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Hepatitis E Virus Infection in Taiwan: Prevalence of Neutralizing Anti-HEVne Positive Serum

Abstract

【Purpose】Hepatitis E virus infection is an important communicable disease in Asia and mainland China. The infection is characterized by the appearance of cholestatic jaundice and a high fatality among pregnant women. No outbreaks of the infection have yet been recorded in Taiwan. To ascertain the likelihood of HEV infection and its routes of transmission in Taiwan, the present study used a newly developed enzyme immunoassay to detect HEV specific neutralizing antibodies (anti-HEVne) in serum in order to assess the prevalence of hepatitis E virus infection.【Materials and Method】A series of ORF2 (open reading frame 2) with high specificity to anti-HEVne, aa578-aa607, was used as a reagent to test antibodies. The subjects tested were divided into 4 age groups: 4-5 years (200 persons, average age 4.4 years), 20-40 years (250 persons average age 32.5 years), 40-60 years 300 persons, average age 53.1 years), 60-80 years 300 persons, average age 71.9 years), and 300 pregnant women, (average age 33 years). 【Results】The newly developed anti-HEVne assay as found to be very

specific for the testing of HEV infection. The anti-HEV positive rates in the different age groups were, 2% (5-6 years), 8% (20-40years), 13% (40-60 years), and 11% (60-80 years). The antibody was present in about 6% of pregnant women. The findings suggested that HEV infection was higher in the 40-60 age group. According to this study, the average infection rate of the population was found to be 8.9%.【Discussion】The present study used a newly developed method to detect anti-HEV positive rates in the sera of the general population. In suspected imported cases, mainland China (56%) was considered a major source of infection. It was also speculated that the infection was spread in a low circulation/sporadic way in Taiwan. Among the groups studied, the anti-HEVne positive rate was higher in the 40-60 age group. In general, positive rates were higher in the older age groups. However, since anti-HEVne gradually disappears several years after infection, whether elderly people were more likely to be re-infected remains to be studied further.

Introduction

Hepatitis E virus infection (HEV) occurs all over the world (Figure 1); it is, however, more prevalent in India, Myanmar, China, the former Soviet Union, and Mexico. Sporadic cases have also been reported elsewhere including some Asian countries, northern Africa, central Africa and Central and South America. Generally speaking, sporadic cases found in North America, Europe and Australia occurred in persons who had visited or whose origins were from epidemic areas. The belief that HEV does not exist in developed countries has been challenged. The infection is now defined as a major emerging communicable disease[1,2,3].

HEV infection is an acute hepatitis. Closer contacts with the southeastern coasts of China in recent years have made its prevention more important. Like hepatitis A virus infection, the severity of hepatitis E virus infection increases with age. However, the infection is more prevalent in young people. They are usually infected from primary sources, and rarely from secondary sources. Unlike hepatitis A, hepatitis E is less likely to be transmitted among susceptible family members or persons in close contact with the patients. In some recent reports, children were infected with HAV and HEV concurrently, resulting in acute liver failure[2,3,4,5]. Though there are sub-clinical infections, the infection is characterized clinically by severe cholestatic jaundice and a high fatality among pregnant women in the 7-9th gestational months. The pathogenic mechanism is still unknown. Reports suggest that the infection could induce severe inflammation and necrosis of liver and gall bladder cells[47,48]. In contrast to these serious symptoms, there is also a likelihood of sub-clinical infection and this should not be overlooked [36].

The HEV genome is a single ply RNA(positive sense) about 7.5 kb long (see Figure 2; 3). It has three ORFs (conserved open reading frames). ORF1 is located at the 5' end of the genome. It encodes a protein molecule of about 1,693 amino acids. The functions of the domains of the protein molecule formed later through proteolytic cleavage could be associated with the cloning of the virus, such as RNA-dependent RNA polymerase. OFR2 is located at the 3' end of the genome. It produces capsid protein. The shorter ORF3 partly covers ORF1 and ORF2. The functions of the genetic products of its 123 amino acids are unknown. The protein will later be phosphorylated and is probably connected with cytoskeleton. That the product of ORF3 will eventually be integrated with the external sheath of the virus is not yet

supported by study results. The virus is similar in gene sequence to *caliciviruses* or other viruses such as Norwalk agent and SSRVs (small, round-structured viruses). They all are spread via the fecal-oral route. However, whether HEV can be grouped under the Caliciviridae family should be re-evaluated, for there are still differences in viral characteristics between this virus and other viruses of that family[4].

