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IMMUNOREACTIVE ANTIGENS OF HEPATITIS E VIRUS

The present invention relates generally to molecules such as peptides, polypeptides and proteins which carry epitopes and in particular B cell epitopes from antigenic proteins encoded by Hepatitis E Virus. These molecules are preferentially immunoreactive to convalescent or acute phase circulating antibodies to the Hepatitis E Virus and are useful in the development of diagnostic, therapeutic and prophylactic agents for Hepatitis E Virus.

10 Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

15 Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

20 Viral hepatitis results from infection with one of at least five very different viral agents. Available serological tests allow the diagnosis of acute hepatitis due to infection with Hepatitis A Virus (HAV) and Hepatitis B Virus (HBV). HBV is required for propagation of the delta agent, or Hepatitis D Virus (HDV); this co-infection results in a high proportion of cases progressing to chronic active hepatitis. The clinical and
25 diagnostic exclusion of HAV and HBV has led to the recognition of other hepatitides that were formerly grouped together as non-A, non-B hepatitis [(NANBH)] (Prince *et al.*, 1974; Feinstone *et al.*, 1975; Tabor, 1985). NANBH is caused by more than one viral agent and can be transmitted by either parenteral or fecal/oral routes (Bradley, 1990a; Reyes and Baroudy, 1991).

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The cloning of a blood-borne agent, termed Hepatitis C Virus (HCV), led to the development of a specific assay for circulating antibody to HCV (Choo *et al.*, 1989; Kuo

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et al., 1989; Kubo *et al.*, 1989, Maeno *et al.*, 1990). This assay predominantly detects infections at the chronic stage, but has facilitated the identification of HCV as the cause of up to 90% of parenterally transmitted NANBH. A second epidemiologically distinct form of NANBH has been shown to occur in both epidemic and sporadic patterns in developing countries and is referred to as enterically transmitted non-A, non-B hepatitis (ET-NANBH) due to its water-borne mode of virus transmission and presumed enteric route of infection (Khuroo, 1980; Wong *et al.*, 1980). ET-NANBH has been documented in India, Pakistan, Burma, USSR, Costa Rica, Mexico and countries in Africa where epidemic outbreaks can generally be traced to fecal contamination of drinking water. (Bradley and Maynard, 1986; Bradley, 1990b). The causative viral agent was previously shown to passage successfully in cynomolgus macaques (cyno) and tamarins with typical liver enzyme elevations and recovery of morphologically similar 27 to 34 nm virus like particles from the feces of clinical specimens and experimental animals (Balayan *et al.*, 1983; Andiaparidze *et al.*, 1986; Bradley *et al.*, 1987; Arankalle *et al.*, 1988).

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Reyes *et al.* (1990) recently reported the isolation of a partial cDNA clone from the virus responsible for ET-NANBH, and termed the newly identified agent the Hepatitis E Virus (HEV). The expressions "enterically transmitted non-A, non-B hepatitis", "ET-NANBH", "Hepatitis E Virus" and "HEV" are used interchangeably herein to refer to a virus or type of virus which is capable of causing infectious hepatitis from contaminated water, which is transmissible and capable of passage in cynomologus macaques and tamarins, which is serologically distinct from HAV, HBV, HCV and HDV and which comprises a genomic nucleotide sequence, a portion of which, at least is homologous to or substantially similar to or capable of hybridizing under low stringency conditions to the all or part of the nucleotide sequences set forth in Figure 1 and/or Figure 2. Preferred homologies and a definition of stringency conditions are set forth below.

The HEV clone was from a Burma isolate of the virus and hybridised with cDNA made from five other distinct geographic isolates. These molecular epidemiological findings are consistent with the available serologic data based on the use of immune electron microscopy and immunofluorescence blocking studies that indicate a single major agent is responsible for the majority of ET-NANBH seen worldwide (Purcell and



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★ ★ Ticehurst, 1988; Bradley *et al.* 1988a; Krawczynski and Bradley, 1989). Tam *et al.*
(1991) subsequently reported on the molecular cloning of the complete HEV (Burma; B)
viral clone together with the deduced amino acid sequences.

5 HEV has a single stranded genome of polyadenylated RNA of positive polarity
which encodes three open reading frames (ORF's) designated ORF1, ORF2 and ORF3.
Open reading frame 2 and ORF3 are partially overlapping in different reading frames and
are thought to encode structural proteins of the virus. Open reading frame 1, on the other
hand, encodes replicative proteins and overlaps ORF3 by one nucleotide.

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Despite the availability of immunoassays for the detection of antibodies to some
strains of HEV, it has been observed that IgM and IgG antibody titres wane rapidly
following infection. Consequently, it has proven difficult to interpret serological surveys
from both endemic and non-endemic areas. Furthermore, the relationship between
15 antibody interactivity and immunity to reinfection remains unclear. These and other
factors have delayed the development of suitable diagnostic and therapeutic protocols for
the HEV, for which a need clearly exists. In particular, it would be most beneficial to
develop an assay which could distinguish between acute phase antibodies (contemporary
antibodies) generated in response to HEV infection and convalescent phase antibodies,
20 which remain in the circulatory and/or secretory system after infection and may contribute
to immunity to reinfection.

In work leading up to the present invention, the inventors sought to identify
epitopes, and in particular B cell epitopes, on peptides, polypeptides and proteins encoded
25 within the HEV genome in order to improve upon current diagnostic procedures and to
further develop therapeutic and prophylactic compositions for HEV. In accordance with
the present invention, peptides, polypeptides and proteins, were recombinantly expressed
from nucleic acid molecules derived from ORFs in the HEV genome. The epitopic and
in particular B cell epitopic regions within these molecules were identified on the basis
30 of interactivity to antibodies specific to HEV, thereby forming the basis for a new range
of diagnostic, therapeutic and prophylactic procedures for HEV. Interestingly, the
inventors have determined that one portion of a molecule encoded by an ORF may inhibit

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or otherwise reduce the immunointeractivity of another portion of the same molecule. They have further determined that the inhibitory effect may be overcome by using non-full length molecules or reducing the inhibitory effect by physical or chemical processes.

5 Accordingly, one aspect of the present invention provides a recombinant molecule encoded by a sequence of nucleotides selected from the list consisting of open reading frame (ORF) 2 and ORF 3 of Hepatitis E Virus (HEV) or a mutant or derivative of said ORF2 or ORF3. More particularly, the present invention provides a recombinant molecule encoded by a sequence of nucleotides comprising "ORF3" or a part of "ORF2"
10 and which molecule is preferentially immunologically interactive in either convalescent phase or acute phase antibodies to HEV are conveniently described respectively as those regions of the HEV genome beginning at nucleotide 5106 extending 369 bases and terminating at nucleotide 5474 (ORF3) and that region beginning at nucleotide 5147 extending 1980 bases and terminating 68 bases upstream of the poly(A) tail (ORF2) using
15 the numbering system of Tam *et al.* (1991).

 The term "recombinant molecule" in this context includes a peptide, polypeptide, protein or a chemical equivalent thereof. The recombinant molecule may or may not be glycosylated. A recombinant molecule also extends to a fusion between an HEV derived
20 peptide, polypeptide or protein and a peptide, polypeptide or protein of non-HEV origin such as but not limited to glutathione-S-transferase (GST) or its derivatives, polylysine, polyhistidine, thioredoxin or any other molecule capable of bioaffinity chromatography.

 In accordance with the present invention, it has been surprisingly discovered that
25 recombinant molecules encoded by ORF2 and in particular the 3' end of ORF2 and ORF3 are capable of interacting with convalescent phase antibodies to HEV whereas recombinant molecules encoded by the 5' end of ORF2 are interactive more preferentially with acute phase antibodies generated to the virus particle *per se*. According to this aspect of the present invention, there is provided a recombinant peptide or polypeptide
30 which is preferentially immunologically reactive with convalescent phase antibodies generated during HEV infection. In a related aspect of the present invention, there is provided a recombinant peptide or polypeptide which is preferentially immunologically

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interactive with acute phase antibodies generated during HEV infection.

As used herein, the 3' end of an ORF (e.g. ORF2) is considered the carboxy (C)-terminal end portion of the molecule encoded by the ORF and the 5' end is considered the amino (N)-terminal end portion.

More particularly, it has further been surprisingly discovered that the 5' (i.e. the C-terminal) end portion of ORF2 exhibits greater immunointeractivity to acute phase antibodies to HEV and the 3' (i.e. N-terminal) end portion of ORF2 exhibits greater immunointeractivity to convalescent phase antibodies to HEV, when present on the same molecule, however, the N-terminal end portion appears to inhibit or reduce the immunointeractivity of the C-terminal end portion.

Accordingly, another aspect of the present invention contemplates a recombinant molecule encoded by a sequence of nucleotides comprising ORF3 or a non-full length ORF2 derived from the 3' end of ORF2 of HEV and wherein said recombinant molecule is preferentially interactive with convalescent phase antibodies to HEV. In a related aspect there is provided a recombinant molecule encoded by a sequence of nucleotides comprising a non-length ORF2 derived from the 5' end of ORF2 of HEV and wherein said recombinant molecule is preferentially interactive with acute phase antibodies to HEV.

In one embodiment the sequence of nucleotides is from the 5' end of ORF2 and encodes a molecule more interactive with acute phase antibodies relative to convalescent phase antibodies. In another embodiment, the nucleotide sequence is from ORF3 or the 3' end of ORF2 and encodes a molecule more interactive with convalescent phase antibodies relative to acute phase antibodies. The preferred nucleotide sequence of the 5' end of ORF2 includes the clones 2.3 and 2.4 defined in Figure 5.

The recombinant molecule may also be encoded by a nucleotide sequence comprising the full length ORF2 but modified to permit interactivity to both convalescent and acute phase antibodies to HEV. Such modification includes chemical and physical

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treatments to expose epitopes, for example by denaturing followed by renaturing of the molecule or the use of antagonists to prevent the inhibition of, for example, the N-terminal end portion over the C-terminal end portion binding to HEV antibodies.

