G.G., Tsarev, S.A., 2004. Virus Taxonomy. VIIIth Report of the ICTV. Elsevier/Academic Press, London, 851–855.
Favorov, M.O., Fields, H.A., Purdy, M.A., Yashina, T.L., Aleksandrov, A.G., Alter, M.J., Yarasheva, D.M., Bradley, D.W., Margolis, H.S., 1992. Serologic identification of hepatitis E virus infections in epidemic and endemic settings. J. Med. Virol. 36 (4), 246–250.
Favorov, M.O., Khudyakov, Y.E., Fields, H.A., Khudyakova, N.S., Padhye, N., Alter, M.J., Mast, E., Polish, L., Yashina, T.L., Yarasheva, D.M., et al., 1994. Enzyme immunoassay for the detection of antibody to hepatitis E virus based on synthetic peptides. J. Virol. Methods 46 (2), 237–250.
Favorov, M.O., Khudyakov, Y.E., Mast, E.E., Yashina, T.L., Shapiro, C.N., Khudyakova, N.S., Jue, D.L., Onischenko, G.G., Margolis, H.S., Fields, H.A., 1996. IgM and IgG antibodies to hepatitis E virus (HEV) detected by an enzyme immunoassay based on an HEV-specific artificial recombinant mosaic protein. J. Med. Virol. 50 (1), 50–58.
Ferguson, M., Walker, D., Mast, E., Fields, H., 2002. Report of a collaborative study to assess the suitability of a reference reagent for antibodies to hepatitis E virus. Biologicals 30 (1), 43–48.
Fogeda, M., de Ory, F., Avellón, A., Echevarría, J.M., 2009. Differential diagnosis of hepatitis E virus, cytomegalovirus and Epstein-Barr virus infection in patients with suspected hepatitis E. J Clin. Virol. 45 (3), 259–261.
Goldsmith, R., Yarbough, P.O., Reyes, G.R., Fry, K.E., Gabor, K.A., Kamel, M., Zakaria, S., Amer, S., Gaffar, Y., 1992. Enzyme-linked immunosorbent assay for diagnosis of acute sporadic hepatitis E in Egyptian children. Lancet 339 (8789), 328–331.
Goto, K., Ito, K., Sugiura, T., Ando, T., Mizutani, F., Miyake, Y., Kawabe, Y., Sugiyama, K., Togari, H., 2006. Prevalence of hepatitis E virus infection in Japanese children. J Pediatr. Gastroenterol. Nutr. 42 (1), 89–92.
Herremans, M., Bakker, J., Duizer, E., Vennema, H., Koopmans, M.P., 2007. Use of serological assays for diagnosis of hepatitis E virus genotype 1 and 3 infections in a setting of low endemicity. Clin. Vaccine Immunol. 14 (5), 562–568.
Hu, W.P., Lu, Y., Precioso, N.A., Chen, H.Y., Howard, T., Anderson, D., Guan, M., 2008. Double-antigen enzyme-linked immunosorbent assay for detection of hepatitis E virus-specific antibodies in human or swine sera. Clin. Vaccine Immunol. 15 (8), 1151–1157.
Huang, W., Zhang, H., Harrison, T.J., Lang, S., Huang, G., Wang, Y., 2008. Cross-protection of hepatitis E virus genotypes 1 and 4 in rhesus macaques. J. Med. Virol. 80 (5), 824–832.
Huang, W.J., Zhang, H.Y., Harrison, T.J., Lan, H.Y., Huang, G.Y., Wang, Y.C., 2009. Immunogenicity and protective efficacy in rhesus monkeys of a recombinant ORF2 protein from hepatitis E virus genotype 4. Arch. Virol. 154 (3), 481–488.

Please cite this article in press as: Khudyakov, Y., Kamili, S., Serological diagnostics of hepatitis E virus infection. Virus Res. (2011), doi:10.1016/j.virusres.2011.06.006

Huang, S., Zhang, X., Jiang, H., Yan, Q., Ai, X., Wang, Y., Cai, J., Jiang, L., Wu, T., Wang, Z., Guan, L., Shih, J.W., Ng, M.H., Zhu, F., Zhang, J., Xia, N., 2010. Profile of acute infectious markers in sporadic hepatitis E. PloS One 5 (10), e13560.