Thus far, four genotypes of HEV have been identified. However, the genetic diversity of HEV, that is the number of genotypes and the animals that are susceptible to the virus, needs further investigation. The prototype of HEV came from a case from Myanmar (named Myanmar genotype). Later, more viruses of high homology with this genotype were isolated from Asia and Africa. The virus isolated from a Mexican patient in the 1986 Telixtac outbreak (named Mexican genotype) is different from the Myanmar genotype. Recently, the HEV virus strains of the US, after phylogenetic analysis, were found to be a different genotype (named the US genotype). The HEV isolated in China comes in two groups, one group is similar to the Myanmar genotype, and the other group is a new genotype (named the Chinese genotype). Other HEV genotypes have yet to be discovered[6-13].

HEV infections occur more often in developing countries. Assays in the past indicate, however, that infection occurs in developed countries as well. Surveillance of the infection in Taiwan also leads one to the same conclusion. Whether imported assays can be used to detect indigenous genotypes is doubtful. Although there are commercial assays for the detection of IgG antibodies, because of genetic mutation and differences in reagent antigens in clinical molecular diagnosis, the detection of virus infection is not yet

standardized. The current assays use reorganized antigens developed from the Myanmar and the Mexican genotypes or synthetic peptides. These genotypes are relatively common. Current laboratory testing does not include detection of the US and the Chinese genotypes. Reports of sero-epidemiological studies of HEV vary significantly with the assays used. Therefore, using these assays to determine the prevalence of HEV infection in Taiwan may be controversial.

Enzyme immunoassays and RT-PCR can be used for diagnosis to some extent. There are not yet any commercial assays for detecting HEV antigens. Researchers can, however, use RT-PCR techniques to detect them. However, as the variation of the antigen fragments is so large, the RT-PCR findings can be false-negative. At present, clinical trials of the vaccines are still in their initial stages[41,47]. Thus improvement of hygiene and sanitation is the most effective and practical method at present of preventing HEV infection.[23]. Studies have suggested that the virus is common to men and animals. Animals, particularly swine, are important sources or reservoirs of HEV infection. These sources or reservoirs still exist in developed countries; they probably are causes of some sporadic cases[17,18].

Recently, Schofield et al. reported that the aa578-aa607 series in the external sheath of virus (ORF2) are important structures in inducing anti-HEVne, they are also of high homology with the amino acid series of several genotypes thus far identified[18,19]. The purpose of the present study was to use synthetic peptides to detect the prevalence of anti-HEVne in the sera of the general population and to compare these findings with the findings of previous studies, as well as to ascertain the prevalence of HEV infection in the general population and its routes of transmission.

Materials and Method

The Groups Studied

Specimens for study came from the Tainan Blood Center, Taipei Blood Center, Taipei Municipal Chunghsiao Hospital, Ilan Poai Hospital, Changhua Christian Hospital, Pingtung Christian Hospital, and DOH Penghu Hospital. Table 1 shows the groups studied.

Table 1. The Groups Studied

Group	Age	No	Average Age	Sex Ration (M/F)
1	4-5 years	100	4.4	1.13(53/47)
2	20-40 years	250	32.5	1.17(135/115)
3	40-60 years	300	53.1	1.33(171/129)
4	60-80 years	300	71.9	0.85(138/162)
5	Pregnant women	300	33	0(0/300)
	Total	1250	44.7	

The Controls

Sera of acute hepatitis B and C patients, chronic hepatitis B and C carriers, acute hepatitis A patients, acute enterovirus infection patients, and acute gastritis patients were collected separately from the Tainan Blood Center, Taipei Blood Center, Taipei Municipal Chunghsiao Hospital, Ilan Poai Hospital, Changhua Christian Hospital, Pingtung Christian Hospital, and DOH Penghu Hospital. The serum of a native infected with acute hepatitis E in India/Pakistan was used as the positive control[22]. The serum of a child who had been infected with enterovirus but not hepatitis was used as the negative control.