5 "Acute and "convalescent" phases are used in their broadest sense to mean respectively the period during infection (acute) and the period beginning from after the peak of infection extending through a convalescent period to a period post infection. The acute phase of infection can conveniently be considered, for example, to be approximately 3 to 6 months following exposure to HEV and the convalescent phase is generally after
10 this period.

Yet another aspect of the present invention is directed to a recombinant polypeptide comprising first and second amino acid sequences wherein said first amino acid sequence is encoded by a nucleotide sequence selected from or within ORF2 and
15 ORF 3 of HEV and wherein said second amino acid sequence is a non-HEV encoded peptide, polypeptide or protein such as but not exclusively GST. Preferably, the first amino acid sequence is encoded by ORF3 or the 3' end of ORF2 and is interactive with convalescent phase antibodies to HEV. Alternatively, the first amino acid sequence is from the 5' end portion of ORF2 and is preferably interactive with acute phase antibodies
20 to HEV.

The presence of selectively or preferentially immunologically reactive antigens provides a means of distinguishing between past (convalescent) and acute (contemporary) phase infection with HEV. Accordingly, the present invention also extends to a method
25 for distinguishing between convalescent and acute phase infection with HEV in an individual which method measures the reactivity of serum to immune interactive recombinant molecule encoded by ORF2 and/or ORF3 or more preferably parts thereof. Most preferably, these parts include the 3' end portion of ORF2 or the 5' end portion of ORF2 or is a chemically modified form of a full length molecule such as to expose
30 epitopes on both the N- and C- termini.

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The present invention also provides an isolated nucleic acid molecule such as DNA or cDNA molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a peptide, polypeptide or protein interactive with acute phase antibodies to HEV. In an alternative embodiment, the peptide, polypeptide or protein is interactive with both convalescent and/or acute phase circulating antibodies to HEV. Accordingly, this aspect of the present invention provides a recombinant molecule carrying a B cell epitope of a polypeptide or protein encoded by ORF2 or ORF3 or more preferably a part thereof. More particularly, the sequence of nucleotides is selected from a sequence comprising or within nucleotides 6326 to 7129 (ORF2) and 5106 to 5474 (ORF3) in the HEV genome using the nucleotide numbering system of Tam *et al* (1991) or a nucleotide region substantially equivalent thereto. Preferably, the nucleotide sequence is operably linked to an expression control sequence such as in the form of an expression vector.

The recombinant molecule according to this aspect of the present invention is generally an isolated nucleic acid molecule comprising DNA (eg cDNA or genomic DNA) or mRNA. The recombinant molecule may be isolated directly from the HEV genome or may be generated *in vitro* by, for example, the stepwise addition of nucleotides or groups of nucleotides. The present invention further extends to a host cell such as bacterium, yeast, mammalian or insect cell transformed with such a recombinant molecule. A preferred mammalian cell is the Chinese Hamster Ovary (CHO) cell line. A preferred bacterium is *E. coli*. Preferably, the nucleic acid molecules are DNA, at least parts of which have a nucleotide sequence substantially corresponding to all or part of the nucleotide sequence shown in Figures 1 (SEQ ID NO: 1) or 2 (SEQ ID NO: 3) or a part, fragment, derivative, homologue or analogue thereof or one or more sequences complementary thereto. The present invention, however also extends to any single or multiple nucleotide substitutions, deletions and/or additions to the sequence shown in Figures 1 or 2 and which still encode an epitopic region of the HEV genome or fragment or derivative thereof having the requisite antigenic profile and interactive with antibodies to HEV. Most preferably, the nucleotide sequence encode either the 3' end of ORF2 or the 5' end of ORF2 such that the nucleotide sequence encodes a peptide or polypeptide which interacts preferably with convalescent phase antibodies or acute phase antibodies,



respectively.

Furthermore, when the nucleic acid molecule is RNA, the ribonucleotide sequence will, in a preferred embodiment, be substantially complementary to one or more of the nucleotide sequences shown in Figures 1 or 2 a part, fragment, derivative, homologue or analogue thereof. Another aspect of this invention is directed to a synthetic (e.g. recombinant) peptide, polypeptide or protein which is immunologically interactive with convalescent phase antibodies generating during HEV infection. Furthermore, the invention is further directed to a synthetic (eg: recombinant) peptide, polypeptide or protein which is immunologically interactive with acute and convalescent circulating antibodies generated during HEV infection.

Such synthetic peptides, polypeptides or proteins may, for example, be prepared by recombinant means such as by the expression of a host cell transformed with the recombinant molecules described above. The peptide, polypeptide or protein may be fused to another peptide, polypeptide or protein. Alternatively, it may be prepared by chemical synthesis, such as by the Merrifield solid-phase synthesis procedure. Furthermore, although synthetic or fragments thereof represent a preferred embodiment, the present invention also extends to biologically pure preparations of the naturally occurring epitopes or their fragments. By "biologically pure" is meant a preparation of at least 60%, preferably at least 70%, more preferably at least 80% and still more preferably at least 90% by weight peptide, polypeptide or protein.

Another aspect of the present invention contemplates a peptide, polypeptide or protein carrying a B cell epitope interactive with antibodies to HEV, said peptide, polypeptide or protein being encoded by a sequence of nucleotides capable of hybridising under low stringency conditions to one or more regions of the nucleotide sequence set forth in Figure 1 (SEQ ID NO: 1) or 2 (SEQ ID NO: 3).

More particularly, one aspect of this embodiment is directed to a nucleic acid molecule comprising a sequence of nucleotides which:



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- (i) encodes a peptide, polypeptide or protein interactive with acute and/or convalescent phase antibodies to HEV; and
- (ii) is capable of hybridizing under low stringency conditions to all or part of the nucleotide sequence set forth in Figure 1 (SEQ ID NO: 1) under low stringency conditions.

In a related aspect, the nucleotide sequence encodes a peptide, polypeptide or protein interactive with acute phase antibodies and is capable of hybridizing under low stringency conditions to all or part of the nucleotide sequence set forth in Figure 1 or Figure 2.

In a preferred embodiment, the nucleotide sequence is capable of hybridizing to the 5' end of ORF2 and encodes a molecule capable of preferential interaction with acute phase antibodies to HEV. Most preferably, the nucleotide sequence is sequence 2.3 or 2.4 of Figure 5.

Another aspect of this embodiment of the present invention provides a recombinant peptide, polypeptide or protein encoded by a nucleotide sequence capable of hybridizing under low stringency conditions to all or part of the nucleotide sequence set forth in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3). This aspect of the present invention includes recombinant or synthetic forms of naturally occurring proteins encoded by the aforementioned nucleotide sequences as well as peptide, polypeptide or protein derivatives thereof which are encoded by modified nucleotide sequences still capable of hybridizing to the nucleotide sequence in Figure 1 (SEQ ID NO: 1) or 2 (SEQ ID NO: 3) and which derivatives are interactive with acute and/or convalescent phase antibodies to HEV.

For the purposes of defining the level of stringency, reference can conveniently be made to Maniatis *et al* (1982) at pages 387-389 which is herein incorporated by reference where the washing step disclosed is considered high stringency. A low stringency is defined herein as being in 4-6X SSC/0.1-0.5% w/v SDS at 37-45°C for 2-3



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hours. Depending on the source and concentration of nucleic acid involved in the hybridization, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 1-4X SSC/0.25-0.5% w/v SDS at $\geq 45^{\circ}\text{C}$ for 2-3 hours or high stringency conditions which are considered herein to be 0.1-
5 1X SSC/0.1% w/v SDS at $\geq 60^{\circ}\text{C}$ for 1-3 hours.

In yet a further aspect, the present invention contemplates a peptide, polypeptide or protein carrying a B cell epitope interactive with antibodies to HEV, said peptide, polypeptide or protein having a substantially similar amino acid sequence to one or more
10 regions of the amino acid sequences set forth in Figure 1 (SEQ ID NO: 2) or 2 (SEQ ID NO: 4). By "substantially similar" is meant a sequence having at least 50%, preferably at least 60%, more preferably at least 75% and still more preferably at least 90% homology (i.e. similarity) with the sequences set forth in Figure 1 (SEQ ID NO: 2) or 2 (SEQ ID NO: 4) at the amino acid level. Preferably, the peptide, polypeptide or protein
15 comprise, the N-terminal encoding region of ORF2 which is preferentially interactive with acute phase antibodies to HEV. Alternatively, the peptide, polypeptide or protein comprises the C-terminal encoding region of ORF2 which is preferentially interactive with convalescent phase antibodies to HEV. In a further alternative, the peptide, polypeptide or protein is a full length ORF encoded molecule but chemically or physically modified
20 or disrupted to expose both the N- and C- terminal epitopes.

The present invention also extends to a method for maintaining interactive conformation of synthetic (eg recombinant) peptides or polypeptides wherein the synthetic peptides or polypeptides are further purified after undergoing a denaturation procedure
25 and subsequently renatured. By way of example the synthetic peptide may be denatured by adding denaturing concentrations of buffers including but not limited to, sodium dodecyl sulphate (SDS), 2-mercaptoethanol, or urea. Purification of denatured protein can be undertaken by procedures well known to the skilled artisan including but not limited to procedures for electrophoretic resolution on polyacrylamide gels or size exclusion
30 chromatography. The denaturing buffers are removed by any number of means including but not limited to dilution, dialysis, chromatography or by binding to a solid support, for example membrane support or microtitre plate.



Accordingly, another aspect of the present invention contemplates a method of enhancing the immunointeractivity of a polypeptide or protein encoded by ORF 2 of HEV, said method comprising chemically or physically modifying the polypeptide or protein to enable both convalescent and acute phase antibodies to HEV to bind to said polypeptide or protein without substantial N-terminal inhibition of C-terminal binding to convalescent phase antibodies to HEV.

In a most preferred embodiment, the present invention extends to naturally occurring or synthetic (eg recombinant) peptide, polypeptide or protein corresponding to an approximately 30KDa protein from the ORF2 region and/or an approximately 14KDa protein from the ORF3 region and to nucleotide sequences coding for same as well as to fragments, derivatives, homologues or immunological relatives thereof. By "derivatives" is meant to include any single or multiple amino acid substitution, deletion and/or addition relative to the naturally occurring sequence or to the sequences as shown in Figures 1 (SEQ ID NO: 2) and 2 (SEQ ID NO: 4) and including any single or multiple substitution, deletion and/or addition to other molecules associated with the peptide or polypeptide including carbohydrate, lipid and/or other proteinacious moieties. Such derivatives, therefore, include glycosylated or non-glycosylated forms or molecules with altered glycosylation patterns.