Husain, M.M., Srivastava, R., Akondy, R., Aggarwal, R., Jameel, S., Naik, S., 2010. Evidence of hepatitis E virus exposure among seronegative healthy residents of an endemic area. Intervirology 54 (3), 139–143.
Ijaz, S., Vyse, A.J., Morgan, D., Pebody, R.G., Tedder, R.S., Brown, D., 2009. Indigenous hepatitis E virus infection in England: more common than it seems. J. Clin. Virol. 44 (4), 272–276.
Im, S.W., Zhang, J.Z., Zhuang, H., Che, X.Y., Zhu, W.F., Xu, G.M., Li, K., Xia, N.S., Ng, M.H., 2001. A bacterially expressed peptide prevents experimental infection of primates by the hepatitis E virus. Vaccine 19 (27), 3726–3732.
Innis, B.L., Seriwatana, J., Robinson, R.A., Shrestha, M.P., Yarbough, P.O., Longer, C.F., Scott, R.M., Vaughn, D.W., Myint, K.S., 2002. Quantitation of immunoglobulin to hepatitis E virus by enzyme immunoassay. Clin. Diagn. Lab. Immunol. 9 (3), 639–648.
Jameel, S., Zafrullah, M., Ozdener, M.H., Panda, S.K., 1996. Expression in animal cells and characterization of the hepatitis E virus structural proteins. J. Virol. 70 (1), 207–216.
Jimenez de Oya, N., Galindo, I., Girones, O., Duizer, E., Escribano, J.M., Saiz, J.C., 2009. Serological immunoassay for detection of hepatitis E virus on the basis of genotype 3 open reading frame 2 recombinant proteins produced in *Trichoplusia ni* larvae. J. Clin. Microbiol. 47 (10), 3276–3282.
Kaur, M., Hyams, K.C., Purdy, M.A., Krawczynski, K., Ching, W.M., Fry, K.E., Reyes, G.R., Bradley, D.W., Carl, M., 1992. Human linear B-cell epitopes encoded by the hepatitis E virus include determinants in the RNA-dependent RNA polymerase. Proc. Natl. Acad. Sci. U.S.A. 89 (9), 3855–3858.
Khudyakov, Y.E., Favorov, M.O., Khudyakova, N.S., Cong, M.E., Holloway, B.P., Padhye, N., Lambert, S.B., Jue, D.L., Fields, H.A., 1994c. Artificial mosaic protein containing antigenic epitopes of hepatitis E virus. J. Virol. 68 (11), 7067–7074.
Khudyakov, Y.E., Khudyakova, N.S., Fields, H.A., Jue, D., Starling, C., Favorov, M.O., Krawczynski, K., Polish, L., Mast, E., Margolis, H., 1993. Epitope mapping in proteins of hepatitis E virus. Virology 194 (1), 89–96.
Khudyakov, Y.E., Lopareva, E.N., Jue, D.L., Crews, T.K., Thyagarajan, S.P., Fields, H.A., 1999. Antigenic domains of the open reading frame 2-encoded protein of hepatitis E virus. J. Clin. Microbiol. 37 (9), 2863–2871.
Khudyakov, Yu E., Favorov, M.O., Jue, D.L., Hine, T.K., Fields, H.A., 1994a. Immunodominant antigenic regions in a structural protein of the hepatitis E virus. Virology 198 (1), 390–393.
Khudyakov, Yu E., Khudyakova, N.S., Jue, D.L., Wells, T.W., Padhya, N., Fields, H.A., 1994b. Comparative characterization of antigenic epitopes in the immunodominant region of the protein encoded by open reading frame 3 in Burmese and Mexican strains of hepatitis E virus. J. Gen. Virol. 75 (Pt 3), 641–646.
Khuroo, M.S., 2010. Seroepidemiology of a second epidemic of hepatitis E in a population that had recorded first epidemic 30 years before and has been under surveillance since then. Hepatol. Int. 4 (2), 494–499.
Khuroo, M.S., 2011. Discovery of hepatitis E: the epidemic non-A, non-B hepatitis 30 years down the memory lane. Virus Res..
Khuroo, M.S., Kamili, S., Dar, M.Y., Moecklii, R., Jameel, S., 1993. Hepatitis E and long-term antibody status. Lancet 341 (8856), 1355.