The Enzyme-Linked Immunosorbent Assays (ELISAs/EIA) Method

The present study used the aa578-aa607 sequences of the ORF2 most specific to anti-HEVne as the reagent. The decision to use the peptide sequences came after a comparison of five indigenous HEV ORF2 sequences isolated by the laboratory with some already known sequences isolated from other eight virus strains or genotypes. Synthetic peptides were used for the coating of the Nunc immunoplate. Synthetic peptides (Laboratory of Molecular Diagnosis, Tuebingen) were used after dilution with 1% DMSO following the standard operational procedures instructions.

Each hole of the Nunc immunoplate (Denmark) could absorb 500 ng synthetic peptides (dissolved in phosphate-buffered saline; PBS). Antigens were maintained at room temperature overnight. Antibodies were added to the immunoplate after it had been washed twice with PBS. The procedure for the testing of antibody reactions was: 10 μl of the serum specimens to be tested and 200 μl PBSTA (PBS with 0.05% Twenn 20/1.25 Albumin) were added to the Nunc immunoplate. It was maintained at 37℃ for two hours, washed with 200 μl of PBST (PBS with 0.05% Twenn 20) five times, 100 μl of HRP-conjugated human IgG or IgA (1:6000) was added, and maintained at 37℃ for a half hour. It was washed with 200 μl of PBSTA five more times , and 100 μl of 0.1% O-phenylenediamine (OPD) and 0.03% Hydrogen Peroxide-Citric Acid were added, then left in a dark room at room temperature for 30 minutes; 50 μl of stop solution (2N Sulphuric Acid) was added to arrest color change. Finally, the lab results were confirmed by absorption of 490 nm Optical Density.[49].

Reading of the results was performed by using the average value of serum optical density plus 500 samples of the sera of three negative controls as the

screening value. When the optic density of the samples was larger than or equal to the screening value, the result was considered to be positive; it was negative when the value was smaller. Reading of positive samples was performed after twice repeated testing.

The present study also used a commercial anti-HEV reagent as the control. Laboratory procedures followed the standard operational procedures instructions.

Detection of HEV RNA

An ingredient specific to the HEV genome was used for reverse-transcriptase-polymerase chain reaction (RT-PCR) to detect HEV RNA. After serum specimens were transported frozen to the laboratory, they were kept in a freezer at -20℃. Qiagen RNA extracts (QIAGEN Ltd., Crawley, UK) were used to isolate RNA directly from plasma (or serum). The isolated RNA was dissolved in 50 µl buffer. A starter specific to HEV was used to isolate HEV-RNA in 50 µl PCR mixture (including 5 µl of isolated DNA, 1xPCR buffer, 0.2 mM deoxynucleoside triphosphates, 2 mM magnesium chloride, 0.5 U Tag polymerase), together with Perkin Elmer (PE 4800) for amplification through reverse transcription. The polymerase chain reaction was carried out with 2 runs (nested). Primer concentrations in both PCR runs were adjusted to 10pmole. The ingredients used are as follows:

Table 2. HEV RNA Genome Specific Ingredients

Ingredient Code	Ingredient Sequence	Use	Cloning Gene
EORF2	5'-ACAGAATTRATTTCGTCGG-3'	External sense	ORF2
EORF2	5'-TTT TTT TTT TTT TTT TTT T-3'	External antisense	ORF2
EORF2	5'-TTGTCTCGGCCAATGGCGA-3'	Internal sense	ORF2

EORF2	5'-TTT TTT TTT CCA GGG AGC -3'	Internal antisense	ORF2
EORF123	5'- GGGTTGACAAATGTTGCG-3'	External sense	ORF123
EORF123	5'-GTT GTG AAA CGA CAT CG-3'	External antisense	ORF123
EORF123	5'-TGT TTA TGG AGT TAG CCC-3'	Internal sense	ORF123
EORF123	5'-GAA TCA ACC CTG TCA CCC-3'	Internal antisense	ORF123
EHVR	5'-AAR ACC TTC MGS ACG WCG -3'	External sense	HVR
EHVR	5'-GAG CAA GTC TCC CGG TAS GC-3'	External antisense	HVR
EHVR	5'-AGC GSC WTT CGC TGA CCG G –3'	Internal sense	HVR
EHVR	5'-GGT ASG CWG CCT CAA GCC T-3'	Internal antisense	HVR