20

The present invention is also directed to a method for distinguishing past (i.e. convalescent phase) or contemporary (i.e. acute phase) infection with HEV whether or not by natural infection or following vaccination, whereby the antisera from infected individuals are preferentially immunologically distinguished by interactive peptides of ORF3 or the N-terminus of ORF2. Alternatively, such antisera are preferably immunologically distinguished by interactive peptides of the C-terminal end portion of ORF2.

25

The present invention also contemplates a method for the detection of antibodies associated with HEV which method comprises contacting a peptide or polypeptide corresponding to all, or an antigenic portion of the epitopic regions of the HEV genome or a derivative thereof with a biological sample from a patient to be tested for a time and

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under conditions sufficient for a complex to form between the peptide or polypeptide and an antibody reactive to HEV and then detecting the complex. Preferably, the biological sample is serum, a blood derived component such as whole blood, serum or plasma or a secretory component such as saliva. Even more preferably, the peptide or polypeptide is immobilised onto a solid support before, during or after contact with the serum. Methods of detection are well known and include colorimetric, fluorometric and radioactive procedures. Other detection means can also be used such as involving immunoblotting. An example of such immunoblotting techniques includes the well known Western Blot technique. This assay can be varied in any number of ways without departing from the scope of the present invention. Another assay contemplated herein to be useful is the AGEN red cell agglutination assay as described by Wilson *et al.* (1991).

The present invention also extends to the use of peptides or polypeptide corresponding to the epitopic regions of the HEV genome or antigenic fragments thereof, as antigens in a diagnostic test for HEV, or for screening asymptomatic individuals by detection or determination of the titre of antibodies in a patient's serum, for example using enzyme-linked immunoassay or radio immunoassay (RIA) technology or an agglutination assay using antigen-coated beads or the like.

This aspect of the present invention may conveniently be carried out by the detection and/or determination of the titre of antibodies in a biological sample (e.g. whole blood, plasma, serum or saliva) from a human subject, said method comprising contacting said sample with peptides or polypeptides corresponding to epitopic regions of the HEV genome or a fragment or derivative thereof for a time and under conditions sufficient for a complex to form between the peptide or polypeptide and an antibody reactive to HEV and then detecting the complex and/or amount of peptide or polypeptide which has been bound in the complex. Preferably, the peptide or polypeptide is immobilised onto a solid support before, during or after contact with the sample and the peptides or polypeptides are as hereinbefore defined.

Alternatively, HEV infection may be detected or at least a negative result re-confirmed by screening for HEV associated immune complexes. It is possible, for

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example, that a negative antibody result could have been caused by antibodies forming complexes with HEV thereby not being available for binding in the aforementioned assay. To conveniently detect HEV immune complexes, serum or other biological fluid is contacted with an anti-HEV antibody (e.g. a monoclonal antibody) for a time and under
5 conditions sufficient for a HEV-antibody immune complex to bind. Preferably, the anti-HEV antibody is first immobilised onto a solid support. An anti-immunoglobulin antibody, generally with a label or other reporter molecule attached, is then used to screen for the antibody component of the HEV complex.

10 One skilled in the art will immediately recognise that the assays as contemplated herein may be modified without departing from the scope of the present invention. All such modifications and variations of these assays are encompassed by the present invention.

15 The presence of HEV convalescent and/or acute phase antibodies in a subject's biological fluid (such as whole blood, plasma, serum or saliva) can be detected using a wide range of immunoassay techniques such as those described in US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. This includes both single-site and two-site, or "sandwich", assays of the non-competitive types, as well as in the traditional competitive
20 binding assays. Sandwich assays are particularly useful and include ELISA dip-stick assays and Immunofluorometric assays (IFA). Competitive binding assays include radioimmunoassays (RIA). Western blot assays may also be used. The choice of assay will depend on the sensitivity required, materials available and the biological sample employed.

25

A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in one assay, an unlabelled peptide, polypeptide or protein (hereinafter abbreviated to "polypeptide") capable of preferably binding to acute or convalescent phase antibodies to HEV is immobilised onto a solid
30 substrate and the biological sample to be tested for HEV antibodies brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of a polypeptide-antibody primary complex, an



immunoglobulin specific antibody, labelled with a reporter molecule capable of producing a detectable signal, is then added and incubated, allowing time sufficient for the formation of a secondary complex of polypeptide-antibody-labelled antibody. Any unreacted material is washed away, and the presence of the VPF is determined by observation of a signal produced by the reporter molecule on the second antibody. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparing with a control sample containing known amounts of HEV antibody. Variations of this assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound polypeptide, or a reverse assay in which the labelled antibody and sample to be tested are first combined, incubated and then added simultaneously to the bound polypeptide. These techniques are well known to those skilled in the art, and the possibility of variations will be readily apparent. The antibodies used above may be monoclonal or polyclonal.

In this assay, the HEV derived polypeptide or antigenic parts thereof is either covalently or passively bound to a solid surface. The solid substrate is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microtitre plates, or any other surface suitable for conducting an immunoassay such as a dipstick. The binding processes are well-known in the art and generally consist of cross-linking, covalently binding or physically adsorbing the molecule to the insoluble carrier.

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, produces an analytically identifiable signal which allows the detection of an HEV polypeptide-antibody complex. The most commonly used reporter molecules in this type of assay include enzymes, fluorophores and radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the antimmunoglobulin antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β -galactosidase



and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change.

5 Alternatively, fluorescent compounds, such as fluorescein, anthranide such as europium and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour
10 visually detectable with a light microscope. As in an enzyme immunoassay (EIA), the fluorescent labelled antibody is allowed to bind to the polypeptide in the polypeptide-antibody complex. After washing off the unbound reagent, the remaining complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both
15 very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotopes, chemiluminescent molecules or bioluminescent molecules may also be employed. It will be readily apparent to the skilled technician how to vary the procedure to suit the required purpose.

20 Accordingly, one aspect of the present invention contemplates a method of detecting convalescent and/or acute phase antibodies in HEV in a biological sample, said method comprising the steps of contacting said biological sample with an immobilised polypeptide preferably interactive with acute phase or convalescent phase antibodies to HEV for a time and under conditions sufficient for a polypeptide-antibody complex to
25 form and subjecting said complex to a detecting means. The latter complex may be detected by, for example, the addition of an antimmunoglobulin antibody labelled with a reporter molecule.

 Alternatively, a competitive immunoassay may be used. The most convenient
30 assay of this type is a radioimmunoassay (RIA). In another assay, the AGEN red cell agglutination test may be employed (see Wilson *et al.* 1991).



The invention also extends to use of the peptides and/or polypeptides, or fragments, or derivatives of the present invention in the treatment or prophylaxis of patients.

5 As contemplated herein prophylaxis may be achieved in a patient by immunising a patient with a vaccine containing antigens corresponding to the epitopic regions of the HEV genome and a suitable adjuvant sufficient to elicit an antibody response in a patient. Methods for production of vaccines are well known to those skilled in the art and by way of example a convenient reference is Remington's Pharmaceutical Sciences, 17th ed.,
10 Mack Publishing Co., Easton, Pennsylvania, USA. Preferred HEV antigens include the N-terminal region of ORF2 or the C-terminal region of ORF2.

Due to the nature of the epitopic region(s) of the HEV antigen it should therefore be possible to discriminate between naturally infected individuals and vaccinated
15 individuals.

The present invention encompasses other forms of kits and diagnostic assays including a kit comprising a container adapted to contain a synthetic peptide or polypeptide corresponding to the epitopic region of the HEV genome or its fragments,
20 derivatives, homologues and/or immunological relatives. Preferred antigens are the N-terminal region of ORF2 and the C-terminal region of ORF2 of HEV. The kit may contain a second container adapted to contain or receive a sample to be tested. A third container may be present adapted to contain reagents for detecting HEV-antibody complexes. Alternatively, where the kit is to detect HEV immune complexes, the kit may
25 comprise one or more containers (e.g. wells) adapted to contain a HEV specific antibody (e.g. a monoclonal antibody). Additional containers with the kit may then contain receptacles for receiving fluid samples and a labelled antibody.

In further accordance with the present invention, expression of the cDNA insert
30 encoding the epitopic regions of HEV described herein or fragments thereof, may be achieved in a number of different ways.

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As an example, successful expression of the antigens encoding the epitopic regions of HEV as a fusion protein can be achieved using the pGEX vectors which give expression of glutathione S-transferase fusion proteins, using *E. coli* as the host cells. Expression could also be achieved, by way of example, using pEV vectors or the

5 polyhistidine expression vectors again using *E. coli* as the host cells. Any other suitable fusion protein capable of use in a bioaffinity chromatography may also be employed such as polylysine, FLAG and thioredoxin. Alternatively, the epitopic region of HEV may be expressed as a non-fused polypeptide, by using appropriate vector and host cell combinations. Other vector and host cell combinations which can be used in accordance

10 with the present invention including a number of well described yeast shuttle vectors for use in yeast cells, or eukaryotic vectors useful in continuous cell lines, (eg CHO cells) yeast cells and/or insect cells or transgenic animals.

The present invention is applicable to any strain of HEV which has a genomic

15 organisation comprising open reading frames equivalent to ORF2 and ORF3 as defined by Tam *et al* (1991). Of the strains isolated to date, any strain of HEV is useful in the practice of the present invention.

The present invention will now be further described with reference to the

20 following non-limiting Figures and Examples.

In the Figures:

Figure 1 shows the nucleotide and deduced amino acid sequence of the 76kDa

25 protein encoded by Open Reading Frame 2, of the strain of hepatitis E virus isolated from an epidemic in Xinjiang autonomous region of China.

Figure 2 shows the nucleotide deduced amino acid sequences of the 14kDa protein encoded by Open Reading Frame 3 of the HEV genome, of the strain of hepatitis E virus

30 isolated from an epidemic in Xinjiang autonomous region of China.

