

医薬品  
医薬部外品 研究報告 調査報告書  
化粧品

識別番号・報告回数		報告日		第一報入手日 2006年2月13日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①乾燥抗 HBs 人免疫グロブリン ②ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の 公表状況	Epidemiology and Infection, 134(1) 87-93, 2006	公表国 韓国	
販売名 (企業名)	①ヘプスブリン (ベネシス) ②静注用ヘプスブリン-IH (ベネシス)					
研究報告の概要	<p>1998-1999年における、韓国の血友病患者の凝固因子製剤と HAV の感染の因果関係を調査するために、我々は凝固因子製剤と患者について HAV の 1 対 3 症例対照研究と遺伝子学的試験を行った。疫学調査によって、凝固第 VIII 因子製剤の 1 ロットが疫学的に A 型肝炎の患者に、オッズ比が 35.0 又は投与間隔を調整した場合は 38.4 と関係していた。我々は、HAV の RT-PCR によって 7 人の患者に由来する 17 の血清と 124 ロットの凝固因子製剤 (第 VIII 因子及び第 IX 因子) を調べた。HAV が、5 つの凝固因子製剤と 6 人の血清に検出された。凝固第 VIII 因子製剤 1 サンプルの HAV のシーケンスは 3 人の患者の血清から見つかった HAV のシーケンスと一致した。検査と疫学から得られた事実より、凝固因子製剤が 3 人の患者の HAV 感染の原因として、関係していることが示唆された。</p>					<p>使用上の注意記載状況・ その他参考事項等</p> <p>代表として静注用ヘプスブリン-IH の記載を示す。</p> <p>2. 重要な基本的注意 (1)本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査 (NAT) を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗 HBs 抗体を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗 HBs 人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理 (ナノフィルトレーション) を施しているが、投与に際しては、次の点に十分注意すること。</p>
	報告企業の意見				今後の対応	
<p>韓国血友病患者の HAV 感染の感染源として凝固因子製剤が示唆されたとの報告である。万一原料血漿に HAV ウイルスが混入したとしても、EMC をモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。</p>				<p>本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。</p>		

## Detection of hepatitis A virus from clotting factors implicated as a source of HAV infection among haemophilia patients in Korea



Y. M. JEE<sup>1\*†</sup>, U. GO<sup>2†</sup>, D. CHEON<sup>1</sup>, Y. KANG<sup>1</sup>, J.-D. YOON<sup>1</sup>, S.-W. LEE<sup>2</sup>, Y. H. SHIN<sup>2</sup>,  
K.-S. KIM<sup>2</sup>, J.-K. LEE<sup>2</sup>, E.-K. JEONG<sup>3</sup>, B.-K. YANG<sup>2</sup> AND H. W. CHO<sup>4</sup>

<sup>1</sup> Division of Enteric and Hepatitis Viruses, Department of Virology, National Institute of Health, Seoul, Korea

<sup>2</sup> Department of Infectious Disease Control, Center for Disease Control and Prevention, Seoul, Korea

<sup>3</sup> Department of Disease Investigation and Surveillance, Center for Disease Control and Prevention, Seoul, Korea

<sup>4</sup> National Institute of Health, Seoul, Korea

(Accepted 5 April 2005, first published online 22 July 2005)

### SUMMARY

To investigate the causal relationship of blood clotting factors and hepatitis A virus (HAV) infection in haemophilia patients during 1998–1999 in Korea, we performed a 1:3 matched case-control study and molecular detection of HAV from clotting factors and patients. The epidemiological investigation showed that one lot of clotting factor VIII was related epidemiologically to patients with hepatitis A with an odds ratio of 35.0, or 38.4 when adjusted for the interval between injections. We examined 17 sera collected from seven patients and 124 lots of blood clotting factors (factor VIII and factor IV) by HAV reverse transcriptase–polymerase chain reaction (RT–PCR). HAV RNA was detected in five clotting factors and six sera. The HAV sequence of one of the factor VIII samples was identical to the sequences found in three patients' sera. Findings from the laboratory and epidemiological studies suggested that the clotting factor was causally related to HAV infection in three haemophilia patients.

### INTRODUCTION

Hepatitis A virus (HAV), a member of the genus Hepatovirus, family Picornaviridae, is a non-enveloped virus resistant to solvent/detergent treatment. HAV is transmitted generally by the faecal–oral route and could be either foodborne or waterborne. However, on rare occasions it can be transmitted parentally through viraemic blood during the incubation period. Outbreaks of HAV infection among haemophilia patients have been reported in Germany, Belgium, Australia and Italy [1–5].

From October 1998 to March 1999, 57 (4.2%) patients among 1370 registered haemophiliacs were diagnosed with acute hepatitis A. Clotting factors administered to patients were implicated as a source of infection. Detection of HAV in clotting factors can be carried out by molecular methods as HAV does not grow easily in cell culture. Reverse transcription–polymerase chain reaction (RT–PCR) was carried out followed by sequencing to detect HAV in clotting factors and samples from HAV-infected haemophiliacs. There have been reports of clustering of HAV infection detected by molecular methods in haemophiliacs [1–5]. Therefore, a matched case-control study was carried out in haemophilia patients to investigate the causal relationship between the hepatitis A outbreak and administration of clotting factors.