Cloning began after RT-PCR at 50℃ for one hour. Temperatures in the cycles of cloning were: 94℃, 30 seconds; 58℃, 30 seconds; 72℃, 30 seconds, for a total of 25 cycles. Positive specimens would have synthetic PCR products. After dyeing with ethidium bromide, the products could be observed under 2% agarose ultraviolet light. For the specificity of the PCR findings, negative controls were detected repeatedly with the ingredients.

In each experiment, a serum specimen with HEV-RNA (specimen confirmed after gene analysis, numbered E101) and another serum specimen without HEV-RNA (specimen repeatedly confirmed with PCR as negative, numbered E102) were used as the positive and negative controls. The estimate using the method developed by the author was that the amount of HEV-RNA in the specimen was about 10^7-10^8 molecules/ml[(15)].

After purification with QIA (quick PCR purification kit, Qiagen Ltd., Germany), the cloned HEV DNA was directly analyzed for gene sequences. The equipment used was ABI 310 automated DNA sequencer (Applied Biosystems, Forster City, CA) using the fluorescent dye terminator cycle method.

Investigation of Overseas Visits and Statistical Methods

In addition to clinical assessment by physicians, serum positive cases, patients or healthy individuals were also interviewed via telephone with a

questionnaire. The questionnaire contained items on personal background, time of disease onset, clinical symptoms, changes in liver and gall-bladder function tests, and medication. Some important points included in the investigation were whether the acute positive cases had been to any developing countries or epidemic areas (Thailand, or China, for instance), or had had blood transfusion or contact with animals. The statistical method used in the present study was the t-test of Sigma Plot. P value smaller than 0.05 was considered statistically significant.

Results

Usefulness of the Anti-HEVne Reagents

As per Table 3, reagents were not reactive to sera of acute B and C hepatitis patients, chronic B/C hepatitis carriers, acute hepatitis A patients, acute enterovirus and acute gastritis patients, but were reactive to sera of acute hepatitis E patients and recovered hepatitis E patients. In addition, to test for false positive reactions due to high level of jaundice, sera of chronic hepatitis B and C carriers (total bilirubin > 5 mg/dl) were used as controls (items 11 and 12 of Table 3).

In the present study, the acute positive serum collected previously from a patient infected with hepatitis E in India/Pakistan was used as the positive control[(22)]. The serum of a child who had been infected with enterovirus but not hepatitis was used as the negative control. After repeated detection (20 times) of the specimens, the reagents were proved to be highly stable and reproducible. The average optical density of the negative control serums was 0.125; that of the positive controls was 0.876 (see Figure 3). When the reagents were used to detect the sera of the said acute patient after recovery, the serum antibodies had already shown a significant decline (items 6, 7, 8 of Table 3). These facts all indicated that the reagents were useful in the detection of

HEV antibodies and HEV infection. A commercial HEV reagent was also used together with some anti-HEVne reagents to detect sera of some confirmed cases (Table 4). Although no significant differences were found between the two groups of reagents, the commercial one was extremely insensitive to one sample Chinese and one sample of Taiwan serum (items 8 and 13 of Table 4).

Infection Status

Serum positive rates in the groups studied were, respectively, 2% (in the 5-6 age group), 8% (20-40 age group), 13% (40-60 age group), 11% (60-80 age group), and 6% (pregnant women) (Table 5). The results suggested that HEV infection was the highest in the 40-60 age group.

In the first through fourth groups studied, about 70% of the positive results were from male subjects (67/94, 72%); whereas only 30% of the positive subjects were female (27/94, 28%). Men were more likely to be infected than women ($p<0.05$).

