Liver function tests, including transaminase levels (aspartate aminotransferase [ALT] and alanine aminotransferase [AST]) in serum were determined using a Thermo Spectronic spectrophotometer (Helios, Barcelona, Spain).

To determine the correlation between the data obtained from the questionnaire and the laboratory results, odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated using binary logistic regression analysis by means of SPSS version 15.0 statistical software. For the statistical comparison of the seroprevalence obtained in the E and NE groups, the Pearson χ^2 test and Student t test were applied.

All individuals tested negative for the presence of HEV RNA in serum. The overall prevalence of anti-HEV IgG confirmed by immunoblotting was 11.6% (23/198). The seroprevalence of anti-HEV IgG in the E group and in NE group was 18.8% (19/101) and 4.1% (4/97), respectively (Table 1). Values of transaminase enzymes were located within the normal range (ALT: men < 45 IU/L, women < 36 IU/L; AST: < 34 IU/L for men and women) in all individuals. No significant differences in the levels of transaminases were observed between the anti-HEV IgG-positive group (ALT: 22 ± 14; AST: 12 ± 7.5) and the anti-HEV IgG-negative group (ALT: 15 ± 12.2; AST: 11 ± 6.8). The statistical analysis showed a significant association ($P < 0.05$) between the presence of anti-HEV IgG and the consumption of untreated water with an OR value of 5.6 ($P = 0.01$). Additionally, people exposed to swine were observed to be 5.4 times ($P = 0.03$) at risk of having anti-HEV IgG antibodies. Ten (52.6%) of the IgG-positive individuals showed two concomitant risk factors: untreated water consumption and exposure to swine. The χ^2 goodness-of-fit test showed a good fit with the observed and expected frequencies in the E and NE groups ($\chi^2 = 10.4$, $P = 0.01$) and consumption of untreated water ($\chi^2 = 12.9$, $P = 0.01$). No significant differences were observed between the rest of the study parameters.

This is the first study in Spain reporting the prevalence of IgG anti-HEV antibodies in swine workers (18.8%) and in people unexposed to swine (4.1%). The increased risk (5.4 times at risk) of having IgG anti-HEV observed in swine workers in this work is not surprising, taking into account the high number of farms (76%) and pigs (23%) testing positive for HEV RNA in the same area.[14] This datum is higher than the OR (1.46) reported by Meng and others[6] in 2002 in the only study that calculated the risk for a veterinarian to be positive for IgG anti-HEV. The fact that the values of transaminases were similar between positive and negative individuals suggests that HEV might be responsible for subclinical infections, because none of the participants reported any past clinical signs of acute hepatitis. The factors triggering the development of an acute or a subclinical hepatitis E infection remain obscure in industrialized countries. Some authors point to several contributing factors such as age,[15] preexisting hepatopathy,[16] and the genotype of the strain.[17]

It has been reported for autochthonous hepatitis E in developed regions that swine isolates from genotype 3 are more related to human strains from the same geographic region than to swine strains from different areas. Moreover, HEV strains circulating in Spanish swine farms are highly homologous with Spanish human strains, which raises the possibility of HEV transmission from swine to humans.[18] HEV has been suggested to be a zoonotic infection where pigs play an important role in the spreading of the disease. HEV is capable of crossing the species barrier, as has been shown by means of experimental infections in pigs with a human HEV strain and in non-human primates with a swine HEV strain.[19]

The results obtained in this study support the link between the presence of anti-HEV antibodies and direct contact with swine, as reported by several authors. Thus, in the United States,[6,7] significant prevalences between veterinarians working with swine (26% and 10.9%, respectively) and unexposed people (18% and 2.4%, respectively) were reported. Similar results were described in The Netherlands, Moldova, and Taiwan,[8–10] with values for those exposed to swine of 11%, 51%, and 27% versus 2%, 24.7%, and 2.4%, respectively. In contrast, studies in Sweden[11] found no significant differences be-

TABLE 1
Characteristics and risk factors of the studied population according to the presence or absence of anti-HEV IgG

	Anti-HEV IgG positive	Anti-HEV IgG negative	P	OR	95% CI
Sex					
Male	21 (20.8%)	80 (79.2%)	0.01	0.08	0–0.3
Female	2 (2%)	95 (97.9%)			
Age (years)	38.2 ± 10.4	26 ± 9.0			
ALT	22 ± 14	15 ± 12.2			
AST	12 ± 7.5	11 ± 6.8			
RNA-HEV	0 (0%)	0 (0%)			
Consume raw vegetables					
No	2 (7.4%)	25 (92.6%)	0.46	1.75	0.3–7.9
Yes	21 (12.3%)	150 (87.7%)			
Consume raw shellfish					
No	23 (11.6%)	175 (88.4%)	0	0	0
Yes	0 (0%)	0 (0%)			
Consume untreated water					
No	13 (7.8%)	154 (92.2%)	0.01	5.6	12.2–14.5
Yes	10 (32.2%)	21 (67.8%)			
Travel abroad					
No	18 (13.2%)	118 (86.8%)	0.29	0.6	0.2–1.6
Yes	5 (8%)	57 (92%)			
Exposure to swine					
No	4 (4.1%)	93 (95.9%)	0.03	5.4	1.7–16.5
Yes	19 (18.8%)	82 (81.2%)			