\* Author for correspondence: Y. M. Jee, M.D., Ph.D., Division of Enteric and Hepatitis Viruses, Department of Virology, National Institute of Health, 5 Nokbun-dong, Eunpyung-gu, Seoul, Korea.  
(Email: ymeejee@nih.go.kr)

† These authors contributed equally to this paper.

## MATERIALS AND METHODS

### Epidemiological investigations

The incidence of hepatitis A was investigated during October 1998 and March 1999. The hospital charts of HAV IgM-positive patients among 1370 haemophiliac patients registered by the Korea Haemophilia Foundation were reviewed. A 1:3 matched case-control study of the patients diagnosed with hepatitis A by HAV IgM test from October 1998 to February 1999 with a record of clotting factor prescriptions was carried out. Of 57 patients with acute HAV, 33 patients whose prescription records were available and received clotting factors were included in this study. An age-matched control group was selected from the registered haemophilia patients who were not diagnosed as having HAV infection by serological tests after October 1998 but who received clotting factors during this period (Table 1).

The lots of clotting factors that were prescribed 10–60 days before the hepatitis A infection onset were investigated. Telephone enquiries were carried out to assess other risk factors such as eating uncooked shellfish, travel history, living in closed settings, drinking untreated water and contact with patients with jaundice. Odds ratios (OR) and 95% confidence intervals (CI) for injecting clotting factors and other risk factors were calculated.

### Virological investigation

Seventeen sera were tested for the presence of HAV from seven haemophiliac patients and 124 lots of blood clotting factors (both factor VIII and factor IX) which were used during 1998. To prevent any cross-contamination, patients' sera and blood clotting factors were processed separately. Slot and Southern blot hybridization were used to confirm the presence of the HAV genome and the amplified PCR fragments were sequenced.

Total RNA was extracted using Tri-reagent (Molecular Research Centre Inc., Cincinnati, OH, USA) as described in the manufacturer's instructions (Product cat. no. TR-118) from 500  $\mu$ l of the clotting factors and from 50–100  $\mu$ l of the patients' sera, followed by isopropanol precipitation at  $-20^{\circ}\text{C}$  overnight. Random primers were used for reverse transcription of extracted RNA which was performed at  $37^{\circ}\text{C}$  for 60 min. The enzyme was subsequently inactivated at  $95^{\circ}\text{C}$  for 5 min. For PCR testing, a 247-bp fragment of the VP3/VP1 region of the HAV

Table 1. Age distribution of hepatitis A infections in the general population compared with the haemophiliac group

Age group (years)	General population	Haemophilia group
1–4	3	2
5–9	12	4
10–14	13	8
15–19	14	7
20–24	9	6
25–29	5	4
30–34	1	—
35–39	—	—
40–44	—	—
45–49	—	—
50–54	—	—
55–59	—	—
60–64	—	—
> 65	—	—
Undefined	—	—
Total	57	31

genome spanning nucleotides 2167–2413 was amplified [6–8] by cycling at  $94^{\circ}\text{C}$  for 1 min,  $55^{\circ}\text{C}$  for 1 min and  $72^{\circ}\text{C}$  for 1 min, repeated 35 times and followed by a 7-min extension at  $72^{\circ}\text{C}$ .

Slot and Southern blot hybridization were performed using a digoxigenin-labelled oligonucleotide probe corresponding to the internal sequence of the VP1 region of HAV (nucleotides 2224–2243). A total of 124 lots of blood clotting factors were tested to confirm the specificity of the PCR amplification and to detect positive samples.

Amplified PCR products were cloned using the TOPO TA cloning kit (Invitrogen Life Technologies, Carlsbad, CA, USA) and sequenced on the ABI Prism 377 automatic sequencer Long Ranger gel. The sequencing reaction was performed in a DNA Thermal Cycler 480 (PerkinElmer, Applied Biosystems, Foster City, CA, USA) by running 25 cycles at  $96^{\circ}\text{C}$  for 30 s,  $50^{\circ}\text{C}$  for 15 s and  $69^{\circ}\text{C}$  for 4 min. The sequences of the PCR products were analysed and compared with other isolates. Nucleotide sequences were analysed using the MegAlign program of Lasergene, DNASTar Inc., Madison, WI, USA. We used the Clustal method to group sequences into clusters by examining the distance between all pairs. The relationship of the sequences is shown in a dendrogram (see Fig.) The length of each pair of branches represents the distance between sequences. Units indicate the numbers of substitution events.