Of the 16 who had been to epidemic areas, nine of them (56%) had been to mainland China, four (25%) to India, two (12%) to Pakistan, and one (6%) to Vietnam for a stay of more than two weeks. Their clinical and laboratory findings are shown in Table 6.

Discussion

The serum anti-HEVne IgG positive rate of groups of people undergoing routine physical examination using the ORF2 antigen of the present study was 8.9% (112/1250). Different antigens are of different specificity and sensitivity in the detection of viruses. It should be noted that the above positive rate was the result of detection by the antigen used in the present study. According to the literature, HEV sequences consist of some variants[8-12]. Using different antigens of varying sensitivity would certainly yield different results. Any

findings, particularly when compared with other test findings, should be scrutinized. The antigen used in the present study was a major neutralizing segment of HEV; the antibodies thus detected should be anti-HEVne that could neutralize HE virus.

The present study used a neutralizing antibody reagent that could produce peptides in the ORF2 structural zone of hepatitis E after infection. The reagent thus was only specific to the detection of this neutralizing antibody. In theory, any neutralizing antibody detected is only one of many protective antibodies produced against the virus antigens. That the positive rate thus detected was lower than that of previous studies was expected. However, since it was antibody that could neutralize virus antigens, persons with such antibody should have immunological protection against HEV infection. The present study found that the anti-HEVne assays were basically not different from the commercial assay, although the latter was the least sensitive to one Chinese and one Taiwan virus strain. It was most probable that the antigen used in the commercial reagent could not simulate the conformational epitopes of some specific Chinese and Taiwan virus strains. This is understandable. As mentioned earlier, the current commercial reagents are reorganized antigens or synthetic peptides developed primarily from the Mexican and the Myanmar genotypes. These are relatively common genotypes. Detection of the US, Chinese, and Taiwan genotypes is not accomplished by the current commercial reagents. Locally manufactured anti-HEVne assays are indigenous and comparative. The findings so obtained were thus different from findings of other authors using commercial assays[(50)].

By using this newly developed testing method, the present study obtained some anti-HEV positive rates in serum. Positive rates in pre-school children and people who had never been abroad (2%) suggested the likelihood of the

virus existing in the local environment. The virus is probably being sporadically transmitted within the country. In the groups studied, anti-HEVne was the highest in the 40-60 age group. Previous follow-up of patients showed that recovery from acute hepatitis E was probably associated with high titers of anti-HEV antibodies. Antibody titers gradually decline over time[35]. This finding suggested that elderly people could possibly be re-infected.

There have been reports of the positive rates of HEV IgG among groups of Taiwanese.[16,17,30,31,32]. Those findings are somewhat different from findings of the present study. The prevalence rate obtained in the present study was lower than that of previous studies[21,24]. The differences were due probably to sampling procedures, different localities, and specificity of assays used. The neutralizing antibodies so detected were part of the anti-HEV of the hosts after infection. That the serum positive rates obtained by the present study were lower than those of other studies was expected. In well-defined local cases, anti-HEV IgA and anti-HEV IgM were shown to very specific for acute phase of HEV infection, in addition to presence of HEV RNA (data not shown). Furthermore, it is therefore a meaningful intention to define these two serological markers and diagnostic tools in the near future.

According to Author's observation, in the early phase of severe cases of hepatitis E virus infection, increased bilirubin level, HEV RNA and anti-HEV IgM/IgA are important parameters. In a very short consequent course, abnormal liver function, and high titer of anti-HEV IgG appear as serological markers. Clinical and pathological expressions of HEV infection are varied, from sub-clinical, mild gall bladder disease, cholestasis, to severe hepatitis. Previously, there has been indirect proof of asymptomatic or sub-clinical infection of HEV[28]. Recently, Nicand et al. also reported asymptomatic or sub-clinical cases of HEV infection and proved that they could shed viral

nucleic acid[36]. Thus far, clinical reports on HEV infection in Taiwan are relatively few[25]. In previous studies, although clinically significant cholestasis[5] and mild cholecystitis[33] could be screened by using sudden high-titer IgG antibody positive and/or virus nucleic acid positive and/or nuclear medicine scanning[45,46,48], some serum positive healthy individuals were unable to give a history indicating infection. What percentage of HEV infected cases are asymptomatic or have sub-clinical disease remains to be clarified. The present study focused primarily on serum positive rates and likely routes of infection; information concerning acute cases and their clinical characteristics by using the screening method of this study will be reported later.