Khuroo, M.S., Rustgi, V.K., Dawson, G.J., Mushahwar, I.K., Yattoo, G.N., Kamili, S., Khan, B.A., 1994. Spectrum of hepatitis E virus infection in India. J. Med. Virol. 43 (3), 281–286.
Krawczynski, K., Bradley, D.W., 1989. Enterically transmitted non-A, non-B hepatitis: identification of virus-associated antigen in experimentally infected cynomolgus macaques. J. Infect. Dis. 159 (6), 1042–1049.
Krawczynski, K., Meng, X.J., Rybczynska, J., 2011. Pathogenetic elements of hepatitis E and animal models of HEV infection. Virus Res..
Kuniholm, M.H., Purcell, R.H., McQuillan, G.M., Engle, R.E., Wasley, A., Nelson, K.E., 2009. Epidemiology of hepatitis E virus in the United States: results from the Third National Health and Nutrition Examination Survey, 1988–1994. J. Infect. Dis. 200 (1), 48–56.
Lal, S.K., Tulasiram, P., Jameel, S., 1997. Expression and characterization of the hepatitis E virus ORF3 protein in the methylotrophic yeast, *Pichia pastoris*. Gene 190 (1), 63–67.
Legrand-Abravanel, F., Thevenet, I., Mansuy, J.M., Saune, K., Vischi, F., Peron, J.M., Kamar, N., Rostaing, L., Izopet, J., 2009. Good performance of immunoglobulin M assays in diagnosing genotype 3 hepatitis E virus infections. Clin. Vaccine Immunol. 16 (5), 772–774.
Li, F., Riddell, M.A., Seow, H.F., Takeda, N., Miyamura, T., Anderson, D.A., 2000a. Recombinant subunit ORF2.1 antigen and induction of antibody against immunodominant epitopes in the hepatitis E virus capsid protein. J. Med. Virol. 60 (4), 379–386.
Li, F., Torresi, J., Locarnini, S.A., Zhuang, H., Zhu, W., Guo, X., Anderson, D.A., 1997. Amino-terminal epitopes are exposed when full-length open reading frame 2 of hepatitis E virus is expressed in *Escherichia coli*, but carboxy-terminal epitopes are masked. J. Med. Virol. 52 (3), 289–300.
Li, F., Zhuang, H., Kolivas, S., Locarnini, S.A., Anderson, D.A., 1994. Persistent and transient antibody responses to hepatitis E virus detected by western immunoblot using open reading frame 2 and 3 and glutathione S-transferase fusion proteins. J. Clin. Microbiol. 32 (9), 2060–2066.
Li, S.W., Zhang, J., Li, Y.M., Ou, S.H., Huang, G.Y., He, Z.Q., Ge, S.X., Xian, Y.L., Pang, S.Q., Ng, M.H., Xia, N.S., 2005. A bacterially expressed particulate hepatitis E vaccine: antigenicity, immunogenicity and protectivity on primates. Vaccine 23 (22), 2893–2901.
Li, T.C., Zhang, J., Shinzawa, H., Ishibashi, M., Sata, M., Mast, E.E., Kim, K., Miyamura, T., Takeda, N., 2000b. Empty virus-like particle-based enzyme-linked immunosorbent assay for antibodies to hepatitis E virus. J. Med. Virol. 62 (3), 327–333.
Liang, J.H., Dai, X., Dong, C., Meng, J.H., 2010. A single amino acid substitution changes antigenicity of ORF2-encoded proteins of hepatitis E virus. Int. J. Mol. Sci. 11 (8), 2962–2975.
Lin, C.C., Wu, J.C., Chang, T.T., Chang, W.Y., Yu, M.L., Tam, A.W., Wang, S.C., Huang, Y.H., Chang, F.Y., Lee, S.D., 2000. Diagnostic value of immunoglobulin G (IgG) and IgM anti-hepatitis E virus (HEV) tests based on HEV RNA in an area where hepatitis E is not endemic. J. Clin. Microbiol. 38 (11), 3915–3918.
Ma, H., Song, X., Li, Z., Harrison, T.J., Zhang, H., Huang, W., Hao, W., Kong, W., Wang, Y., 2009. Varying abilities of recombinant polypeptides from different regions of hepatitis E virus ORF2 and ORF3 to detect anti-HEV immunoglobulin M. J. Med. Virol. 81 (6), 1052–1061.