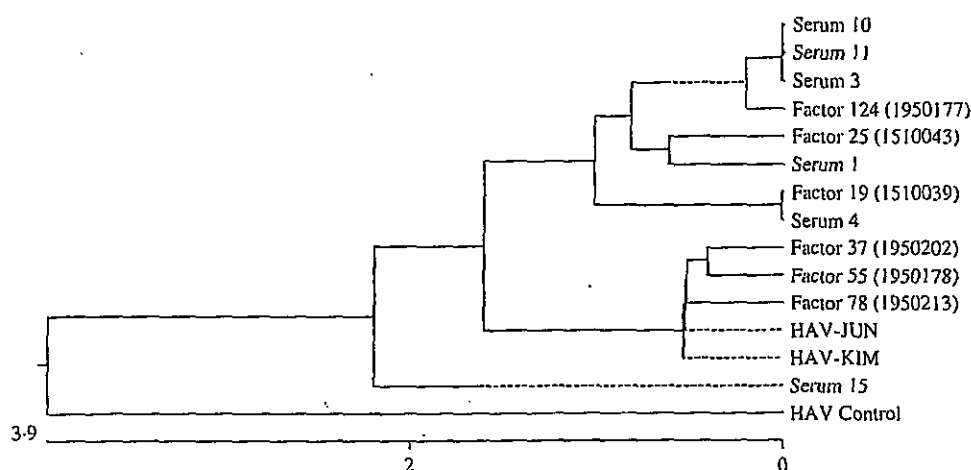


Fig. Nucleotide sequences of the HAV strains detected from clotting factors and patients. The phylogenetic analysis of HAV sequences detected from haemophiliac patients and blood products shows that the sequences of HAV from patient nos. 2, 4, 5 and one blood product (factor VIII, lot no. 1950177) are identical, as are HAV sequences from patient no. 6 and another product (factor IX, lot no. 1510039). The HAV sequence from patient no. 1 is closely related to that from factor IX (lot no. 1510043). MegAlign of the DNASTar aligns multiple sequences by drawing a histogram of consensus strength at the top of each alignment panel using one of two algorithms: the Jotun Hein method or the Clustal method. We used the Clustal method to group sequences into clusters by examining the distance between all pairs. The relationship of the sequences was shown in dendrograms. The length of each pair of branches represents the distance between sequences.

## RESULTS

### Epidemiological investigations

The incidence of hepatitis A among registered haemophiliac patients was the highest in the 15–19 years age group, followed by the 10–14 and 5–9 years age groups. Examination of regional incidence showed that haemophilia patients in Daejeon had the highest attack rate, although patients were found all over the country except Jeju. Table 2 shows the hepatitis A attack rates among haemophiliacs and non-haemophiliacs by region and Table 3 shows the age distribution.

The hepatitis A incidence rates were 4.5, 3.2 and 0% among patients with haemophilia type A ( $n=1122$ ), type B ( $n=189$ ), and other clotting factor disorders ( $n=59$ ) respectively. Cases of hepatitis A among haemophilia patients were first identified in the first week of October 1998 and peaked in mid October to the final week of October 1998. Another peak (six patients) was detected in December 1998 to January 1999, and four of these patients had type B haemophilia.

The main clinical symptoms of the 50 haemophilia patients whose records were available for review were right upper quadrant abdominal pain, jaundice, fever and gastrointestinal symptoms such as loss of appetite, nausea and vomiting. Their liver function test results also showed high values for serum glutamic-oxaloacetic transaminase (SGOT), serum

glutamic-pyruvic transaminase (SGPT), alkaline phosphatase, total bilirubin and direct bilirubin.

From the analysis of risk factors, we have found that one lot (lot no. 1950177) of clotting factor VIII was related significantly to the outbreak of hepatitis A among haemophilia A patients (OR 35, 95% CI 11.3–108.9). An interval of fewer than 10 days between injections was related to a higher incidence of hepatitis A when compared with an interval of more than 10 days (OR 4.2, 95% CI 1.1–15.8). Other factors such as consumption of raw shellfish or other seafood within 2 months of symptom onset, travel or living in a group were not related to a higher incidence of hepatitis A (Table 4). The OR of one lot (lot no. 1950177) of clotting factor VIII was 38.4 (95% CI 8.8–168.2) when the effect of the interval between injections was adjusted.

The incidence of hepatitis A and relative risks of the groups who did or did not receive clotting factors from the injection records of 437 individuals was evaluated. A significantly high level of relative risk (46.9,  $P<0.01$ ) was found in one lot (lot no. 1950177) (Table 5). Statistical analysis was not possible as data were available for only two patients who were injected with factor IX. Although they were injected with lot nos. 1510037, 1510038 and 1510039, the factor IX product of lot no. 1510039 showed the most significant difference in exposure ratios between the case group and the control group.

Table 2. Comparison of attack rates of hepatitis A among haemophiliac and non-haemophiliac patients by region

Region	Haemophilia patients			Hepatitis A attack rate among haemophilia patients (1/100 000)	General population Attack rates among males in the general population (/100 000)*
	Enrolled haemophilia patients	Hepatitis A patients among haemophilia patients	Hepatitis A attack rate among haemophilia patients (%)		
Seoul	326	14	4.3	4294	5.4
Busan	82	4	4.9	4878	0.4
Incheon	60	2	3.3	3333	8.1
Daejeon	43	3	7.0	6977	7.1
Daegu	64	2	3.1	3125	1.0
Gwangju	45	2	4.4	4444	1.0
Gyeonggi-do	253	10	4.0	3953	4.6
Gangwon-do	47	2	4.3	4255	2.3
Chungcheongnam-do	47	2	4.3	4255	2.6
Chungcheongbuk-do	49	1	2.0	2041	1.6
Gyollanam-do	60	1	1.7	1667	0.2
Gyollabuk-do	71	3	4.2	5263	1.3
Gyeongsangnam-do	105	6	5.7	5714	0.0
Gyeongsangbuk-do	95	5	5.2	5263	0.3
Jeju-do	12	0	0.0	0	—
Total	1370	57	4.2		

\* Attack rates among males in the general population (%): July 1996 to June 1998.