Figure 3 shows a Western blot of sera taken at differing time points from an

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infection of a rhesus monkey (*Macaca mulatta*) with the HEV virus using the 56kDa and/or 39kDa fusions proteins or GST as antigen.

Figure 4 shows a comparison of the results obtained using the Western blot of the present invention to screen sera samples from an Australian Aboriginal population versus results obtained with a commercially available EIA for HEV (Genelabs Diagnostics, Singapore).

Figure 5 shows the cloning strategy for the recombinant HEV-GST fusion proteins herein described. The cDNA fragments of 1658, 830 and 380 bp were generated after reverse transcription and PCR of HEV RNA extracted from the bile of an experimentally infected monkey. All DNA fragments were purified by agarose gel electrophoresis and Prep-A-Gene at each step.

Figure 6 shows the cloning strategies for (A): HEV cDNA clones; (B): recombinant HEV-GST fusion proteins ORF3, ORF2.1 and ORF2.2; and (C): the recombinant HEV-GST fusion proteins ORF2, ORF2.3 and ORF2.4. The recombinant HEV-GST fusion proteins herein described. The cDNA fragments of 1658, 830 and 380 bp were generated after reverse transcription and PCR of HEV RNA extracted from the bile of an experimentally infected monkey, while other PCR products were generated from the clones pGEX1-AC3 and pGEX1-AC2.2. All DNA fragments were purified by agarose gel electrophoresis and Prep-A-Gene at each step.

Figure 7 shows a Western blot of human acute hepatitis sera against recombinant GST-ORF2.1 and GST-ORF3 proteins. Approximately equal masses of each recombinant protein and unfused GST were mixed and electrophoresed on a 10% w/v acrylamide gel, proteins transferred to nitrocellulose, and the membrane blocked with casein. The membrane was then incubated with a 1:500 dilution of patient sera or 1:10,000 dilution of HRPO-streptavidin (marker lane), and immune complexes detected using HRPO-conjugated anti-human IgG and enhanced chemiluminescence. Lanes: MW, biotinylated molecular weight markers (sizes indicated at left); 1-10, acute hepatitis A; 11-18, acute hepatitis B; 19-23, acute hepatitis E. The positions of reactive degradation products of



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GST-ORF2.1 and GST-ORF3 (closed and open circles, respectively), GST and each fusion protein are indicated at right.

Figure 8 shows a Western blot of GST-ORF2.1 and GST-ORF3 with sera from three rhesus macaques experimentally infected with HEV and subsequently rechallenged with aliquots of the original inoculum. (A), (B), (C) monkeys M31, M32 and M33, respectively. The days of rechallenge are indicated above each figure, and the days of serum collection below each figure. Lane MW, biotinylated molecular weight markers (sizes indicated at left). Note that reactivity to GST-ORF2.1 remains at high levels and is boosted following rechallenge, whereas reactivity to GST-ORF3 declines with time.

Figure 9 is a Western blot of GST-ORF2.1 and GST-ORF2.2 with sera from infected macaques. GST and fusion proteins GST-ORF2.1 and GST-ORF2.2 were mixed in equal proportions, electrophoresed and transferred essentially as in Figure 3. Serum samples are from the animals shown in Figure 8. (A), (B), (C) monkeys M31, M32 and M33, respectively. Lane MW, biotinylated molecular weight markers (sizes indicated at left) Note that reactivity to GST-ORF2.1 is weaker than that to GST-ORF2.2 during the acute phase of infection, but is boosted after rechallenge and subsequently remains at higher levels than that to GST-ORF2.2.

20

Figure 10 is a Western blot of (a) GST-ORF2, GST-ORF2.1, (b) GST-ORF 2.3 and GST-ORF2.4, with sera from infected macaques. GST and fusion proteins were mixed in equal proportions, electrophoresed and transferred essentially as in Figure 3. Serum samples are from the animals shown in Figure 8: (A), (B), (C), monkeys M31, M32 and M33. Lane MW, biotinylated molecular weight markers. Note that the reactivity of GST-ORF2 appears to lie in the N-terminal sequences overlapping with GST-ORF2.3 and GST-ORF2.4: breakdown products of GST-ORF2 as well as the shorter GST-ORF2.3 and GST-ORF2.4 fusion proteins are strongly reactive with acute phase sera.

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EXAMPLE 1

HEPATITIS E VIRUS AND ANTIGENS THEREFROM

The strain of HEV used in these studies was isolated from infected individuals
5 from the Xinjiang autonomous region of China during an epidemic between September
1986 and April 1988.

Aye TT *et al* (1992) reported that an HEV strain isolated from infected individuals
from the Xinjiang autonomous region of China had 93.9% nucleotide sequence homology
10 with the Burmese strain (Tam *et al.*, 1989).

Virus was cultured by infecting rhesus monkeys with fecal samples from infected
individuals, using known methods (Cao, X-Y *et al*, 1991). The monkeys were acutely
infected, with the HEV being excreted into the bile and faeces of the monkey.

15

The viral antigens were isolated as follows using reverse transcriptase PCR.:

- (a) Extraction of RNA from bile. Bile specimens were collected directly from the
gall bladder of an infected rhesus monkey at day 7-14 after inoculation. An
20 aliquot of 10 μ l of bile was mixed with 25 μ l nuclease free water and 465 μ l GIT
(4M guanidine thiocyanate, 25mM sodium citrate, 0.5% v/v sarkosyl, 0.1M 2-
mercaptoethanol). An aliquot of 50 μ l of 3M sodium acetate (pH5.2) was added
to the mixture above followed by extraction with 450 μ l phenol/chloroform (5:1).
After separation by centrifugation, the upper phase was transferred to a fresh tube
25 containing 2 μ l of 10 mg/ml tRNA. The RNA was precipitated in 750 μ l of ice-
cold isopropanol at -20°C for a minimum of 2 hours, then pelleted at 13,000 rpm
for 15 minutes, washed with 80% v/v ethanol and repelleted as above. The dried
pellet was resuspended in 10 μ l of nuclease free water and incubated at 65°C for
10 minutes, then stored at 4°C ready for RT-PCR. A similar method may be used
30 on faeces.

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- (b) **Primers.** Two pairs of primers were designed to isolate the whole fragment of ORF3 and one third of the 3' end of ORF2 (ORF2.1), respectively. These primers were modified to contain *Eco*R1 restriction sites to facilitate cloning. The nucleotide sequences of the primers, with the HEV-specific sequences underlined, are:

For ORF3: 5'-GTGAATTCCATGAATAACATGTCTTTTGC-3'(forward)
(SEQ ID NO: 5)

10 5'-GTGAATTCTTAGCGGCGCGGCCCCAGCT-3'(reverse)
(SEQ ID NO: 6)

For ORF2: 5'-GTGAATTCCAGCTGTTCTACTCCCGTCC-3'(forward)
(SEQ ID NO: 7)

15 5'-GTGAATTCGCGCACTTCGTTTATTTGATGTT-3'(reverse)
(SEQ ID NO: 8)

A third primer pair was designed to amplify approximately two thirds of ORF2, terminating 27 nucleotides from the carboxy terminus (ORF2.0) This PCR product did not contain modified restriction enzyme sites, and instead was cloned using the 3' A overhang.

ORF2.0: 5'-TCGTAGACCTACCACAGCTG-3' (forward)
25 (SEQ ID NO: 9)

5'-TCTTAAGGCGCTGAAGCTCA-3' (reverse)
(SEQ ID NO: 10)

30 These primer sets were designed on the basis of published sequences for HEV.

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To extend the collection of cDNA clones (Figure 6C), the first primer pair was designed to amplify the amino-terminal 330bp of ORF2 (ORF2.3). These primers were modified to contain BamH or EcoR 1 restriction sites to facilitate cloning. The nucleotide sequences of primers, with the HEV-specific sequences underlined, are:

5 P4740: 5'-GTGGATCCATGCGCCCTCGGCCTATTTTGT-3' (forward)

(SEQ ID NO: 11)

P3566: 5'-GTGAATTCTTAGCGGCGCGGCCCCAGCT-3' (reverse)

(SEQ ID NO: 12)

The second primer pair was designed to amplify 440bp fragment of ORF2
10 which overlaps AC2.3.

P2: 5'-TCGTAGACCTACCACAGCTG-3' (forward)

(SEQ ID NO: 13)

P5019: 5'-CACAAGCTCAGAGGCTATGCCG-3' (reverse)

(SEQ ID NO: 14)

15

(c) RT-PCR. Prior to PCR amplification, the RNA was reverse transcribed into cDNA. To each RNA sample, 10µl of RT Master Mixture (4µl 25mM MgCl₂, 2.0µl 10XRT buffer, 2.0µl 10mM dNTP mixture, 1.0µl 200ng/µl reverse primer, 0.5µl rRNasin, 0.5 µl AWV RT) was added. After a 60 minute
20 incubation at 42°C, the reaction was boiled for 5 minutes and quenched in chip ice for 5 minutes.

For the PCR reaction, 30µl of PCR Master Mixture (19.1µl nuclease free water, 3.6µl 25mM MgCl₂ 3.0µl 10XTaq Buffer (500 mM KCl, 100mM Tris pH 9.0, 1% v/v Triton X-100), 3.0 µl 2mM dNTP, 1.0µl forward primer, 0.3µl
25 AmpliTaq DNA polymerase (Promega) was mixed with the cDNA reaction and overlayed with 50 µl mineral oil. The amplifications were carried out in a thermocycler (DNA Thermal Cycler 480, PERKIN ELMER CETUS) with the following cycling program: 94°C denaturaton for 1 minute, 55°C annealing
30 from 1 minute and 72°C extension from 2 minutes. A total of 40 cycles were processed followed by a final extension at 72°C for 7 minutes.