Generally speaking, hepatitis E, transmitted via the fecal-mouth route, is a major pathogenic cause of acute hepatitis particularly in developing countries. From the present study, it is clear that the existence of anti-HEVne positive sera is evidence of HEV infection among the Taiwanese population. The 16 cases that could be followed-up had been to either developing countries or epidemic areas. According to this study, mainland China (56%, 9/16) was still the major source of infection[38], corresponding to the findings of Wu et al. (using a commercial assay with viral antigens derived from foreign strains) [29]. It is therefore recommended that visitors to mainland China should take precautions by not eating raw food nor drinking tap water[23,34]. Other cases such as positive cases who had never been abroad or the 2% prevalence rate in pre-school children suggest the likelihood of indigenous HEV infection in Taiwan.

The virus can infect many animals (monkeys[37], swine, rodents, chickens [52] etc.); infection in Taiwan can be associated with animals, particularly swine[25,26,47]. In 1% of the blood of swine in Taiwan, virus nucleic acid RNA can be detected[25]. The feces excreted by these animals are likely to contain

viruses that can contaminate water sources and the environment. Hsieh et al.[26] reported that pork meat dealers in Taiwan had a high serum positive rate of 26.7%. Because the samples studied were small, the findings remain to be verified further. Since the viremia phase of virus in blood after infection is long, and there are asymptomatic infections, the possibility of blood-borne route of infection should also not be excluded[42,43,44]. The question concerning whether hepatitis E virus infection play a causal role in the disease development in Taiwan or not deserve further investigation.

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Figure 1. Geographic Distribution of Hepatitis E [51]

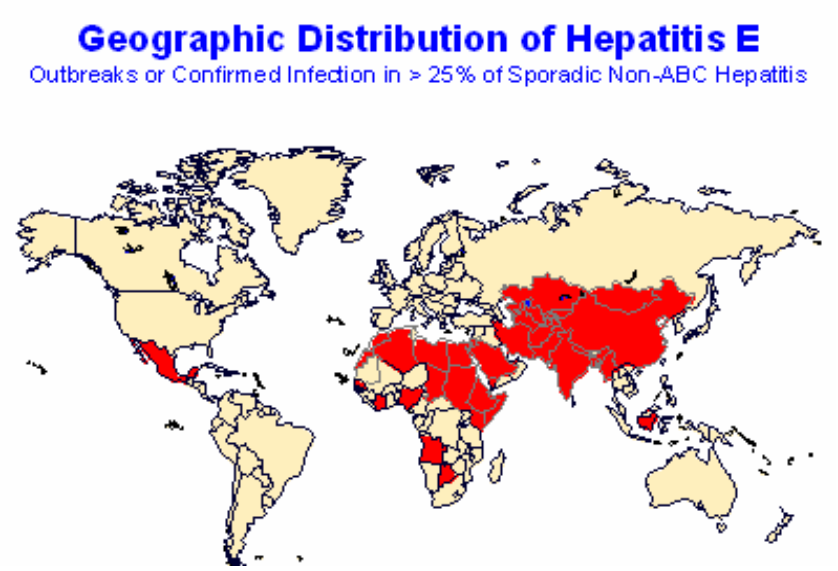

Figure 2. Permutation and Functions of HEV RNA Genome[3]

Positions of the three Open Reading Frames (ORFs): 1) ORF1, non-structural region contains genes such as methyltransferase (MT), potential protease (Pro), helicase and replicase (RNA-dependent RNA polymerase) domains; 2) ORF2, structural region contains genes such as amino-terminal signal peptide of structural protein and glycosylation sites; 3) ORF3, located between ORF1 and ORF2, functions unknown.