Table 3. Comparison of attack rates of hepatitis A among haemophiliac and non-haemophiliacs by age group

Age group (years)	Haemophilia patients			Hepatitis A attack rate among haemophilia patients (1/100 000)	General population Attack rates among males in the general population (/100 000)*
	Enrolled haemophilia patients	Hepatitis A patients among haemophilia patients	Hepatitis A attack rate among haemophilia patients (%)		
1-4	100	3	3.0	3000	0.4
5-9	222	12	5.4	5405	1.5
10-14	189	13	6.9	6878	4.6
15-19	193	14	7.3	7254	6.1
20-24	180	9	5.0	5000	8.0
25-29	144	5	3.5	3472	7.5
30-34	131	1	0.8	763	5.2
35-39	70	—	—	—	2.2
40-44	60	—	—	—	0.6
45-49	32	—	—	—	0.2
50-54	12	—	—	—	0.3
55-59	16	—	—	—	0.1
60-64	4	—	—	—	0.3
> 65	9	—	—	—	0.2
Undefined	8	—	—	—	—
Total	1370	57	4.2		

\* Attack rates among males in the general population (%): July 1996 to June 1998.

Table 4. Odds ratio (OR) and 95% confidence intervals (CI) of products among haemophilia A patients

Variables	Exposure rate among cases (n=31)	Exposure rate among controls (n=92)	OR	95% CI*
<b>Factor VIII lot no.</b>				
1950172	3.2%	1.1%	3.0	0.2-50.0
1950173	6.5%	3.3%	2.0	0.3-12.9
1950174	6.5%	6.5%	1.0	0.2-5.2
1950175	9.7%	9.8%	1.0	0.3-3.9
1950176	3.2%	12.0%	0.2	0.0-2.0
1950177	71.0%	6.5%	35.0	11.3-108.9
1950178	16.1%	14.1%	1.2	0.4-3.6
1950179	25.8%	18.5%	1.5	0.6-4.0
1950180	35.5%	22.8%	1.9	0.8-4.5
1950181	29.0%	20.0%	1.7	0.7-4.3
1950182	29.0%	17.4%	1.9	0.8-5.0
1950183	6.5%	4.4%	1.5	0.3-8.7
1950184	9.7%	10.9%	0.9	0.2-3.4
1950185	3.2%	3.3%	1.0	0.1-1.0
<b>Other risk factors</b>				
Injection interval period of <10 days	85.7%	58.7%	4.2	1.1-15.8
Drinking unboiled tap water at home	29.2%	38.8%	0.6	0.2-1.8
Drinking unboiled tap water at school	55.0%	60.0%	0.8	0.3-2.4
Eating raw shellfish and seafood	5.3%	17.9%	0.3	0.0-2.1
Travel history to endemic region	17.4%	28.4%	0.5	0.2-1.8
Past history of living in groups	12.5%	9.0%	1.5	0.3-6.3
History of using common kitchen	4.4%	9.1%	0.5	0.1-4.1

#### Virological examinations

Among the 17 sera from seven HAV IgM-positive patients and 124 blood clotting factors (factor VIII and factor IX), HAV RNA was detected in five clotting factors and 11 HAV-positive sera (Tables 6 and 7). HAV RNA was detected in six out of seven patients tested. Five of these six patients and six clotting factors were positive by slot and Southern blot hybridization (Table 6). The analysis of the HAV sequences found in the clotting factors and sera showed that the HAV detected in one of the factors (factor VIII, lot no. 1950177) had 100% sequence homology with HAV found in three patients' sera (Fig.). This result coincides with the results of the epidemiological analysis in which it was found that those three patients were injected with lot no.

1950177 and this factor was highly related to the HAV infection in haemophilia patients with the high OR (Table 4).

#### Phylogenetic analysis

The phylogenetic analysis of HAV sequences detected from haemophiliac patients and blood products showed that the HAV sequences from sera 3 (patient no. 4), 10 (patient no. 5), 11 (patient no. 2) and one blood product (factor VIII, lot no. 1950177) were closely related to each other, as were HAV sequences from serum 4 (patient no. 6) and another product (factor IX, lot no. 1510039) (Fig.). The HAV sequence from patient no. 1 was closely related to that of factor IX (lot no. 1510043). However, the HAV sequence

Table 5. *Relative risks of factor VIII among haemophilia patients*

Factor VIII lot no.	Attack rate among non-exposed patients	Attack rate among exposed patients	Relative Risks
1950172	1.8	6.4	3.5**
1950173	3.2	0.0	0.0
1950174	3.0	2.9	1.0
1950175	2.7	5.1	1.9
1950176	3.3	0.0	0.0
1950177	0.5	23.9	46.9***
1950178	2.8	5.4	2.0
1950179	3.3	1.3	0.4
1950180	3.1	2.4	0.8
1950181	2.1	7.8	3.6**
1950182	2.0	6.6	3.3**
1950183	3.3	0.0	0.0
1950184	2.3	5.7	2.5
1950185	2.4	4.8	2.0

\*\*  $P < 0.05$ , \*\*\*  $P < 0.001$ .