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EXAMPLE 2

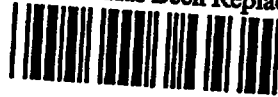
CONSTRUCTION OF EXPRESSION VECTORS

The plasmids and cloning strategy used in this study are shown in Figures 5 and 6. PCR products were separated on a 1.0% v/v agarose gel. Fragments of about 380 bp (ORF3), 830 bp (ORF2.1) and 1658 bp (ORF2.0) were recovered by cutting the bands out of the gel. The DNA was purified using the Pre-A-Gene kit (Bio-Rad, USA). Fragments ORF3 and ORF2.0 were digested with *Eco*R1 and the restriction fragments (370bp or 820 bp) were separated on a gel and purified as above.

Approximately 6ng of ORF3 or 16 ng of the ORF 2.1 DNA were ligated with 10 ng of pGEX1 vector *Eco*R1 cut, dephosphorylated, (Pharmacia Biotech, USA) to give the plasmids pGEX1-AC3 and pGEX1-AC2.1, respectively, transformed into *E. coli* strain NM522 (Pharmacia Biotech, USA) using the Gene Pulser (Bio-Rad, USA), and plated on LB-ampicillin. Plasmids were prepared from transformants by minipreparation and following *Eco*R1 digestion were examined for inserts of the expected sizes. Fragment ORF2.0 (20 ng) was ligated with 50 ng of dephosphorylated vector pCR (Invitrogen, San Diego, Ca) utilizing the T overhangs on plasmid and A overhangs on PCR product to give the plasmid pCR-AC2.0. After transformation and selection of recombinants, purified plasmids were subjected to partial *Eco*R1 digestion and the resulting fragment of 1670 bp purified and ligated into pGEX1 as above to give plasmid pGEX1-AC2.0. This plasmid and pGEX1-AC2.1 were digested to completion with *Kpn*I and *Bam*H1. The restriction fragment of 1390 bp from pGEX1-AC2.0 was then ligated with the fragment from pGEX1-AC2.0 containing the pGEX 1 sequence together with the ORF2 sequences downstream of the *Kpn*I site, to generate plasmid pGEX1-AC2.2.

The plasmids and cloning strategy used in this study are shown in Figures 5 and 6. Three clones, pGEX1-AC3, pGEX1-AC2.1 and pGEX1-AC2.2, were constructed in this laboratory earlier. DNA from plasmid pGEX1-AC3 and pGEX1-AC2.2 were extracted by minipreparation and used as templates for PCR to amplify two overlapped fragments. A PCR product from pGEX1-AC3 (ORF2.3), which contains 330bp nucleotides of 5' end of ORF2, was digested with *Bam*H1 and *Eco*R1

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and ligated with pGEX1, to generate plasmid pGEX1-AC2.3. Another PCR product from pGEX1-AC2.2 was digested with Pvu 2 and EcoR1(360bp) and ligated with the restriction fragment of 312bp from ORF2.3 after BamH1 and Pvu2 digestion, as well as pGEX1. The resulting plasmid(pGEX1-AC2.4) contains first 672bp nucleotides of ORF2. pGEX1-AC2.4 was digested with EcoR1 then ligated with the EcoR1 fragment of 1328bp from pGEX2.2 to give plasmid pGEX-AC2, which contains full length of ORF2.

Selected colonies from each transformant were then induced with isopropylthio- β -D-galactoside (IPTG) and examined for expression of fusion proteins of the expected sizes.

EXAMPLE 3

PRODUCTION OF GST FUSION PROTEINS

15

The transformants of pGEX1-AC3, pGEX1-AC2.1 and -2.2, and pGEX1 (as a control) were grown for 5 h in L-Broth medium (LB) containing 50 μ g/ml of ampicillin. An aliquot of 0.5 μ l of the overnight culture was inoculated into 200 ml of LB plus ampicillin and incubated for 6 hr. IPTG (200 μ l of 100 mM stock) was added and the culture was grown overnight. Cells were pelleted at 4000 rpm for 15 min in a bench centrifuge. The pellet was washed in 10mM Tris (pH 7.5) and spun as before. For each gram of packed cells, 3 μ l of lysis buffer (50 mM Tris pH 8.0, 1 mM EDTA, 100 mM NaCl), 4 μ l of 100 mM phenylmethylsulfonylfluoride (PMSF) and 80 μ l of 10 mg/ml lysozyme were added. The suspension was incubated at room temperature for 30 min then 37° for an additional 30 min. The solution was sonicated for 3 min. and centrifuged at 4000 rpm for 15 min. The pellet (containing most of the fusion proteins) was washed with ice-cold 1M NaCl, 10 mM Tris pH 7.5. The protein pellet was resuspended in 3 ml of 10mM Tris (pH 7.5), and stored in aliquots at -20°.

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EXAMPLE 4**SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS**

Each of the fusion proteins (GST-ORF3, GST-ORF2.1, GST-ORF2.2, GST-ORF2.3, GST-ORF2.4) was thoroughly resuspended in 50 mM Tris (pH 6.8), 2% v/v SDS, 5% v/v 2-mercaptoethanol, 8% v/v glycerol, 0.01% v/v bromophenol blue. In each experiment, unfused GST (prepared as above) was mixed with the fusion proteins before electrophoresis. The protein mixture was heated at 100° for 5 min and loaded on SDS-PAGE gels in a mini-gel apparatus (Bio-Rad, USA) with a separating gel of 10% w/v acrylamide, 0.267% w/v bis-acrylamide, using the discontinuous buffer system of Laemmli (2% w/v SDS, 5% v/v 2-mercaptoethanol, 8% v/v glycerol, 50 mM Tris[pH 6.8], 0.01% w/v bromophenol blue). The gels were electrophoresed at 75V for 20 min then 150V until the dye reached the bottom of the gel. The proteins were transferred to nitrocellulose membranes (Hybond-C extra, Amersham) on a Semi-Dry Transfer Cell (Bio-Rad, USA) in transfer buffer (0.15M glycine, 0.025M Tris [pH 8.5], 20% v/v methanol). Biotinylated marker proteins (Bio-Rad) were run in reference lanes.

EXAMPLE 5**WESTERN BLOT**

The nitrocellulose membranes were blocked with 0.1M Tris (pH7.5), 0.1M NaCl, 0.3% v/v tween-20 (TBST) containing 3% w/v casein and 0.01% w/v sodium azide at room temperature for 1 hr. Test sera were diluted 1:500 in TBST plus 1% w/v casein and preabsorbed with 1% w/v GST lysate suspension at 37° for 1.5 hr. The diluted sera were incubated at 35° with agitation for 1 hr with the membrane, using the Mini-Proean Multi Screen apparatus (Bio-Rad, USA). The membranes were washed three times for 5 min in TBST, incubated with horseradish peroxidase (HRPO)-conjugated rabbit anti-human IgG (diluted 1:5000; DAKO Co. Denmark) at 35° for 1 hr, then washed as before. Biotinylated markers were detected using HRPO-conjugated streptavidin (diluted 1:10,000; Amersham). Enzyme complexes were detected by enhanced chemiluminescence (ECL; Amersham). The membranes were

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soaked in ECL reagents for 1 min, then placed between acetate sheets and exposed to Hyperfilm ECL (Amersham, USA) for 30 sec to 2 min at room temperature.

EXAMPLE 6

5 TEST SERA FOR WESTERN BLOT

Human acute hepatitis sera

Sera from other viral hepatitis cases in Australia were used as negative controls: 10 acute hepatitis A (positive for anti-HAV IgM, Abbot Diagnostics), 18
 10 acute hepatitis B (positive for anti-HBc IgM, Abbot Diagnostics) and 58 hepatitis C (positive in second generation HCV EIA, Abbot Diagnostics). Sixty-six sera were collected from acute phase patients in Xinjiang, China during the Hepatitis E epidemic of 1988.

15

Serial sera from infected monkeys

Serum specimens were collected approximately twice weekly from 7 rhesus monkeys (M27, M31, M32, M33, M34, M35, M36) experimentally infected with HEV. Samples were stored at -20°.

20

EXAMPLE 7

EXPRESSION OF HEV-GST FUSION PROTEINS

Clones of putative HEV structural proteins were constructed in the highly
 25 efficient pGEX expression system, giving rise to proteins fused with GST (normal molecular mass of 26 kDa). The clones studied represent the complete sequence of ORF3 (GST-ORF3), and partially overlapping sequences within ORF2 (GST-ORF2.1, GST-ORF2.2, GST-ORF2.3, GEST-ORF2.4) as shown in Figures 5 and 6. After induction with IPTG, bacterial lysates were found to contain unique proteins of about
 30 37, 39.5, 49, 56, 86 and 97 kDa, consistent with the predicted molecular masses of these translated sequences. It should be noted that pGEX1-AC2, pGEX1-AC2.2 and pGEX1-AC2.1 encode proteins having common C-termini (representing the extreme

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C-terminus of ORF2). pGEX1-AC2.1 encodes 273 amino acids, that is the most C-terminal one-third of ORF2, while pGEX1-AC2.2 has a N-terminal extension of 294 amino acids, and pGEX1-AC2 has a further extension of 93 amino acids and therefore represents 660 amino acids or the full-length ORF2. It should be further noted that

5 pGEX1-AC2, pGEX1-AC2.3 and pGEX1-AC2.4 encode proteins having common N-termini (representing the extreme N-terminus of ORF2). pGEX1-AC2.3 encodes 1509 amino acids, while pGEX1-AC2.4 has a C-terminal extension of 110 amino acids and pGEX1-AC2 has a further extension of 400 amino acids or the full-length ORF2, as above.

10

EXAMPLE 8**DETECTION OF ANTI-HEV BY WESTERN BLOT**

To select conditions for a sensitive diagnostic assay and to examine the dynamics of antibody responses to each fusion protein, western blots were conducted

15 to detect anti-HEV IgG in sera from acute-phase hepatitis patients and from experimentally infected monkeys.