Ma, Y., Lin, S.Q., Gao, Y., Li, M., Luo, W.X., Zhang, J., Xia, N.S., 2003. Expression of ORF2 partial gene of hepatitis E virus in tomatoes and immunoactivity of expression products. World J. Gastroenterol. 9 (10), 2211–2215.
Mansuy, J.M., Legrand-Abravanel, F., Calot, J.P., Peron, J.M., Alric, L., Agudo, S., Rech, H., Destruel, F., Izopet, J., 2008. High prevalence of anti-hepatitis E virus antibodies in blood donors from South West France. J. Med. Virol. 80 (2), 289–293.
Mast, E.E., Alter, M.J., Holland, P.V., Purcell, R.H., 1998. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. Hepatology 27 (3), 857–861.
Meky, F.A., Stoszek, S.K., Abdel-Hamid, M., Selim, S., Abdel-Wahab, A., Mikhail, N., El-Kafrawy, S., El-Daly, M., Abdel-Aziz, F., Sharaf, S., Mohamed, M.K., Engle, R.E., Emerson, S.U., Purcell, R.H., Fix, A.D., Strickland, G.T., 2006. Active surveillance for acute viral hepatitis in rural villages in the Nile delta. Clin. Infect. Dis. 42, 628–633.
Meng, J., Dai, X., Chang, J.C., Lopareva, E., Pillot, J., Fields, H.A., Khudyakov, Y.E., 2001. Identification and characterization of the neutralization epitope(s) of the hepatitis E virus. Virology 288 (2), 203–211.
Meng, J., Pillot, J., Dai, X., Fields, H.A., Khudyakov, Y.E., 1998. Neutralization of different geographic strains of the hepatitis E virus with anti-hepatitis E virus-positive serum samples obtained from different sources. Virology 249 (2), 316–324.
Meng, X.J., 2010. Hepatitis E virus: animal reservoirs and zoonotic risk. Vet. Microbiol. 140 (3–4), 256–265.
Miyamura, T., 2011. Hepatitis E virus infection in developed countries. Virus Res..
Mushahwar, I.K., 2008. Hepatitis E virus: molecular virology, clinical features, diagnosis, transmission, epidemiology and prevention. J. Med. Virol. 80, 646–658.
Myint, K.S., Endy, T.P., Gibbons, R.V., Laras, K., Mammen Jr., M.P., Sedyaningsih, E.R., Seriwatana, J., Glass, J.S., Narupiti, S., Corwin, A.L., 2006a. Evaluation of diagnostic assays for hepatitis E virus in outbreak settings. J. Clin. Microbiol. 44 (4), 1581–1583.
Myint, K.S., Endy, T.P., Shrestha, M.P., Shrestha, S.K., Vaughn, D.W., Innis, B.L., Gibbons, R.V., Kuschner, R.A., Seriwatana, J., Scott, R.M., 2006b. Hepatitis E antibody kinetics in Nepalese patients. Trans. R. Soc. Trop. Med. Hyg. 100 (10), 938–941.
Myint, K.S., Guan, M., Chen, H.Y., Lu, Y., Anderson, D., Howard, T., Noedl, H., Mammen Jr., M.P., 2005. Evaluation of a new rapid immunochromatographic assay for serodiagnosis of acute hepatitis E infection. Am. J. Trop. Med. Hyg. 73 (5), 942–946.
Naot, Y., Remington, J.S., 1980. An enzyme-linked immunosorbent assay for detection of IgM antibodies to T*oxoplasma gondii*: use for diagnosis of acute acquired toxoplasmosis. J. Infect. Dis. 142 (5), 757–766.
Nicand, E., Grandadam, M., Teyssou, R., Rey, J.L., Buisson, Y., 2001. Viraemia and faecal shedding of HEV in symptom-free carriers. Lancet 357 (9249), 68–69.
Obriadina, A., Meng, J.H., Ulanova, T., Trinta, K., Burkov, A., Fields, H.A., Khudyakov, Y.E., 2002. A new enzyme immunoassay for the detection of antibody to hepatitis E virus. J. Gastroenterol. Hepatol. 17 (Suppl. 3), S360–S364.