Table 6. *Detection of HAV genome from clotting factors*

Sample no.	Lot no.	HAV RT-PCR	Slot/Southern blot hybridization
19	1510039 (factor IX)	+	+
25	1510043 (factor IX)	-	+
37	1950177 (factor VIII)	+	+
55	1950178 (factor VIII)	+	+
78	1950202 (factor VIII)	+	+
124	1950213 (factor VIII)	+	+

from patient no. 3 did not match sequences from any blood products we examined. Sequences from two 1998 Korean isolates (HAV-JUN, HAV-KIM) and a HAV control (HM 175 strain) used as positive controls were not closely related to sequences from patients, although three lots of factors (lot nos. 1950202, 1950178, 1950213) were relatively related closely to two 1998 Korean isolates (HAV-JUN, HAV-KIM).

## DISCUSSION

An outbreak of hepatitis A infection was investigated in a group of haemophiliacs in Korea in 1998. A peak in the incidence of hepatitis A was detected from May to June 1998, and the incidence decreased gradually

Table 7. *Detection of HAV genome from patients*

Patients	No. of tested sera	Serial nos. of sera	Number of HAV RT-PCR positive sera	Serial nos. of positive sera
1	1	1	1	1
2	2	6, 11	2	6, 11
3	4	2, 7, 13, 15	2	2, 15
4	3	3, 12, 14	1	3
5	4	5, 8, 9, 10	4	5, 8, 9, 10
6	1	4	1	4
7	2	16, 17	0	
Total no.	17		11	

during the latter half of the year. By comparing the incidence of hepatitis A among the general population and haemophilia patients, a significant increase in hepatitis A incidence was detected among the haemophilia patients. The epidemiological study also implicated factor VIII (lot no. 1950177) as a causal agent of this outbreak. Another product (factor IX, lot no. 1510039) was only used in two haemophilia patients, therefore, it was not possible to analyse the relative risk of this lot.

The HAV sequences detected from patient nos. 2, 4, 5 and lot no. 1950177 of factor VIII were identical and closely related to sequences of HAV isolates in the United States. The plasma pool of lot no. 1950177 was imported from the United States. In addition, the HAV sequences from serum 4 (patient no. 6) and clotting factor IX (lot no. 1510039) were identical. Clotting factors used in Korea are produced by the solvent/detergent method to inactivate bloodborne viruses and it has been reported that HAV can survive this procedure [9].

Although there were reports of HAV strains detected in Korea during 1994–2000, the sequences of these strains could not be compared with the strains from haemophilia patients and from clotting factors because of differences in the amplified regions [10–12]. Comparison of nucleotide sequences from the Korean strains with foreign strains revealed that there were more nucleotide substitutions among the foreign strains.

A causal relationship was found between the injection of blood clotting factors and an outbreak of hepatitis A among haemophilia patients. During 1991–1993, similar cases were reported from other countries including Italy, Germany, Ireland and Belgium where the solvent/detergent method was used

to inactivate bloodborne viruses. We recommend that all haemophilia patients in Korea should be vaccinated against HAV following this outbreak.

## REFERENCES

1. Mannuccio P, Gdovin S, Gringeri A, et al. and the Italian Collaborative Group. Transmission of Hepatitis A to patients with haemophilia by factor VIII concentrates treated with organic solvent and detergent to inactivate viruses. *Ann Intern Med* 1994; 120: 1-7.
2. Chudy M, Budek J, Keller-Stanislawski B, et al. A new cluster of Hepatitis A infection in haemophiliacs traced to a contaminated plasma pool. *J Med Virol* 1999; 57: 91-99.
3. Soucie JM, Robertson BH, McCaustland KA, Evatt BL. Hepatitis A virus infections associated with clotting factor concentrate in the United States. *Transfusion* 1998; 38: 573-579.
4. Robertson BH, Alter MJ, Bell BP, et al. Hepatitis A virus sequence detected in clotting factor concentrates associated with disease transmission. *Biologicals* 1998; 26: 95-99.
5. Robertson BH, Friedberg D, Normann A, Graff J, Flehming B, Shouval D. Sequence variability of Hepatitis A virus and factor VIII associated Hepatitis A infections in haemophilia patients in Europe. An update. *Vox Sang* 1994; 67 (Suppl 1): 39-45.
6. Paul AV, Tada H, von der Helm K, et al. The entire nucleotide sequence of the genome of human Hepatitis A virus (isolate MBB). *Virus Res* 1987; 8: 153-171.
7. Aulaire-Marchais V, Robertson BH, Aubineay-Ferre V, et al. Direct sequencing of Hepatitis A virus strain isolated during an epidemic in France. *Appl Environ Microbiol* 1995; 61: 3977-3980.
8. Robertson BH, Khanna B. Epidemiologic patterns of wild type Hepatitis A virus determined by genetic variation. *J Infect Dis* 1991; 163: 286-292.
9. Hollinger FB, Emerson SU. Hepatitis A virus. *Fields virology*. Philadelphia, USA: Lippincott Williams and Wilkins, 2001, chapter 26: 799-840.
10. Kim JS, Roh SC, Jeoung C, Kim SH. Detection of Hepatitis A virus by reverse transcription-polymerase chain reaction and sequence analysis of an outbreak of Hepatitis A. *J Korean Mil Med Assoc* 2001; 32: 112-119.
11. Byun KS, Kim JH, Song KJ, et al. Molecular studies: Hepatitis A virus and hepatectomy. *J Gastroenterol Hepatol* 2001; 16: 519-524.
12. Kwon OS, Byun KS, Yeon JE, et al. Detection of Hepatitis A viral RNA in sera of patients with acute Hepatitis A. *J Gastroenterol Hepatol* 2000; 15: 1043-1047.