Detection of anti-HEV in human acute hepatitis sera

Sera from well-documented cases of acute hepatitis A, B, C and E were

20 assayed by western blot (Figure 6, Table 1). The results show that none of 10 Hepatitis A and 18 Hepatitis B sera contained IgG against GST-ORF3 or GST-ORF2.1, but 3 of 58 hepatitis C were weakly reactive to GST-ORF2.1 and one of these was also reactive to GST-ORF3. Sixty-one of 66 (92%) acute HEV sera from Xinjiang contained anti-HEV IgG that bound specifically to the GST-ORF2.1 band but

25 not to the negative control (GST). Of these, 55 were also positive for IgG to GST-ORF3, and one serum was reactive with GST-ORF3 but not with GST-ORF2.1. Nine of nineteen sera collected between 3 and 9 months after suspected HEV were reactive against GST-ORF2.1, but only 3 of these were active with GST-ORF3. When tested using a commercial EIA based on ORF3 of the Burmese and Mexican strains, only

30 one of the convalescent sera was reactive and this serum was reactive against both GST-ORF2.1 and GST-ORF3.



Both fusion proteins were, therefore, immunoreactive by Western blot and resulted in a high detection rate of anti-HEV IgG in epidemic cases of HEV in China, with a high level of specificity indicated by the low reactive rate in other forms of viral hepatitis. Some HEV samples strongly positive for antibody to GST-ORF2.1 or 3 were also reactive with degradation products of the respective fusion proteins (indicated with closed and open circles respectively in Figure 6 and subsequent figures), but no sera showed reactivity with the control GST protein band.

Detection of anti-HEV in serial sera from rhesus monkeys

Rhesus monkeys were inoculated intravenously with a Chinese strain of HEV, and displayed an increase in ALT at 7-26 days indicating acute hepatitis. Serial blood samples were tested for anti-HEV IgG by Western blot, and the results for one monkey (M27) are shown in Figure 7. In this monkey, antibodies to both GST-ORF2.1 and GST-ORF3 were detected at day 16, with anti-GST-ORF2.1 continuing to increase throughout the study period whereas anti-GST-ORF3 reached a peak at 71 days and then declined. Degradation products of both fusion proteins were sometimes detected in Western blots, as indicated with open circles (GST-ORF3) or closed circles (GST-ORF2.1) in Figure 7 and subsequent figures.

All 7 monkeys seroconverted since antibody to GST-ORF2.1 was detected at day 14-21, immediately after ALT elevation. Antibody to GST-ORF3 was first detected at the same time or 7 days after first detection of GST-ORF2.1 in four monkeys, but was weakly detectable in only one serum sample from each of 2 monkeys and in no samples from the remaining monkey. Antibody to GST-ORF2.1, therefore, appears to be a more consistent marker of infection in macaques, as was also observed with samples from man (Table 1).

Two monkeys were rechallenged at days 53, 107 and 316, and a third monkey at days 107 and 316 after initial infection. All monkeys have shown biochemical evidence of hepatitis after primary inoculation and less severe hepatitis after the first rechallenge. Only one (M32) of two monkeys appeared to be infected after the second rechallenge, suggesting the acquisition of partial protective immunity after each

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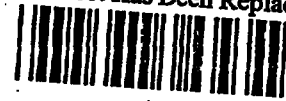
infection. Coincident with this partial immunity, we detected antibody to GST-ORF2.1 in each monkey (Figure 8), remaining at high levels throughout the study and sometimes being boosted after rechallenge. The boosted reactivity may result from limited viral replication or at least partly from viral antigen present in the inocula. It should be noted, however, that in both M31 and M32 no further increase in reactivity was detected following a third rechallenge, which suggests that the increased reactivity observed previously was due to virus replication.

Antibody to GST-ORF3 was detected at high levels in M31 and M32 between days 17 and approximately 60, but waned thereafter. Interestingly, antibody to GST-ORF3 was boosted after the second rechallenge in monkey 32, and in monkey 33 the only sample positive for this antibody was after rechallenge. The reactivity with GST-ORF3 closely reflects that observed using a commercial HEV EIA based on ORF3 synthetic proteins, in which the day 151 sample was also the only reactive sample from M33.

Comparison of GST-ORF2.1 and 2.2

The fusion protein GST-ORF2.1 contains only the carboxy-terminal one-third of the coding sequences from HEV ORF2. In order to examine whether additional sequences from ORF2 would show greater reactivity, sera from monkeys M31, M32 and M33 were reacted with GST-ORF2.1 and GST-ORF2.2, having an N-terminal extension of 294 amino acids. In the acute phase of infection, the extended protein was indeed more strongly reactive, although no samples were positive only for this protein. However, interestingly, the peak reactivity of the extended protein was in fact much lower than that of GST-ORF2.1 in each of the animals. In addition, the antibody was found to decline with time and was only weakly boosted after each rechallenge.

These two proteins were also used to screen some of the sera from patients with acute hepatitis A, B or E as described above, with no difference observed in the proportion of reactive sera, although in most cases the signal for GST-ORF2.2 was stronger than that for GST-ORF2.1, as also seen during the acute phase in monkeys



(Fig. 9).

GST fusion proteins GST-ORF2.1, GST-ORF2.2 and GST-ORF2 are partially overlapping with common carboxy termini (Figure 5, Figure 6). As shown in Figures 3 and 7-10, the shortest of these, GST-ORF2.1, is the most reactive with convalescent hepatitis E sera and therefore is the best protein for expression of the epitopes which are involved in protection from reinfection.

GST fusion proteins GST-ORF2, GST-ORF2.3 and GST-ORF2.4 are partially overlapping with common amino term (Figure 5). As shown in Figure 10, the reactivity in sera from experimentally infected macaques against the full-length GST-ORF2 closely approximates that seen with the shorter GST-ORF2.3 and GST-ORF2.4 proteins: that is, they are most reactive with acute phase sera and poorly reactive with convalescent phase sera. The reactivity of full-length GST-ORF2 therefore lies within the region b5147 to 5477 in ORF2 according to Tam, and no reactivity is seen against the sequences b6326 to 7145 which overlap with GST-ORF2.1. The sequences b5147 to 5477 as in GST-ORF2.3 or GST-ORF2.4 are therefore suitable for the detection of acute phase antibodies to HEV, and on the basis of the greater reactivity in macaque M33 compared to that against GST-ORF3, we claim that these proteins will enhance the detection of acute hepatitis E virus infection.

Taken together with the lower reactivity of GST-ORF2.2 and GST-ORF2 compared with GST-ORF2.1 (Figure 9, Figure 10), this again suggests that the inclusion of amino terminal sequences of ORF2 inhibits the reactivity of carboxy terminal sequences which may be included in the same protein. It would be expected that they might also reduce the immunogenicity of similar proteins, and we therefore claim that proteins containing sequens between b5147 and 6325 are suitable for diagnostic reagents but not for vaccines unless they can be demonsrated not to interfere with the reactivity of the sequences 6326 to 7145.

It is further claimed that antibodies to GST-ORF3 and/or GST-ORF2.3 and/or GST-ORF2.4 could be used to detect clinical or subclinical infection with HEV in



vaccine recipients who have antibodies to GST-ORF2.1 as a result of active or passive immunisation.

EXAMPLE 9

5

Recent infection can be diagnosed on the basis of antibody to ORF3 and/or increasing titre of antibody to C-terminus ORF2 and/or antibody to the N-terminal part of ORF2, while past infection of HEV can be diagnosed by the presence of antibody to ORF2 with or without antibody to ORF3. Antibody to ORF3 is expected to be present
10 only in a proportion of individuals infected with the virus, and not in individuals who are exposed to viral antigen in the form of an inactivated or live, attenuated vaccine. This is also likely to be true for antibodies to ORF1.

EXAMPLE 10

15

DEVELOPMENT OF VACCINES AGAINST HEV

Conventional approaches to recombinant vaccines generally rely on using either synthetic peptides which are small enough to be chemically synthesised, or recombinant or fusion proteins representing as large a portion of the antigen of interest as possible.
20 Consequently, most work on HEV has previously focussed on full-length open reading frame (ORF) 2. However, a key feature of the present invention is the disparity between the length of recombinant HEV proteins and their reactivity. Recombinant protein, GST-ORF2.1, representing only the C-terminal 1/3rd of ORF2, is in fact far more reactive with convalescent sera than is a full length GST-ORF2 protein. Furthermore, the reactivity of
25 the full-length protein is most likely attributable to the N-terminal sequences, as its reactivity with convalescent sera almost exactly mirrors that of such N-terminal proteins (Figure 10). Full-length ORF2, therefore, does not efficiently present the epitopes found in GST-ORF2.1, which are likely to be important for protective humoral immunity.

30

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The

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invention also includes all of the steps, features, compositions and compounds referred to our indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Table 1. Detection of anti-HEV IgG by Western Blotting

	Infection	Number	Number positive (%)		Total (%) ^a
			GST-ORF2.1	GST-ORF3	
5	Hepatitis A	10	0	0	0
	Hepatitis B	18	0	0	0
	Hepatitis C	58	3 (5)	1 (2)	3 (5)
	Hepatitis E (acute) ^b	66	61 (92)	56 (85)	62 (94)
10	Hepatitis E (convalescent) ^c	19	9 (47)	3 (16)	9 (47)

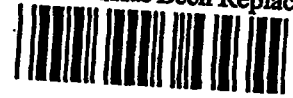
^a Positive for GST-ORF2.1 and/or GST-ORF3.

^b Less than three months after onset of disease

15 ^c Three to nine months after onset of disease



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BIBLIOGRAPHY

ANDIAPARIDZE, A.G., BALAYAN, M.S. SAVINOV, A.P., BRAGINEKY, D.M., POLESCHIJK, V.F. and ZAMYATINA, N.A. (1986). Fecal-Oral transmitted non-A, non-B hepatitis induced in monkeys. *Vopr. Virusol.* 1, 73-80.

ARANKALLE, V.A., TICEHURST, L., SREENIVASAN, M.A. *et al.* (1988). Aetiological association of a virus-like particle with enterically transmitted non-A, non-B hepatitis. *Lancet* 1, 550-554.

AYE, T.T. *et al* (1992). Sequence comparison of the capsid region of hepatitis E viruses isolated from Myanmar and China *Microbial-Immunol* 36(6) 615-21.

BALAYAN, M.S., ANDJAPARIDZE, A.G., SAVINBKAYA, S.S., *et al.* (1983). Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology* 20, 23-31.

BRADLEY, D.W. (1990a). Hepatitis non-A, non-B viruses become identified as hepatitis C and E viruses. *Prog. Med. Virol.* 37, 101-135.