Pan, J.S., Zhang, K., Zhou, J., Wu, C., Zhuang, H., Zhou, Y.H., 2010. Application of truncated immunodominant polypeptide from hepatitis E virus (HEV) ORF2 in an assay to exclude nonspecific binding in detecting anti-HEV immunoglobulin M. J. Clin. Microbiol. 48 (3), 779–784.
Paul, D.A., Knigge, M.F., Ritter, A., Gutierrez, R., Pilot-Matias, T., Chau, K.H., Dawson, G.J., 1994. Determination of hepatitis E virus seroprevalence by using recombinant fusion proteins and synthetic peptides. J. Infect. Dis. 169 (4), 801–806.
Purdy, M.A., Carson, D., McCaustland, K.A., Bradley, D.W., Beach, M.J., Krawczynski, K., 1994. Viral specificity of hepatitis E virus antigens identified by fluorescent antibody assay using recombinant HEV proteins. J. Med. Virol. 44 (2), 212–214.
Purdy, M.A., Khudyakov, Y.E., 2011. The molecular epidemiology of hepatitis E virus infection. Virus Res..
Purdy, M.A., McCaustland, K.A., Krawczynski, K., Spelbring, J., Reyes, G.R., Bradley, D.W., 1993. Preliminary evidence that a trpE-HEV fusion protein protects cynomolgus macaques against challenge with wild-type hepatitis E virus (HEV). J. Med. Virol. 41 (1), 90–94.
Purdy, M.A., McCaustland, K.A., Krawczynski, K., Tam, A., Beach, M.J., Tassopoulos, N.C., Reyes, G.R., Bradley, D.W., 1992. Expression of a hepatitis E virus (HEV)-trpE fusion protein containing epitopes recognized by antibodies in sera from human cases and experimentally infected primates. Arch. Virol. 123 (3–4), 335–349.
Qi, Z., Cui, D., Pan, W., Yu, C., Song, Y., Cui, H., Arima, T., 1995. Synthesis and application of hepatitis E virus peptides to diagnosis. J. Virol. Methods 55 (1), 55–66.
Riddell, M.A., Li, F., Anderson, D.A., 2000. Identification of immunodominant and conformational epitopes in the capsid protein of hepatitis E virus by using monoclonal antibodies. J. Virol. 74 (17), 8011–8017.
Robinson, R.A., Burgess, W.H., Emerson, S.U., Leibowitz, R.S., Sosnovtseva, S.A., Tsarev, S., Purcell, R.H., 1998. Structural characterization of recombinant

Please cite this article in press as: Khudyakov, Y., Kamili, S., Serological diagnostics of hepatitis E virus infection. Virus Res. (2011), doi:10.1016/j.virusres.2011.06.006

hepatitis E virus ORF2 proteins in baculovirus-infected insect cells. Protein Expr. Purif. 12 (1), 75–84.
Sanford, B.J., Dryman, B.A., Huang, Y.W., Feagins, A.R., Leroith, T., Meng, X.J., 2011. Prior infection of pigs with a genotype 3 swine hepatitis E virus (HEV) protects against subsequent challenges with homologous and heterologous genotypes 3 and 4 human HEV. Virus Res..
Schofield, D.J., Glamann, J., Emerson, S.U., Purcell, R.H., 2000. Identification by phage display and characterization of two neutralizing chimpanzee monoclonal antibodies to the hepatitis E virus capsid protein. J. Virol. 74 (12), 5548–5555.
Seriwatana, J., Shrestha, M.P., Scott, R.M., Tsarev, S.A., Vaughn, D.W., Myint, K.S., Innis, B.L., 2002. Clinical and epidemiological relevance of quantitating hepatitis E virus-specific immunoglobulin M. Clin. Diagn. Lab. Immunol. 9 (5), 1072–1078.
Srinivasappa, J., Saegusa, J., Prabhakar, B.S., Gentry, M.K., Buchmeier, M.J., Wiktor, T.J., Koprowski, H., Oldstone, M.B., Notkins, A.L., 1986. Molecular mimicry: frequency of reactivity of monoclonal antiviral antibodies with normal tissues. J. Virol. 57 (1), 397–401.
