3 考察

3.1 ブタのB型肝炎ウイルスについて

動物の B 型肝炎ウイルスの検出については中国においても複数の報告がされているが、食肉処理したブタ体内のB型肝炎ウイルスの検出に関する報告は稀有なケースである[15]。本試験では既存の研究を基礎としつつ、PCR 技術および透過電子顕微鏡技術を応用し、食肉処理したブタの血清および肝臓中の HBV 抗原に対する検査を行った。電子顕微鏡を用いてネガティブ染色サンプルを観察した結果、ELISA 法にて HBV の表現抗原に強陽性を示す血清サンプルに、ヒト HBV の Dane 粒子および小球状粒子に形態およびサイズの類似するウイルス粒子の存在が認められた。ヒト HBV キャリアの血清中においては、通常、小球状粒子が多数を占め、Dane 粒子は少数であるが、本試験では電子顕微鏡による観察を行ったネガティブ染色サンプル中に数多くの Dane 粒子が存在する結果となった(図 3 を参照のこと)。

現在、海外において禽獣の HBV に関する報告はなく、中国においては HBV 検査試薬を用いた血清マーカーおよび関連抗原の検査、ならびにウイルスの形態および遺伝子の S 領域などに対する研究に従事する研究者は存在するものの、動物の HBV に対する分子ウイルス学およびその病原性に関する研究、ヒトの HBV との関連性に関する研究は非常に少ない。本試験において HBV S 領域のプライマーを用いてブタの血清および肝臓中より予測断片を検出し、シークエンシング分析を行った結果、HBV の相同性は実に 98～100%に達することが明らかになった。本試験において検出を行った断片の占める割合は HBV 全遺伝子の約 9%に過ぎないが、少なくとも一定レベルにおいてブタ HBV とヒト HBV が高い相同性を有することを証明した。

一般的には、禽獣の B 型肝炎ウイルスはヒトに対する病原性を持たないと認識されているが、動物に対する病原性の有無、ならびに食肉および食肉加工製品を介して人体に摂取された後にこれに対する免疫反応を引き起こす可能性の有無については、現在もなお不明である。中国には 1.2 億人の B 型肝炎キャリアが存在すると見られ、この高い感染率に禽獣の B 型肝炎ウイルスが何らかの関連を持つか否かについては、今後さらに研究を進める価値がある。

HBV は現在においても体外での培養が不可能であり、また宿主領域が極めて狭いことから、適切な小型動物を動物モデルとした病原、発症機序、ワクチンおよび治療薬に対する研究はなく、さらに倫理的理由からヒトを除く霊長類動物モデルの使用は制限を受ける[1]。禽獣の B 型肝炎の発見は、肝向性ウイルス学に新たな研究対象を追加するのみならず、肝向性ウイルスの起源、進化、持続感染、発症機序、慢性ウイルス性肝細胞ガンの起源など各方面の研究に新たな研究対象を提供する。

3.2 E型肝炎について

多数の研究を通じ、HE は人畜共通感染症であること、経口感染すること、またブタが重要なウイルスキャリアであることが明らかになった。日本、インドなどでは加

熱不十分な食用ブタレバーおよびブタ肉の摂取による HEV 感染が報告されている。日本、米国の研究においてはブタと接触する職業に従事する人員群の血清抗 HEV 抗体はその他職業に従事する人員群よりも高く、また養豚場周辺の汚水中から HEV が検出されたことも明らかになった[16~18]。曹海俊[19]らが、浙江地域においてブタの食肉処理および販売に従事する人員群の HEV 感染状況について調査を行った結果、浙江省にてブタの食肉処理および販売に従事する人員群の 77.25%が HEV 陽性であり、1992 年に全国 13 省および市において実施された HE 血清流行病調査中の 1～59 歳人口に占める陽性率(17.2%)をはるかに上回ることが明らかになった。さらに別の報告では、中国の月齢 4 ヶ月以上のブタにおける血清抗体陽性率の平均が 40%にのぼり、ブタの飼育者の血清抗体陽性率に至っては 100%に達することも明らかになっている。その他タイでは、月齢 3 ヶ月以上のブタにおける陽性率が 9～20%に達し、ブタの飼育者の陽性率は 71%にのぼる。上記の研究結果は、ヒトの HE 陽性検出率とブタに接触する職業への従事者の間に一定の関連性があること、ならびに HE は人畜相互間の感染症であることを証明するものである。