OR = odds ratio; CI = confidence interval; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

tween those exposed (13%) and unexposed to swine (9.3%), and in Italy,[12] prevalences of 3.3% in swine farmers and 2.9% in people without occupational exposure to swine were reported. The high variation among the prevalences described above might be caused by differences in sample size, country of origin, and the diagnostic assay used. In this context, it has been described that there are significant sensitivity variations in developed countries depending on the type of ELISA kit used, as well as immunoblotting confirmation of the ELISA-positive samples. The data obtained by Herremans and others[20] in 2007 suggest that there are few differences in the sensitivity of ELISAs based in genotype 1 or 3 antigens. Therefore, the number of false negatives in the healthy population is expected to be low. In our study, to minimize the possibility of false positives and yield more accurate prevalence results, positive samples were confirmed by means of an immunoblot assay (Recomblot HEV; Mikrogen).

Regarding other risk factors studied in this work, an elevated prevalence (32.2%) and risk (OR = 5.6) in people who reported consumption of untreated water from water fountains in the countryside was recorded. The relationship between untreated water consumption and exposure to swine in swine workers is not surprising because the farms are located in the countryside where untreated water fountains are numerous. Additionally, it is very common among farmers to fertilize cultivated fields with manure from swine farms, which could infiltrate down through the ground, contaminating subterranean water and reaching to the water fountains. However, this hypothesis needs to be confirmed by further studies detecting HEV in water fountains.

The seroprevalence observed in other industrialized countries such as the United Kingdom,[19] Italy,[21] France,[22] New Zealand,[23] and Brazil,[24] with 6.3%, 2.6%, 3.2%, 4%, and 2.3%, respectively, was lower than the value reported in our study. The overall percentage found in this study (11.6%) is also higher than the one observed by Mateos and others[25] (2.8%) and the rate obtained by Buti and others (7.3%)[26] in a normal Spanish population. These cannot be properly compared with the data obtained in this study because of the high number of exposed people (50%). These high prevalences suggest that autochthonous HEV is circulating in Spain, and the infection is underdiagnosed. Although transfusion-transmitted HEV is probably much too rare to sustain HEV transmission, it should be taken into account that HEV is spread through uncertain routes, and the potential risk of transfusion-transmitted HEV infection should be considered.[27]

In conclusion, this is the first study in Spain reporting a high prevalence of IgG anti-HEV antibodies in swine workers. These data support that HEV infection should be treated as a vocational illness in swine workers. Therefore, systematic application of hygiene measures in this group is highly recommended to avoid the exposition to this virus.

Received October 16, 2007. Accepted for publication January 3, 2008.

Financial support: This project was supported by UCH-CEU (PRUCH 06/21), EVES (053/2005), and Generalitat Valenciana (GV05/132).

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医薬品　研究報告・調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	血小板		研究報告の公表状況	Transfusion(United States)Jul2008,48(7)p1368-75.	公表国 米国	
販売名(企業名)	－					

研究報告の概要	使用上の注意記載状況・その他参考事項等
HEV の輸血伝染のリスクは低いと思われるが、これまでに非流行国において 5 例の輸血伝染の HEV 感染が報告されており、原因供血者の HEV 感染経路が知られている例はない。 今回、原因供血者の感染経路が確認された最初の症例である、動物原性食品伝染経路を介して HEV に感染した供血者からの輸血伝染によって引き起こされた急性 E 型肝炎の症例を報告する。 HEV に汚染されていた血小板が、血清中 HEV マーカー陰性の 64 歳の日本人男性非ホジキンリンパ腫患者に輸血された。輸血後 3 週間の肝機能検査は正常であったが、約 22 日後に ALT 値が一過性に 67 IU/L まで上昇し、血清中に HEV が検出され、急性 E 型肝炎と診断された。原因となった供血者は供血の約 1 ヵ月前に親族 12 名と焼肉レストランでブタの肝臓や腸などを食べており、血液サンプル中の HEV マーカーを検査したところ、13 例中 7 例に抗 HEV 抗体が検出された。 これまでの日本における数例の E 型肝炎症例は動物原性食品伝染経路を介して生じたことを示唆しており、最近の研究では HEV は加熱不活性化に対して中等度の耐性を有することが示されている。 抗 HCV 抗体検査開始後は HCV 感染に対して ALT 検査はほとんど貢献しないことから、米国などでは ALT スクリーニングは中止されているが、今回の症例は HEV が存在する血液を排除する上で ALT 検査が貢献することを示唆している(日本赤十字血液センターでは ALT 検査を実施している)。 血液原性の HEV 伝染のリスクを抑制する最も効果的な予防策は、供血の HEV をスクリーニングすること、または病原体の不活性化を実施することである。日本赤十字社は日本人血液供血者における HEV 感染の疫学的研究、および北海道における HEV の NAT スクリーニングの実行可能性試験を計画している。	2004 年の感染例に関する報告であり、北海道赤十字血液センター管内における献血者の HEV 保有状況の調査結果などについては、薬事・食品衛生審議会運営委員会(2006 年 1 月 26 日開催)で報告されている。