Takahashi, M., Kusakai, S., Mizuo, H., Suzuki, K., Fujimura, K., Masuko, K., Sugai, Y., Aikawa, T., Nishizawa, T., Okamoto, H., 2005a. Simultaneous detection of immunoglobulin A (IgA) and IgM antibodies against hepatitis E virus (HEV) is highly specific for diagnosis of acute HEV infection. J. Clin. Microbiol. 43 (1), 49–56.
Takahashi, M., Nishizawa, T., Tanaka, T., Tsatsralt-Od, B., Inoue, J., Okamoto, H., 2005b. Correlation between positivity for immunoglobulin A antibodies and viraemia of swine hepatitis E virus observed among farm pigs in Japan. J. Gen. Virol. 86 (Pt 6), 1807–1813.
Tam, A.W., Smith, M.M., Guerra, M.E., Huang, C.C., Bradley, D.W., Fry, K.E., Reyes, G.R., 1991. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. Virology 185 (1), 120–131.
Tanaka, E., Matsumoto, A., Takeda, N., Li, T.C., Umemura, T., Yoshizawa, K., Miyakawa, Y., Miyamura, T., Kiyosawa, K., 2005. Age-specific antibody to hepatitis E virus has remained constant during the past 20 years in Japan. J. Viral Hepat. 12 (4), 439–442.
Teo, C.G., 2006. Hepatitis E indigenous to economically developed countries: to what extent a zoonosis? Curr. Opin. Infect. Dis. 19 (5), 460–466.
Teshale, E.H., Howard, C.M., Grytdal, S.P., Handzel, T.R., Barry, V., Kamili, S., Drobeniuc, J., Okware, S., Downing, R., Tappero, J.W., Bakamutumaho, B., Teo, C.G., Ward, J.W., Holmberg, S.D., Hu, D.J., 2010. Hepatitis E epidemic, Uganda. Emerg. Infect. Dis. 16 (1), 126–129.
Thomas, D.L., Yarbough, P.O., Vlahov, D., Tsarev, S.A., Nelson, K.E., Saah, A.J., Purcell, R.H., 1997. Seroreactivity to hepatitis E virus in areas where the disease is not endemic. J. Clin. Microbiol. 35 (5), 1244–1247.
Tokita, H., Harada, H., Gotanda, Y., Takahashi, M., Nishizawa, T., Okamoto, H., 2003. Molecular and serological characterization of sporadic acute hepatitis E in a Japanese patient infected with a genotype III hepatitis E virus in 1993. J. Gen. Virol. 84 (Pt 2), 421–427.
Tsarev, S.A., Tsareva, T.S., Emerson, S.U., Kapikian, A.Z., Ticehurst, J., London, W., Purcell, R.H., 1993. ELISA for antibody to hepatitis E virus (HEV) based on complete open-reading frame-2 protein expressed in insect cells: identification of HEV infection in primates. J. Infect. Dis. 168 (2), 369–378.
Tuuminen, T., Seppanen, H., Pitkanen, E.M., Palomaki, P., Kapyaho, K., 1999. Improvement of immunoglobulin M capture immunoassay specificity: toxoplasma antibody detection method as a model. J. Clin. Microbiol. 37 (1), 270–273.
Ulanova, T.I., Obriadina, A.P., Talekar, G., Burkov, A.N., Fields, H.A., Khudyakov, Y.E., 2009. A new artificial antigen of the hepatitis E virus. J. Immunoassay Immunochem. 30 (1), 18–39.
Wang, Y., Zhang, H., Li, Z., Gu, W., Lan, H., Hao, W., Ling, R., Li, H., Harrison, T.J., 2001. Detection of sporadic cases of hepatitis E virus (HEV) infection in China using immunoassays based on recombinant open reading frame 2 and 3 polypeptides from HEV genotype 4. J. Clin. Microbiol. 39 (12), 4370–4379.
Wedemeyer, H., Pischke, S., 2011. Hepatitis: Hepatitis E vaccination – is HEV 239 the breakthrough? Nat. Rev. Gastroenterol. Hepatol. 8 (1), 8–10.
Xing, L., Wang, J.C., Li, T.C., Yasutomi, Y., Lara, J., Khudyakov, Y., Schofield, D., Emerson, S.U., Purcell, R.H., Takeda, N., Miyamura, T., Cheng, R.H., 2011. Spatial configuration of hepatitis E virus antigenic domain. J. Virol. 85 (2), 1117–1124.