BRADLEY, D.W. (1990b). Enterically transmitted non-A, non-B hepatitis. *Br Med. Bull.* 46, 442-461.

BRADLEY, D.W., and MAYNARD, I.E. (1986). Etiology and natural history of post-transfusion and enterically-transmitted non-A, non-B hepatitis, *Sem. Liver Dis.* 6, 56-66.

BRADLEY, D.W., KRAWCZYNSKI, K., COOK, E.H., Jr, *et al.* (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. USA* 84, 6277-6281.

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BRADLEY, D.W., KRAWCZYNSKI, K., COOK, E.H., Jr, *et al.* (1988a). Enterically transmitted non-A, non-B hepatitis: Etiology of disease and laboratory studies in non human primates in "Viral Hepatitis and Live Disease" (A. J. Zuckerman, Ed.). pp 138-147. A.R. Liss, New York.

CAO, X, MA, X, LIU Y, *et al.* Epidemiological and etiological studies on enterically transmitted non-A, non-B hepatitis in the south part of Xinjiang, China. *Chinese Journal of Experimental and Clinical Virology*, 1989, 3(2): 1-9.

CHOO, G.L., KUO, G., WEINER, A.J., OVERBY, L.R., BRADLEY, D.W., and HOUGHTON, M., (1989). Isolation of cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 244, 359-362.

FEINSTONE, S.M., KAPIKIAN, A.Z., PURCELL, R.H., ALTER, H.J., and HOLLAND, P.V. (1975). Transfusion associated hepatitis not due to viral hepatitis type A or B. *N. Engl. J. Med.* 292, 767-770.

KHURROO, M.S. (1990). Study of an epidemic of non-A, non-B hepatitis : Possibility of another human hepatitis virus distent from post-transfusion non-A, non-B type. *Am. J. Med.* 68, 818-824.

KRAWCZYNSKI, K., and BRADLEY, G.W., (1989). Enterically transmitted non-A, non-B hepatitis: Identification of cirus associated antigen in experimentally infected cynomolgus macaques. *J. Infect, Dis.* 159, 1042-1049.

KUBO, Y., TAKEUCHI, K., BOONMAR, S., *et al*, (1989). A cDNA fragment of hepatitis C virus isolated from an implicated donor of post-transfusion non-A, non-B hepatitis in Japan. *Nucleic Acids Res.* 17,10,367-10,372.

KUO, G., CHOO, G.L., ALTER, H.I., *et al*, (1988). An essay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 244, 362-364.



MAENO, M., KAMINAKA, K., SUGIMOTO, H., *et al*, (1990). A cDNA clone closely associated with non-A, non-B hepatitis. *Nucleic Acids Res.* 18, 2685-2689.

MANIATIS *et al* (1982). *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, USA

PRINCE, A.M., BROTMAN, B., and GRADY, G.F., (1974). Long-incubation post-transfusion hepatitis without serological evidence of exposure to hepatitis B virus. *Lancet* 2, 241-246.

PURCELL, R.H., and TICEHURST, J.R., (1988). Enterically transmitted non-A, non-B hepatitis; epidemiology and clinical characteristics. in "Viral Hepatitis and Liver Disease" (A.J. Zuckerman, Ed). pp 131-137, A.R. Liss, New York.

REYES., G.R., and BAROUDY., G.M., (1991). Molecular biology of non-A, non-B hepatitis agents; The hepatitis C and hepatitis E viruses in "Advances in Virus Research" (K. Maramorosch, F.A. Murphy, and A.J. Shatkin, Eds.). Vol 40, pp 55-101. Academic Press, Orlando.

REYES, G.R., PURDY, M.A., KIM, F.P., *et al*. (1990), Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. *Science* 247, 1335-1339.

TABOR, E. (1985). The three viruses of non-A, non-B hepatitis. *Lancet* 1, 743-745.

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, 120-131.

WILSON, K.M., GERAMETTEO, M., RYLATT, D.B., BUNDESEN, P.D., MCPHEE, D.A., KEMP, B.E., (1991) Rapid whole blood assay for HIV-1 seropositivity using an Fab-peptide conjugate, *J. Immunol. Methods* 138, 111-119.

This Sheet Has Been Replaced



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WONG, D.C., PURCELL, R.H., SREENIVABAN, M.A., PRABAO, S.R., and PAVRI, K.M. (1980). Epidemic and endemic hepatitis in India: evidence for a non-A, non-B hepatitis virus aetiology, *Lancet* 2, 876-879.



SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION:
- (i) APPLICANT (Other than U.S.): THE MACFARLANE BURNET CENTRE FOR MEDICAL RESEARCH LIMITED
- (U.S. only): ANDERSON, D.A.
TORRESI, J.
10 LOCARNINI, S.A.
LI, FAN
HUI, ZHUANG
- (ii) TITLE OF INVENTION: IMMUNOREACTIVITY ANTIGENS OF HEPATITIS E VIRUS
- 15 (iii) NUMBER OF SEQUENCES: 14
- (iv) CORRESPONDENCE ADDRESS:
- 20 (A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
25 (F) ZIP: 3000
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
30 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: PCT International
(B) FILING DATE: 23-SEP-1994
35 (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: PM1423
(B) FILING DATE: 24-SEP-1993
40 (A) APPLICATION NUMBER: PM2964
(B) FILING DATE: 15-DEC-1993
- (viii) ATTORNEY/AGENT INFORMATION:
- 45 (A) NAME: HUGHES DR, E JOHN L
(C) REFERENCE/DOCKET NUMBER: EJH/EK
- (ix) TELECOMMUNICATION INFORMATION:
- 50 (A) TELEPHONE: +61 3 254 2777
(B) TELEFAX: +61 3 254 2770



(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1984 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..1983

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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 Leu Pro Ala Pro Pro Pro Gly Gln Pro Ser Gly Arg Arg Arg Gly Arg
 20 25 30

CGC AGC GGC GGT TCC GGC GGT GGT TTC TGG GGT GAC CGG GTT GAT TCT 144
 Arg Ser Gly Gly Ser Gly Gly Gly Phe Trp Gly Asp Arg Val Asp Ser
 35 40 45

CAG CCC TTC GCA ATC CCC TAT ATT CAT CCA ACC AAC CCC TTC GCC CCC 192
 Gln Pro Phe Ala Ile Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Pro
 50 55 60

GAT GTC ACC GCT GCG GCC GGG GCT GGA CCT CGT GTT CGC CAA CCC GCC 240
 Asp Val Thr Ala Ala Ala Gly Ala Gly Pro Arg Val Arg Gln Pro Ala
 65 70 75 80

CGA CCA CTC GGC TCC GCT TGG CGT GAC CAG GCC CAG CGC CCC GCC GTT 288
 Arg Pro Leu Gly Ser Ala Trp Arg Asp Gln Ala Gln Arg Pro Ala Val
 85 90 95

GCC TCA CGT CGT AGA CCT ACC ACA GCT GGG GCC GCG CCG CTA ACC GCG 336
 Ala Ser Arg Arg Arg Pro Thr Thr Ala Gly Ala Ala Pro Leu Thr Ala
 100 105 110

GTC GCT CCG GCC CAT GAC ACC CCG CCA GTG CCT GAT GTT GAC TCC CGC 384
 Val Ala Pro Ala His Asp Thr Pro Pro Val Pro Asp Val Asp Ser Arg
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GGC GCC ATC CTG CGC CGG CAG TAT AAC CTA TCA ACA TCT CCC CTT ACT 432
 Gly Ala Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr
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TCT TCC GTG GCC ACC GGT ACA AAC TTG GTT CTA TAC GCC GCT CCT CTT 480
 Ser Ser Val Ala Thr Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu
 145 150 155 160

AGC CCA CTT CTA CCC CTC CAG GAC GGC ACC AAT ACT CAT ATA ATG GCC 528
 Ser Pro Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala
 165 170 175

ACA GAA GCT TCT AAT TAT GCC CAG TAC CCG GTT GCT CGT GCC ACA ATT 576
 Thr Glu Ala Ser Asn Tyr Ala Gln Tyr Arg Val Ala Arg Ala Thr Ile
 180 185 190

CGC TAC CGC CCG CTG GTC CCC AAC GCT GTT GGT GGC TAC GCC ATC TCC 624
 Arg Tyr Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser
 195 200 205

ATC TCG TTC TGG CCA CAG ACC ACC ACC ACC CCG ACG TCC GTT GAC ATG 672
 Ile Ser Phe Trp Pro Gln Thr Thr Thr Pro Thr Ser Val Asp Met
 210 215 220



	AAT TCA ATA ACC TCG ACG GAT GTT CGT ATT TTA GTC CAG CCC GGC ATA Asn Ser Ile Thr Ser Thr Asp Val Arg Ile Leu Val Gln Pro Gly Ile 225 230 235 240	720
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	GAA CTT GAG TTC CGC AAC CTC ACC CCC GGT AAT ACC AAC ACG CGG GTC Glu Leu Glu Phe Arg Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val 305 310 315 320	960
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15	CAT CGG GTT GCT ATT TCC ACT TAC ACC ACT AGC CTG GGT GCT GGT CCC His Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro 580 585 590			1776
20	GTC TCT ATT TCC GCG GTT GCT GTT TTA GCC CCC CAC TCC GCG CTA GCA Val Ser Ile Ser Ala Val Ala Val Leu Ala Pro His Ser Ala Leu Ala 595 600 605			1824
25	TTG CTT GAG GAT ACC ATG GAC TAC CCT GCC CGC GCC CAT ACT TTC GAT Leu Leu Glu Asp Thr Met Asp Tyr Pro Ala Arg Ala His Thr Phe Asp 610 615 620			1872
30	GAC TTC TGC CCG GAG TGC CGC CCC CTT GGC CTC CAG GGC TGT GCT TTT Asp Phe Cys Pro Glu Cys Arg Pro Leu Gly Leu Gln Gly Cys Ala Phe 625 630 635 640			1920
	CAG TCT ACT GTC GCT GAG CTT CAG CGC CTT AAG ATG AAG GTG GGT AAA Gln Ser Thr Val Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys 645 650 655			1968
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40 (2) INFORMATION FOR SEQ ID NO:2: 1.1