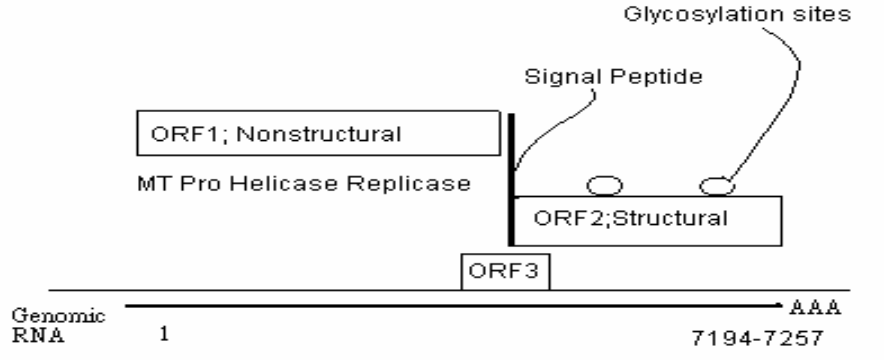

Table 3. Reactions of anti-HEVne reagents to Various Clinical Sera Optical Density 0.589)

Item	Clinical Serum Specimens	No.	Average Optical Density	Result
1	Acute hepatitis B serum	3	0.156	Negative
2	Acute hepatitis C serum	2	0.127	Negative
3	Chronic hepatitis B carrier serum	5	0.122	Negative
4	Chronic hepatitis C carrier serum	5	0.124	Negative
5	Acute hepatitis A serum	2	0.109	Negative
6	Acute hepatitis E serum	2	0.987	Positive
7	One year after recovery from hepatitis E	1	0.754	Positive
8	Two year after recovery from hepatitis E	1	0.554	Positive
9	Acute enterovirus infection serum	1	0.087	Negative
10	Acute gastritis serum	20	0.108	Negative
11	Chronic hepatitis B carrier serum (Total Bilirubin >5mg/dl)	5	0.129	Negative
12	Chronic hepatitis C carrier serum (Total Bilirubin >5mg/dl)	7	0.183	Negative

Figure 3. Optical Density of Positive and Negative Controls

(In the present study, the acute positive serum collected previously from a native who was infected in India/Pakistan[22] as the positive control. The serum of a child who was infected with enterovirus but not hepatitis was used as the negative control. After repeated testing (20 times) of these specimens, the testing was found highly stable and reproducible. The average optical density of the negative control was 0.125; that of the positive control was 0.876.)

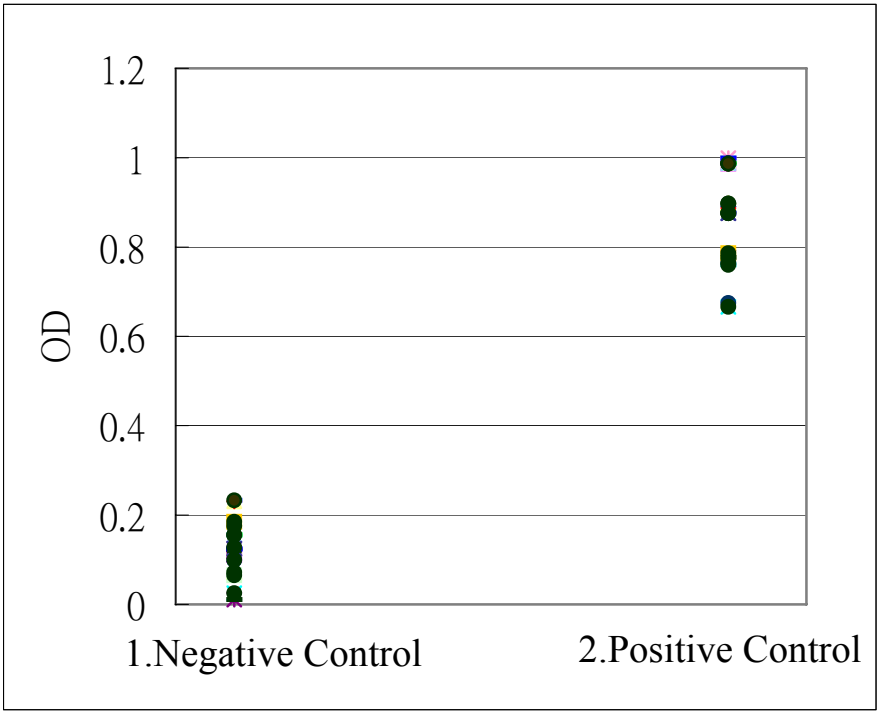

Table 4. Testing of Serums of Confirmed International Cases with anti-HEVne Reagent and One Commercial Reagent

(T1 for commercial reagent; T2 for anti-HEVne reagent; figures are cutoff values of optical density.)