報告企業の意見	今後の対応
輸血による HEV 感染に関する情報である。現在まで、血漿分画製剤による伝播の報告はなく、製造工程中には複数のウイルス不活化除去工程を設けているが、今後とも関連情報の収集に努める。	今後とも同様な情報に留意し、関連情報の収集に努めていく。

A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route

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BACKGROUND: Five cases of transfusion transmission of hepatitis E virus (HEV) have been reported so far. The infection routes of the causative donors remain unclear, however. Also, the progress of virus markers in the entire course of HEV infection has not been well documented.

STUDY DESIGN AND METHODS: Nucleic acid testing was performed by real-time reverse transcription–polymerase chain reaction targeting the open reading frame 2 region of HEV. Full-length nucleotide sequences of HEV RNA were detected by direct sequencing.

RESULTS: Lookback study of a HEV-positive donor revealed that the platelets (PLTs) donated from him 2 weeks previously contained HEV RNA and were transfused to a patient. Thirteen relatives including the donor were ascertained to enjoy grilled pork meats together in a barbecue restaurant 23 days before the donation. Thereafter, his father died of fulminant hepatitis E and the other 6 members showed serum markers of HEV infection. In the recipient, HEV was detected in serum on Day 22 and reached the peak of 7.2 log copies per mL on Day 44 followed by the steep increase of alanine aminotransferase. Immunoglobulin G anti-HEV emerged on Day 67; subsequently, hepatitis was resolved. HEV RNA sequences from the donor and recipient were an identical, Japan-indigenous strain of genotype 4. HEV RNA was detectable up to Day 97 in serum, Day 85 in feces, and Day 71 in saliva.

CONCLUSION: A transfusion-transmitted hepatitis E case by blood from a donor infected via the zoonotic food-borne route and the progress of HEV markers in the entire course are demonstrated. Further studies are needed to clarify the epidemiology and the transfusion-related risks for HEV even in industrialized countries.

Hepatitis E virus (HEV) infection has been considered to occur mainly via fecal-oral transmission and is an important public health concern in developing countries.[1] In industrialized countries including Japan, cases have been rarely reported and hepatitis E has been regarded as an imported infectious disease from its endemic areas. Recently, however, increasing numbers of sporadic cases have been reported,[2-11] some of which resulted from infection via a zoonotic food-borne route by consumption of raw or undercooked meats of wild boar, wild deer, or farmed pig that was contaminated with HEV.[8-11]

In 2004, we reported the first molecularly confirmed case of transfusion transmission of HEV.[12] The infection route in the causative donor was not very clear, however. Thereafter, at least four cases of transfusion transmission of HEV have been reported in Japan, the United Kingdom,

ABBREVIATIONS: FAM = 6-carboxyfluorescein; HEV = hepatitis E virus; ORF = open reading frame; PSL = predonisolone; TAMRA = 6-carboxy-tetramethylrhodamine.

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Received for publication September 5, 2007; revision received January 20, 2008, and accepted January 20, 2008.

doi: 10.1111/j.1537-2995.2008.01722.x

TRANSFUSION 2008;48:1368-1375.

and France,[13-16] where hepatitis E is nonendemic and HEV infection routes remained to be obscure.

Here, we report a case of acute hepatitis E caused by transfusion transmission from the donor who was infected with HEV via a zoonotic food-borne manner. To our knowledge, this is the first case in which the infection route of the causative donor has been confirmed. Also, in this report, we describe, for the first time, the virus kinetics and changes of anti-HEV in serum, prospectively monitored from latent period of infection until convalescence, accompanied by disease progression in the patient.