## 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 2006. 2. 21	新医薬品等の区分 該当なし	機構処理欄
一般的名称	新鮮凍結人血漿	研究報告の公表状況	Sadler GJ, Mells GF, Shah NH, Chesner IM, Walt RP. J Med Virol. 2006 Feb 15;78(4):473-475.	公表国	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社)			英国	
研究報告の概要	<p>○英国にE型肝炎 英国国内で感染したE型肝炎の症例が8例報告された。これらの症例はバーミンガム市内の病院で、2005年に5ヶ月を越える期間にわたって発見された。E型肝炎は英国ではまれな疾患と考えられており、通常は流行地への渡航後に発症する。これまでに文献で報告されたE型肝炎の英国国内感染症例は5例しかない。この一連の報告はE型肝炎の国内での発生増加を示している。急性肝炎の患者すべてに、渡航歴とは無関係にHEVの可能性を考慮する必要がある。</p>				使用上の注意記載状況・ その他参考事項等
					新鮮凍結血漿「日赤」  血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク
報告企業の意見		今後の対応			
英国国内で感染したE型肝炎の症例が、バーミンガム市内の病院で8例発見されたとの報告である。		日本赤十字社では、厚生労働省科学研究「本邦に於けるE型肝炎の診断・予防・疫学に関する研究班」と共同して、献血者におけるHEV感染の疫学調査を行っている。北海道における輸血HEV感染報告を受け、試験的に北海道では生肉の摂取の有無について問診の有用性を検討し研究的NATを行うなど安全対策を実施している。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。			

2

## UK Acquired Hepatitis E—An Emerging Problem?

G.J. Sadler, G.F.G. Mells, N.H. Shah, I.M. Chesner, and Robert P. Walt\*

Department of Gastroenterology, The Heart of England Foundation Trust, Birmingham Heartlands Hospital, Bordesley Green East, Birmingham, United Kingdom

Eight cases of hepatitis E acquired in the UK are reported. These cases presented to an inner city hospital in Birmingham, UK, over a 5-month period in 2005. HEV is considered unusual in the UK and generally occurs after travel to endemic regions. Only five cases of hepatitis E acquired in the UK have been reported in the literature. This series represents an increase in the local incidence of hepatitis E, particularly that of UK-acquired infection. HEV should be considered in all patients with acute hepatitis, irrespective of travel history. *J. Med. Virol.* 78:473–475, 2006. © 2006 Wiley-Liss, Inc.

**KEY WORDS:** acute hepatitis; jaundice; virus

ered rare in industrialized countries. Eight patients (cases A–H, Table I) presented to the jaundice clinic of a UK inner city hospital over a 5-month period in 2005 and were found to have hepatitis E. All cases had clinical and biochemical features consistent with acute hepatitis. None had any past history of liver disease. None had travelled abroad to an area where HEV is considered endemic. One patient had recently been in contact with a jaundiced individual (case C), one admitted to daily consumption of pork pies (case F), and another to frequent consumption of shellfish (case G). In all cases, serology for HAV, HBV, HCV, and autoimmune (ANA, SMA, and LKM antibodies) hepatitis was negative. Hepatitis could not be attributed to medications. Abdominal ultrasound revealed no focal hepatic abnormality or biliary tract dilatation. All patients have been reviewed at 6 weeks follow-up, and have recovered fully.

### INTRODUCTION

HEV is a common cause of sporadic and epidemic acute hepatitis in the developing world, but is consid-

### RESULTS

TABLE I. Serum Values and HEV Serology for Patients A–H.

	A	B	C	D	E	F	G <sup>a</sup>	H
Age	60	50	36	78	75	61	52	67
Sex	M	F	F	M	M	M	F	F
Race	Caucasian	Caucasian	Asian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
AST (IU/L)	1457	3961	1947	1540	883	2791	1456	1072
ALP (IU/L)	395	307	228	656	230	375	165	305
Bil (μmol/l)	189	248	469	427	75	48	199	381
Alb (g/L)	37	33	36	32	47	43	40	32
ENR	NA	1.2	1.6	NA	NA	NA	NA	1.5
HEV IgM	+	+	+	+	+	+	Equivocal	–
HEV IgG	+	+	+	+	+	Equivocal	+	+
HEV RNA	Not detected	Genotype 3	Genotype 1	Not detected	Not detected	Not detected	Not detected	Not detected
AST at follow up	39	24	17	31	26	44	21	31

NA, not available.

<sup>a</sup>HEV serology only requested on serum taken at 6-week follow-up.

Abbreviations used: HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; ANA, anti-nuclear antibody; SMA, smooth muscle antibody; LKM, liver-kidney-microsomal antibody; AST, aspartate aminotransferase; AlkP, alkaline phosphatase; PCR, polymerase chain reaction.