かつて Meng[20]は異なる月齢のブタ血清から HEV RNA の検出を行ったが、中国内外において食肉処理したブタの肝臓から HEV RNA の検出を行ったという報告はない。本試験では RT-PCR 法を用いて食肉処理したブタ肝臓中の HEV RNA の増幅を行った結果、食肉処理したブタの肝臓中にも HEV RNA の存在が確認された。本試験室における過去の研究において食肉処理したブタ肝臓中の HEV に関連を有する抗原の陽性検出率が 95～100%と高率にのぼることが明らかになり、また食肉処理したブタが精肉製品生産網の末端に組み込まれていることを考慮すると、公衆衛生の見地からも、この問題は決して放置できない問題である。現在のところ、ブタの生肉中より HEV が検出されたという報告はなされていないものの、肝臓中の HEV に関連する抗原の陽性検出率が上記のように高いことが、人体の健康に対する潜在的な脅威となることは確実である。以上より、筆者は食肉処理したブタの検疫において HEV を検査項目として採用することを提案する。

別紙様式第2-1

No. 36

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	機構処理欄
				2008. 8. 18	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Girou E, Chevaliez S, Challine D, Thiessart M, Morice Y, Lesprit P, Tkoub-Scheirlinck L, Soing-Altrach S, Cizeau F, Cavin C, André M, Dahmanne D, Lang P, Pawlotsky JM. Clin Infect Dis. 2008 Sep 1;47(5):627-33.	公表国 フランス	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)					

研究報告の概要

○環境汚染および標準的予防法の非遵守が血液透析装置のC型肝炎感染リスクに及ぼす影響

背景:C型肝炎ウイルス(HCV)感染症の原因の第2位は院内感染である。環境汚染および血液透析装置のHCV交差感染を予防する標準的予防法の非遵守が院内感染に及ぼす影響を検討するため、前向き観察試験を実施した。

方法:フランスの大学病院において長期血液透析を受けている患者を系統的にスクリーニングし、HCV散発感染症例2例が認められた。試験を行い、当該患者の感染が血液透析装置によるものかどうかを判定し、環境汚染と予防のための標準法非遵守が院内感染に影響する可能性を調べた。新規HCV感染症例と、血液およびHCV RNAによる環境汚染、手の衛生と手袋使用ガイドラインの非遵守との関連性を検討した。

結果:患者2例が試験期間中にHCV抗体陽性となった。系統発生解析では、これらの患者の1例が、同一ユニット内で治療を受けている慢性感染患者と相同なウイルス株に感染していることが示された。環境表面検体740検体中82検体(11%)にヘモグロビンが含まれ、6検体(7%)にHCV RNAが含まれていた。手の衛生に関する遵守率は37%(95%信頼区間、35%～39%)であり、患者ケアの直後に手袋をはずしていたのは33%(95%信頼区間、29%～37%)だった。環境表面のヘモグロビンの存在を予測する独立因子は、患者に対する看護師数の少なさ、手の衛生の不良であった。

結論:血液に汚染された表面は、血液透析装置がHCV交差感染の原因である可能性がある。血液透析患者間のHCV交差感染リスクを低減させるためには、手の衛生および手袋使用の厳重遵守と治療手順の徹底が必要である。

報告企業の意見	今後の対応
フランスの大学病院において長期血液透析を受けている患者2名がHCVに感染し、患者に対する看護師数の少なさと手の衛生の不良が、病院内の環境汚染の原因であることが示された。院内感染リスク低減のためには、手の衛生および手袋使用の厳重遵守と治療手順の徹底が必要であるとの報告である。輸血後HCV感染の調査では、院内感染など輸血以外の伝播ルートについて考慮する必要がある。	日本赤十字社では、HCV抗体検査を実施することに加えて、HCVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)及び精度を向上させた新NATシステムを導入した。HCV感染に関する新たな知見等について今後も情報の収集に努める。

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」
新鮮凍結血漿-LR「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

MAJOR ARTICLE

Determinant Roles of Environmental Contamination and Noncompliance with Standard Precautions in the Risk of Hepatitis C Virus Transmission in a Hemodialysis Unit

Emmanuelle Girou,[1,2] Stéphane Chevaliez,[2] Dominique Challine,[2] Michaël Thiessart,[1] Yoann Morice,[2] Philippe Lesprit,[1] Latifa Tkoub-Scheirlinck,[1] Sophan Soing-Altrach,[1] Florence Cizeau,[1] Celine Cavin,[1] Martine André,[3] Djamel Dahmanne,[3] Philippe Lang,[3] and Jean-Michel Pawlotsky[2]