Yamashita, T., Mori, Y., Miyazaki, N., Cheng, R.H., Yoshimura, M., Unno, H., Shima, R., Moriishi, K., Tsukihara, T., Li, T.C., Takeda, N., Miyamura, T., Matsuura, Y., 2009. Biological and immunological characteristics of hepatitis E virus-like particles based on the crystal structure. Proc. Natl. Acad. Sci. U.S.A. 106 (31), 12986–12991.
Yarbough, P.O., Tam, A.W., Fry, K.E., Krawczynski, K., McCaustland, K.A., Bradley, D.W., Reyes, G.R., 1991. Hepatitis E virus: identification of type-common epitopes. J. Virol. 65 (11), 5790–5797.
Yu, C., Engle, R.E., Bryan, J.P., Emerson, S.U., Purcell, R.H., 2003. Detection of immunoglobulin M antibodies to hepatitis E virus by class capture enzyme immunoassay. Clin. Diagn. Lab. Immunol. 10 (4), 579–586.
Zhang, F., Li, X., Li, Z., Harrison, T.J., Chong, H., Qiao, S., Huang, W., Zhang, H., Zhuang, H., Wang, Y., 2006. Detection of HEV antigen as a novel marker for the diagnosis of hepatitis E. J. Med. Virol. 78 (11), 1441–1448.
Zhang, H., Dai, X., Shan, X., Meng, J., 2009. Characterization of antigenic epitopes of the ORF2 protein from hepatitis E virus genotype 4. Virus Res. 142 (1–2), 140–143.
Zhang, J., Gu, Y., Ge, S.X., Li, S.W., He, Z.Q., Huang, G.Y., Zhuang, H., Ng, M.H., Xia, N.S., 2005. Analysis of hepatitis E virus neutralization sites using monoclonal antibodies directed against a virus capsid protein. Vaccine 23 (22), 2881–2892.
Zhang, J.Z., Im, S.W., Lau, S.H., Chau, T.N., Lai, S.T., Ng, S.P., Peiris, M., Tse, C., Ng, T.K., Ng, M.H., 2002. Occurrence of hepatitis E virus IgM, low avidity IgG serum antibodies, and viremia in sporadic cases of non-A, -B, and -C acute hepatitis. J. Med. Virol. 66 (1), 40–48.
Zhang, J.Z., Ng, M.H., Xia, N.S., Lau, S.H., Che, X.Y., Chau, T.N., Lai, S.T., Im, S.W., 2001a. Conformational antigenic determinants generated by interactions between a bacterially expressed recombinant peptide of the hepatitis E virus structural protein. J. Med. Virol. 64 (2), 125–132.
Zhang, M., Emerson, S.U., Nguyen, H., Engle, R.E., Govindarajan, S., Gerin, J.L., Purcell, R.H., 2001b. Immunogenicity and protective efficacy of a vaccine prepared from 53 kDa truncated hepatitis E virus capsid protein expressed in insect cells. Vaccine 20 (5–6), 853–857.
Zhao, C., Li, L., Harrison, T.J., Wang, Q., Song, A., Fan, J., Ma, H., Zhang, C., Wang, Y., 2009a. Relationships among viral diagnostic markers and markers of liver function in acute hepatitis E. J. Gastroenterol. 44 (2), 139–145.
Zhao, K., Liu, Q., Yu, R., Li, Z., Li, J., Zhu, H., Wu, X., Tan, F., Wang, J., Tang, X., 2009b. Screening of specific diagnostic peptides of swine hepatitis E virus. Virol. J. 6, 186.
Zhou, Y.H., Purcell, R.H., Emerson, S.U., 2004. An ELISA for putative neutralizing antibodies to hepatitis E virus detects antibodies to genotypes 1, 2, 3, and 4. Vaccine 22 (20), 2578–2585.
Zhou, Y.X., Lee, M.Y., Ng, J.M., Chye, M.L., Yip, W.K., Zee, S.Y., Lam, E., 2006. A truncated hepatitis E virus ORF2 protein expressed in tobacco plastids is immunogenic in mice. World J. Gastroenterol. 12 (2), 306–312.

Please cite this article in press as: Khudyakov, Y., Kamili, S., Serological diagnostics of hepatitis E virus infection. Virus Res. (2011), doi:10.1016/j.virusres.2011.06.006