\*Correspondence to: Robert P. Walt, Department of Gastroenterology, The Heart of England Foundation Trust, Birmingham Heartlands Hospital, Bordesley Green East, Birmingham B9 5SS, UK. E-mail: bob.walt@uhb.nhs.uk

Accepted 27 October 2005

DOI 10.1002/jmv.20564

Published online in Wiley InterScience  
(www.interscience.wiley.com)

## DISCUSSION

HEV is a non-enveloped, single strand RNA virus, which causes an acute hepatitis similar to Hepatitis A. Infection caused by HEV is more severe however, with a mortality of 0.5%–3%, increasing to approximately 20% in pregnant women [Khuroo et al., 1981; Hussaini et al., 1997]. Only one serotype is recognized, but at least four different genotypes have been identified on the basis of sequence diversity in the viral genome-genotype 1 includes Asia-Africa strains, genotype 2 Mexico, genotype 3 United States and genotype 4 strains from Japan, China, and Taiwan (World Health Organisation website).

HEV is endemic in the developing world, where it causes epidemics of viral hepatitis and accounts for a significant proportion of sporadic cases [Aggarwal and Naik, 1997]. Whilst HEV is considered rare in the developed world, sporadic cases do occur, almost exclusively in travellers returning from endemic areas. However, autochthonous infection has been reported [Tassopoulos et al., 1994; Zaaijer et al., 1995; Kwo et al., 1997; Zanetti et al., 1999; McCrudden et al., 2000; Pina et al., 2000]. In the UK, 311 cases were reported to the CDSA between 1993 and 2002, of which only two were definitely acquired in the UK (Health Protection Agency website).

HEV is transmitted enterically, and outbreaks in the developing world have been linked to massive faecal contamination of water supplies [Naik et al., 1992; Corwin et al., 1999]. Person-to-person spread is rare [Aggarwal and Naik, 1994; Arankalle et al., 2000], possibly because of low faecal excretion of the virus [Nanda et al., 1995] and the need for a large oral dose to cause infection [Skidmore, 1999].

Consumption of contaminated water is unlikely in areas of good sanitation and, if contagion is negligible, this suggests an alternative source of infection.

There is considerable evidence that HEV is a zoonosis. HEV has been isolated from pigs [Meng et al., 1997] and other animals, with high sequence similarity between geographically related porcine and human strains [Banks et al., 2004a; Nishizawa et al., 2003].

Experimental cross-species infection of primates with porcine HEV has been demonstrated [Meng et al., 1998]. Anti-HEV seroprevalence is higher in those who work with swine [Withers et al., 2002]. Furthermore, in regions of Japan, 9 of 10 cases of HEV occur within 2 months of consuming grilled or undercooked pig liver [Yazaki et al., 2003]. More direct evidence comes from a report of several cases of HEV occurring after consumption of uncooked deer meat, in which HEV RNA from patients was identical to that isolated from meat which had been saved [Tei et al., 2003]. Significantly, in the UK up to 85.5% of pigs are anti-HEV positive [Banks et al., 2004b].

Diagnosis of hepatitis E is based on specific antibody. IgM indicates recent infection, but rapidly disappears. However, IgG remains detectable for 2–13 years [Panda and Jameel, 1997]. Progressive reduction in IgG

reactivity and eventual positive to negative seroconversion has been demonstrated [Goldsmith et al., 1992], which may permit re-infection.

Eight cases of hepatitis E are reported. In patients A–F, HEV IgM confirms recent hepatitis E. Patient G was HEV IgM equivocal and patient H IgM negative. However, HEV serology was tested following clinical recovery, HEV IgG was positive, and no alternative cause for acute hepatitis was identified. These patients are therefore most likely to also have suffered hepatitis E.

In these cases, absence of travel to an endemic region suggests UK-acquired infection. None of these patients had any contact with pigs or admitted to the consumption of undercooked meat, but frequent consumption of pork pies or shellfish is a potential source of infection in two patients.

Patient C was found to have a genotype 1 infection. She had been in contact with an individual who had developed jaundice after travel to Pakistan and who was found to be HEV IgG positive, although HEV IgM and RNA negative. Given that genotype 1 strains are localized to Asia and Africa, patient C is likely to have contracted HEV through person-to-person transmission of imported HEV. This has not been reported in the literature.

It is concluded that these cases represent a dramatic increase in the local incidence of UK-acquired HEV infection. This may be occurring on a more national scale. Heightened awareness of this important type of acute viral hepatitis is important. HEV should be sought in all cases of acute hepatitis in the UK, regardless of travel history, once more common causes have been excluded.

## ACKNOWLEDGMENTS

We acknowledge Dr. E Boxall and her colleagues at the Birmingham Public Health Laboratory for all their help and guidance.