45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 660 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

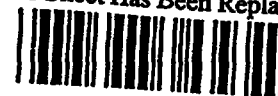
(ii) MOLECULE TYPE: protein

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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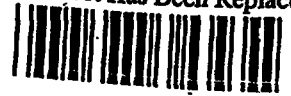
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 Arg Tyr Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser
 195 200 205
 15 Ile Ser Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met
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 225 230 235 240
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 245 250 255
 25 Gly Trp Arg Ser Val Glu Thr Ser Gly Val Ala Glu Glu Glu Ala Thr
 260 265 270
 Ser Gly Leu Val Met Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr
 275 280 285
 30 Thr Asn Thr Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu
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 40 Gly Thr Ala Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp
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 385 390 395 400
 Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
 405 410 415
 55 Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
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 60 Gln Asp Arg Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val
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 70 Ser Val Thr Leu Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg
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 Ser Leu Asp Trp Thr Lys Val Thr Leu Asp Gly Arg Pro Leu Ser Thr
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5 Leu Ser Phe Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn
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565 570 575

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580 585 590

Val Ser Ile Ser Ala Val Ala Val Leu Ala Pro His Ser Ala Leu Ala
595 600 605

15 Leu Leu Glu Asp Thr Met Asp Tyr Pro Ala Arg Ala His Thr Phe Asp
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Gln Ser Thr Val Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 372 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..369

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3: (COPY)

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96	CTC GGC CTA TTT TGC TGT TGC TCC TCA TGT TTC TGC CTA TGC TGC CCG
	Leu Gly Leu Phe Cys Cys Cys Ser Ser Cys Phe Cys Leu Cys Cys Pro
144	CGC CAC CGC CCG GTC AGC CGT CTG GCC GCC GTC GTG GGC GGC GCA GCG
	Arg His Arg Pro Val Ser Arg Leu Ala Ala Val Val Gly Gly Ala Ala
192	GCG GTT CCG GCG GTG GTT TCT GGG GTG ACC GGG TTG ATT CTC AGC CCT
60	Ala Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser Pro
240	TCG CAA TCC CCT ATA TTC ATC CAA CCA ACC CCT TCG CCC CCG ATG TCA
65	Ser Gln Ser Pro Ile Phe Ile Gln Pro Thr Pro Ser Pro Pro Met Ser
288	CCG CTG CCG CCG GGG CTG GAC CTC GTG TTC GCC AAC CCG CCC GAC CAC
	Pro Leu Arg Pro Gly Leu Asp Leu Val Phe Ala Asn Pro Pro Asp His
336	TCG GCT CCG CTT GGC GTG ACC AGG CCC AGC GCC CCG CCG TTG CCT CAC
	Ser Ala Pro Leu Gly Val Thr Arg Pro Ser Ala Pro Pro Leu Pro His

- 36 -

GTC GTA GAC CTA CCA CAG CTG GGG CCG CGC CGC TAA
 Val Val Asp Leu Pro Gln Leu Gly Pro Arg Arg
 115 120

372

This Sheet Has Been Replaced



5 (2) INFORMATION FOR SEQ ID NO:4:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 123 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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 Arg His Arg Pro Val Ser Arg Leu Ala Ala Val Val Gly Gly Ala Ala
 25 35 40 45
 30 Ala Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser Pro
 50 55 60
 Ser Gln Ser Pro Ile Phe Ile Gln Pro Thr Pro Ser Pro Pro Met Ser
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 Pro Leu Arg Pro Gly Leu Asp Leu Val Phe Ala Asn Pro Pro Asp His
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40 (2) INFORMATION FOR SEQ ID NO:5:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: DNA

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(2) INFORMATION FOR SEQ ID NO:6:

60 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

65 (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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- 37 -

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(2) INFORMATION FOR SEQ ID NO:7:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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(2) INFORMATION FOR SEQ ID NO:9:

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(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCTTAAGGCG CTGAAGCTCA

- 38 -

This Sheet Has Been Replaced



(2) INFORMATION FOR SEQ ID NO:11:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
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(2) INFORMATION FOR SEQ ID NO:12:

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(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
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(2) INFORMATION FOR SEQ ID NO:13:

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(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
45 TCGTAGACCT ACCACAGCTG

(2) INFORMATION FOR SEQ ID NO:14:

50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: DNA

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
CACAAGCTCA GAGGCTATGC CG



CLAIMS

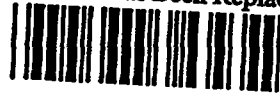
1. A recombinant molecule encoded by a sequence of nucleotides selected from the list consisting of open reading frame (ORF) 3 and a part or portion of ORF2 of Hepatitis E Virus (HEV).
2. A recombinant molecule according to claim 1 wherein said recombinant molecule is interactive with convalescent phase antibodies to HEV.
3. A recombinant molecule according to claim 1 or 2 wherein said recombinant molecule is interactive with acute phase antibodies to HEV.
4. A recombinant molecule according to claim 1 wherein said molecule comprises the N-terminal portion of ORF2.
5. A recombinant molecule according to claim 1 wherein said molecule comprises the C-terminal portion of ORF2.
6. A recombinant molecule according to claim 1 or 2 or 5 wherein said recombinant molecule is encoded by the 3' end portion of the nucleotide sequence set forth in SEQ ID NO: 1.
7. A recombinant molecule according to claim 1 or 3 or 4 wherein said recombinant molecule is encoded by the 5' end portion of the nucleotide sequence set forth in SEQ ID NO: 1.
8. A recombinant molecule encoded by a sequence of nucleotides selected from the list consisting of ORF3 and a part or portion of ORF2 of HEV and wherein said recombinant molecule is preferentially interactive with convalescent phase antibodies relative to acute phase antibodies.



9. A recombinant molecule according to claim 8 wherein said molecule is encoded by the 5' end portion of ORF2 of HEV.
10. A recombinant molecule according to claim 9 wherein the nucleotide sequence corresponds to clones 2.3 and 2.4 set forth in Figure 5.
11. A recombinant polypeptide comprising first and second amino acid sequences wherein said first amino acid sequence is encoded by a nucleotide sequence selected from ORF3 and a part or portion of ORF2 of HEV and wherein said second amino acid sequence is a non-HEV encoded peptide, polypeptide or protein and wherein said first amino acid sequence is interactive with convalescent phase antibodies to HEV and optionally interactive with acute phase antibodies.
12. A recombinant polypeptide according to claim 11 wherein said first amino acid sequence is interactive with convalescent phase antibodies to HEV and is encoded by the 3' region of ORF2 of HEV.
13. A recombinant polypeptide according to claim 11 wherein said first amino acid sequence is interactive with acute phase antibodies to HEV and is encoded by the 5' region of ORF2 of HEV.
14. A recombinant polypeptide according to claim 11 or 12 or 13 wherein said second amino acid sequence is glutathione-S-transferase (GST) or a part or derivative thereof.
15. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a peptide, polypeptide or protein interactive with acute phase antibodies to HEV.
16. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a peptide, polypeptide or protein interactive with convalescent phase antibodies to HEV.



17. An isolated nucleic acid molecule according to claim 15 wherein said nucleotide sequence comprises ORF3 or the 5' end portion of ORF2 of HEV.
18. An isolated nucleic acid molecule according to claim 16 wherein said nucleotide sequence comprises the 3' end portion of ORF2 of HEV.
19. An isolated nucleic acid molecule according to any one of claims 14 to 18 further comprising a second nucleotide sequence encoding or complementary to a sequence encoding a peptide, polypeptide or protein of non-HEV origin.
20. An isolated nucleic acid molecule according to claim 19 wherein the second nucleotide sequence encodes or is complementary to a sequence which encodes GST or a part or derivative thereof.
21. An expression vector having a nucleotide sequence comprising ORF3 or a part of ORF2 of HEV and wherein the expression vector directs expression of a recombinant molecule interactive with acute phase antibodies to HEV.
22. An expression vector having a nucleotide sequence comprising a part of ORF2 of HEV and wherein the expression vector directs expression of a recombinant molecule interactive with convalescent phase antibodies to HEV.
23. A method for detecting HEV interactive antibodies in a biological sample said method comprising contacting a recombinant molecule according to claim 1 or 8 with said biological sample for a time and under conditions sufficient to permit a complex to form with HEV antibodies if present in said biological sample and then detecting the complex.
24. A method according to claim 23 wherein said biological sample is whole blood, plasma, serum or saliva.



25. A method of detecting convalescent phase circulating antibodies to HEV in a biological sample, said method comprising incubating said biological sample with a recombinant molecule comprising a sequence of amino acids encoded by a nucleotide sequence comprises ORF3 or a part of ORF2 of HEV for a time and under conditions sufficient for a complex to form between HEV antibodies if present in said biological sample as said recombinant molecule and then detecting said complex.
26. A method according to claim 25 wherein said biological sample is whole blood, plasma, serum or saliva.
27. A method according to claim 25 wherein said recombinant molecule further comprises a sequence of amino acids from a non-HEV peptide, polypeptide or protein.
28. A method according to claim 27 wherein the further amino acid sequence is GST or a part or derivative thereof.
29. A vaccine to inhibit, reduce or ameliorate the effects of HEV infection in a human, said vaccine comprising a recombinant molecule comprising a sequence of amino acids encoded by the 3' end of ORF2 of HEV and one or more carriers and/or diluents suitable for use in a vaccine.
30. A method for inhibiting, reducing or ameliorating the effects of HEV infection in a human, said method comprising administering to said human an effective amount of a recombinant molecule encoded by the 3' end of ORF 2 of HEV and wherein said recombinant molecule is interactive with convalescent phase HEV antibodies.
31. A kit for detecting antibodies to HEV, said kit comprising in compartmental form a first compartment comprising a peptide, polypeptide or protein encoded by one or more of the following regions:
 - (i) ORF3 of HEV or a part or portion of said ORF3;
 - (ii) 3' end portion of ORF2 of HEV; and
 - (iii) 5' end portion of ORF2 of HEV;

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said kit further comprising one or more compartments comprising agents suitable for detecting antibodies.

This Sheet Has Been Replaced