No	Serum Code	Nationality	Source of Infection	Supplied	T1	T2
1	G001	Germany	Lithuania	Tuebungen Univ., Germany	2.1	1.7
2	G002	Germany	Africa	Tuebungen Univ., Germany	1.9	2.1
3	G003	Germany	India	Tuebungen Univ., Germany	1.2	1.9
4	G004	Germany	Greece	Tuebungen Univ., Germany	2.4	1.8
5	F001	France	Vietnam	Tuebungen Univ., Germany	1.2	2.5
6	F002	Africa	South Africa[37]	Tuebungen Univ., Germany	1.1	1.4
7	F003	Africa	Kenya[37]	Tuebungen Univ., Germany	2.3	2.9
8	T001	Taiwan	Qingdao,China	CDC DOH	0.4	2.1
9	T002	Taiwan	Fujian, China	CDC DOH	1.6	2.4
10	T003	Taiwan	Pakistan	CDC DOH	1.4	1.2
11	T004	Taiwan	India	CDC DOH	2.3	2.5
12	T005	Taiwan	Taiwan	CDC DOH	2.1	0.9
13	T006	Taiwan	Taiwan	CDC DOH	1.7	2.1
14	T007	Taiwan	Taiwan	CDC DOH	0.3	1.1
15	T008	Taiwan	Taiwan	CDC DOH	2.8	2.3

Table 5. Positive Rates of anti-HEVne in Various Groups

No.	Group（No.）	HEV IgG Positive(%)	HEV IgG Negative(%)	Average Age
1	4-5 yrs（100）	2(2)	98(98)	4.4
2	20-40 (250)	20(8)	230(92)	32.5
3	40-60 (300)	33(11)	267(89)	53.1
4	60-80 (300)	39(13)	261(87)	71.9
5	Pregnant women (300)	18(6)	282(94)	33
	Total (1250)	112	1138	44.7

Table 6. Clinical and Laboratory Findings of Acute HEV Patients

(1. M: male, F: female, Pos: positive, Neg: negative;)

(2. Time of specimen collection and laboratory testing: specimens collected and tested shortly after GPT at its peak)

No. and Sex	GPT (U/L)	Bil (mg/dl)	IgG Antibody Anti-HEV	IgM Antibody Anti-HEV	IgA Antibody Anti-HEV	HEVRNA	Time[2]	Traveling
No.1/M	1082	12	Pos	Pos	Pos	Pos	5	India
No.2/M	802	10	Pos	Pos	Pos	Pos	6	China
No.3/M	342	2	Pos	Pos	Pos	Neg	8	Thailanf
No.4/M	253	36	Pos	Pos	Pos	Pos	12	China
No.5/M	86	2	Pos	Pos	Pos	Neg	14	China
No.6/M	358	9	Neg	Pos	Neg	Pos	4	India
No.7/M	36	1	Pos	Pos	Pos	Neg	22	China
No.8/M	1162	23	Pos	Pos	Pos	Pos	4	China
No.9/M	165	1.2	Pos	Pos	Pos	Pos	8	China
No.10/M	45	0.9	Pos	Pos	Pos	Neg	16	Pakistan
No.11/M	43	0.8	Pos	Pos	Pos	Pos	12	India
No.12/M	76	1	Pos	Pos	Pos	Pos	7	China
No.13/M	1238	6.9	Neg	Pos	Neg	Pos	4	China
No.14/M	34	0.2	Pos	Pos	Pos	Neg	19	Pakistan
No.15/M	765	1.8	Pos	Pos	Pos	Neg	24	Vietnam
No.16/M	888	2.8	Neg	Pos	Neg	Pos	3	China