## REFERENCES

- Aggarwal R, Naik SR. 1994. Hepatitis E: Intrafamilial transmission versus waterborne spread. *J Hepatol* 21:718–723.
- Aggarwal R, Naik SR. 1997. Epidemiology of hepatitis E past, present and future. *Trop Gastroenterol* 18:49–56.
- Arankalle VA, Chadha MS, Mehendale SM, Tungatkar SP. 2000. Epidemic hepatitis E: Serological evidence for lack of intrafamilial spread. *Indian J Gastroenterol* 19:24–28.
- Banks M, Bendall R, Grierson S, Heath G, Mitchell J, Dalton H. 2004a. Human and porcine hepatitis E virus strains, United Kingdom. *Emerg Infect Dis* 10:953–955.
- Banks M, Heath GS, Grierson SS, King DP, Gresham A, Girones R, Widen F, Harrison TJ. 2004b. Evidence for the presence of hepatitis E virus in pigs in the United Kingdom. *Vet Rec* 154: 223–227.
- Corwin AL, Tien NT, Bounlu K, Winarno J, Putri MP, Laras K, Larasati RP, Sukri N, Endy T, Sulaiman HA, Hyams KC. 1999. The unique riverine ecology of hepatitis E virus transmission in South-East Asia. *Trans R Soc Trop Med Hyg* 93:255–260.
- Goldsmith R, Yarbrough PO, Reyes GR, Fry KE, Gabor KA, Kamel M, Zakaria S, Amer S, Gaffar Y. 1992. Enzyme-linked immunosorbent assay for diagnosis of acute sporadic hepatitis E in Egyptian children. *Lancet* 339:328–331.

- Health Protection Agency website. [http://www.hpa.org.uk/infections/topics\\_az/travel/pdf/food\\_waterborne.pdf](http://www.hpa.org.uk/infections/topics_az/travel/pdf/food_waterborne.pdf) (Pages 21-22).
- Hussaini SH, Skidmore SJ, Richardson P, Sherratt LM, Cooper BT, O'Grady JG. Severe hepatitis E infection during pregnancy. 1997. *J Viral Hepatol* 4:51-54.
- Khuroo MS, Teli MR, Skidmore S, Sofi MA, Khuroo MI. 1981. Incidence and severity of viral hepatitis in pregnancy. *Am J Med* 70:252-255.
- Kwo PY, Schisuder GG, Carpenter HA, Murphy PJ, Rosenblatt JE, Dawson GJ, Mast EE, Krawczynski K, Balan V. 1997. Acute Hepatitis E by a new isolate acquired in the United States. *Mayo Clin Proc* 72:1133-1136.
- McCrudden R, O'Connell S, Farrant T, Beaton S, Iredale JP, Fine D. 2000. Sporadic acute hepatitis E in the United Kingdom: An underdiagnosed phenomenon? *Gut* 46:732-733.
- Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ, Emerson SU. 1997. A novel virus in swine that is closely related to the human hepatitis E virus. *Proc Natl Acad Sci* 94:9860-9865.
- Meng XJ, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Purcell RH, Emerson SU. 1998. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *J Virol* 72:9714-9721.
- Naik SR, Aggarwal R, Salunke PN, Mehrotra NN. 1992. A large waterborne viral hepatitis E epidemic in Kanpur, India. *Bull World Health Organ* 70:597-604.
- Nanda SK, Ansari IH, Acharya SK, Jameel S, Panda SK. 1995. Protracted viraemia during acute hepatitis E virus infection. *Gastroenterology* 108:225-230.
- Nishizawa T, Takahashi M, Mizuo H, Miyajima H, Gotanda Y, Okamoto H. 2003. Characterization of Japanese swine and human hepatitis E virus isolates of genotype IV with 99% identity over the entire genome. *J Gen Virol* 84:1245-1251.
- Panda SK, Jameel S. 1997. Hepatitis E virus: From epidemiology to molecular biology. *Viral Hepat Rev* 3:227-251.
- Pina S, Buti M, Cotrina M, Piella J, Girones R. 2000. HEV identified in serum from humans with acute hepatitis and sewage of animal origin in Spain. *J Hepatol* 33:842-845.
- Skidmore SJ. 1999. Factors in the spread of hepatitis E. *Lancet* 354:1049-1051.
- Tassopoulos NC, Krawczynski K, Hatzakis A, Katsoulidou A, Delladetsima I, Koutelou MG, Trichopoulos D. 1994. Case report: Role of hepatitis E virus in the etiology of community-acquired non-A, non-B hepatitis in Greece. *J Med Virol* 42:124-128.
- Tei S, Kitajima N, Takahashi K, Mishiro S. 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet* 362:371-379.
- Withers MR, Correa MT, Morrow M, Stebbins ME, Seriwatana J, Webster WD, Boak MB, Vaughn DW. 2002. Antibody levels to the hepatitis E virus in North Carolina swine workers, non-swine workers, swine, and murids. *Am J Trop Med Hyg* 66:384-388.
- World Health Organisation (WHO) website. <http://www.who.int/csr/disease/hepatitis/whocdscsredc200112/en/index.html>.
- Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, Okamoto H. 2003. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *J Gen Virol* 84:2351-2357.
- Zaajer HL, Mauser-Bunschoten EP, ten Veen JH, Kapprell HP, Kok M, van den Berg HM, Lelie PN. 1995. Hepatitis E virus antibodies among patients with hemophilia, blood donors, and hepatitis patients. *J Med Virol* 46:244-246.
- Zanetti AR, Schlauder GG, Romano L, Tanzi E, Fabris P, Dawson GJ, Mushahwar IK. 1999. Identification of a novel variant of hepatitis E virus in Italy. *J Med Virol* 57:356-360.