MATERIALS AND METHODS

Detection and quantitation of HEV RNA

For reverse transcription–polymerase chain reaction (RT-PCR) to detect HEV RNA in the samples, the following oligonucleotides were designed to detect 75 nucleotides of highly conserved sequence in the open reading frame (ORF) 2 region of all HEV genotypes: forward primer 5′-CGGCGGTGGTTTCTGG-3′, reverse primer 5′-AAGG GGTTGGTTGGATGAATA-3′, and mixed probes with a 5′-reporter dye (6-carboxyfluorescein, FAM) and a 3′-quencher dye (6-carboxy-tetramethylrhodamine, TAMRA) and FAM-5′-TGACAGGGTTGATTCTCAGCCCTTCG-3′-TAMRA, FAM-5′-TGACCGGGTTGATTCTCAGCCCTTC-3′-TAMRA, and FAM-5′-TGACCGGGCTGATTCTCAGCCC TT-3′-TAMRA (Sigma-Aldrich Japan, Tokyo, Japan). Nucleic acid was extracted from 200 µL of serum and saliva and from 100 µL of 10 percent (wt/vol) fecal suspension in saline with kits (QIAamp MinElute virus spin kit, Qiagen K.K., Tokyo, Japan; and SMITEST R&D-EX, Medical & Biological Laboratories, Nagoya, Japan). Before extraction, the samples were centrifuged at 6000 × *g* at 4°C for 10 minutes; thereafter the clear supernatant was subjected to nucleic acid extraction. Before RT-PCR, RNA preparation of feces was diluted at 10 times with nuclease-free water to reduce the effect of inhibitors. Twenty microliters of nucleic acid sample was used for each reaction. Each 50 µL of reaction mixture contained 25 µL of 2× RT-PCR kit master mix (QuantiTect Probe RT-PCR kit, Qiagen), 0.5 µL of RT mix (QuantiTect Probe RT-PCR kit, Qiagen), 400 nmol per L each of forward and reverse primer, and 67 nmol per L each of three probes. RT-PCR mixture was incubated at 50°C for 30 minutes and at 95°C for 15 minutes, followed by 50 cycles of 94°C for 15 seconds, and 60°C for 1 minute utilizing a thermocycler (Applied Biosystems 7500, real time PCR system, Applied Biosystems, Tokyo, Japan). HEV nucleic acid testing (NAT) was performed individually. The analytical sensitivity of the HEV NAT was determined to be 25 (13-166) copies per mL (with 95% confidence interval) by logistic analysis. HEV viral load was determined from standard curves generated by using 10^1 to 10^7 copies of HEV RNA per reaction. The HEV quantitation standard was generated by transcribing HEV cDNA of HEV ORF2 region that was cloned into a plasmid (pCRII-TOPO, Invitrogen, Carlsbad, CA), using the in vitro transcription kit (MAXIscript T7 high-yield transcription kit, Ambion, Austin, TX). Purified plasmid DNA was linearized with *Hin*dIII restriction endonuclease and transcribed to yield 717-nucleotide-long RNA transcripts containing 75-nucleotide target sequence.

Phylogenetic analysis of HEV isolates

Entire or nearly entire sequences of HEV isolates were determined as previously described by Takahashi and coworkers.[4] The sequences were aligned together with reported HEV strains with a computer program (CLUSTAL W, Version 1.8).[17] A phylogenetic tree based on the nearly entire HEV RNA sequence was constructed by the neighbor-joining method,[18] and the final tree was obtained by a computer program (TreeView, Version 1.6.6).[19] Bootstrap values were determined by resampling 1000 times of the data sets. The nucleotide sequences isolates HRC-HE14C, JST-KitAsa04C, and JTC-Kit-FH04L reported in this study have been assigned DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB291965, AB291966, and AB291959, respectively.

Detection of serum anti-HEV

Samples were tested for immunoglobulin M (IgM)- and immunoglobulin G (IgG)-class antibodies against HEV using a commercial enzyme-linked immunosorbent assay kit (Viragent HEV-Ab, Cosmic Corp., Tokyo, Japan).[5,20]

Alanine aminotransferase testing

Alanine aminotransferase (ALT) testing was carried out using transaminase-HRII Nisseki/GPT (Wako Pure Chemical Industries Ltd, Osaka, Japan) on an automatic analyzer (ACA5400, Olympus Corp., Tokyo, Japan).

RESULTS

A lookback study of a causative blood donor

Blood from a 39-year-old Japanese male on September 20, 2004, was disqualified because of the elevated ALT level at 236 IU per L and tested for hepatitis viruses because of the abnormal ALT result. His blood sample turned out to be positive for the presence of HEV RNA at 4.8 log copies per mL as well as anti-HEV IgM and IgG and negative for the presence of any marker of hepatitis B virus (HBV) or hepatitis C virus (HCV). A lookback study revealed that his donated blood on September 6, 2004, 2 weeks before the last donation, was positive for the presence of HEV RNA at 3.1 log copies per mL and negative for the presence of IgM- or IgG-class anti-HEV. The HEV isolate, HRC-HE14C, was classified as genotype 4 of a Japan-indigenous strain (Fig. 1). The blood (platelet [PLT] concentrate) donated on