[1]Infection Control Unit, [2]French National Reference Center for Viral Hepatitis B, C, and delta, Department of Virology & INSERM U635, and [3]Nephrology Ward, Henri Mondor Hospital, Assistance Publique–Hôpitaux de Paris, Université Paris 12, Créteil, France

Background. Nosocomial transmission is the second most frequent cause of hepatitis C virus (HCV) infection. A prospective observational study was conducted to assess the roles of environmental contamination and noncompliance with standard precautions in HCV cross-transmission in a hemodialysis unit.

Methods. Patients undergoing chronic hemodialysis in a French university hospital unit were systematically screened, revealing 2 sporadic cases of HCV transmission. An investigation was launched to determine whether the patients were infected in the hemodialysis unit and the possible roles of environmental contamination and noncompliance with standard precautions. We examined possible relationships among new cases of HCV infection, environmental contamination by blood and HCV RNA, and compliance with guidelines on hand hygiene and glove use.

Results. Two patients experienced seroconversion to HCV during the study period. Phylogenetic analyses showed that 1 of these patients was infected with the same strain as that affecting a chronically infected patient also treated in the unit. Of 740 environmental surface samples, 82 (11%) contained hemoglobin; 6 (7%) of those contained HCV RNA. The rate of compliance with hand hygiene was 37% (95% confidence interval, 35%-39%), and gloves were immediately removed after patient care in 33% (95% confidence interval, 29%-37%) of cases. A low ratio of nurses to patients and poor hand hygiene were independent predictors of the presence of hemoglobin on environmental surfaces.

Conclusion. Blood-contaminated surfaces may be a source of HCV cross-transmission in a hemodialysis unit. Strict compliance with hand hygiene and glove use and strict organization of care procedures are needed to reduce the risk of HCV cross-transmission among patients undergoing hemodialysis.

Hepatitis C virus (HCV) infection is a major health problem. Worldwide, >170 million individuals carry the virus, and the infection becomes chronic in ~80% of adult cases. Approximately 20% of patients with chronic HCV infection develop cirrhosis, and the incidence of hepatocellular carcinoma is 4%–5% per year in cirrhotic patients [1].

HCV is principally, if not exclusively, transmitted by blood. Historically, the 2 main routes of transmission have been blood transfusion and injection drug use. Since the implementation, in the United States and Europe, of blood-donor screening with highly sensitive EIAs for anti-HCV antibodies and minipool testing for HCV RNA, the incidence of transfusion-transmitted hepatitis C has decreased to ~1 case per 2 million transfused blood units [2, 3]. In France, 3000–4000 new cases of HCV infection still occur every year [4]. Approximately two-thirds of these cases are related to injection drug use, but nosocomial transmission is the

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Reprints or correspondence: Dr. Emmanuelle Girou, Unité de Contrôle, Epidémiologie et Prévention de l'Infection, Hôpital Henri Mondor, 51 Ave. du Maréchal de Lattre de Tassigny, 94010 Créteil, France (emmanuelle.girou@hmn.aphp.fr).

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second most common source of HCV infection. Most cases of HCV transmission in the hospital setting are attributable to patient-to-patient transmission through invasive procedures, such as insertion of an intravascular catheter, colonoscopy, sharing of dialysis equipment, surgery, and sharing of multidose vials [5–11].

The prevalence of HCV infection is high among patients who undergo hemodialysis, because of both contaminated transfusions before the early 1990s and nosocomial transmission. Several outbreaks and sporadic cases of nosocomial HCV or hepatitis B virus transmission in dialysis units have been linked to poor disinfection of dialysis equipment and to poor compliance with standard infection-control measures [9, 12–18]. However, the exact route and mechanism of transmission were unknown in most cases. Here, we examined the intricate roles of noncompliance with standard precautions, environmental contamination, and low nurse-to-patient ratio in cross-transmission of HCV within a dialysis unit.

PATIENTS AND METHODS

Setting and patients. Henri Mondor University Hospital has a 9-bed hemodialysis unit that mainly treats patients with chronic renal failure. A case of HCV seroconversion was detected by systematic screening during the study period. The study period was defined as the interval between the probable date of infection and the discovery of the index case—that is, January–September 2004. Patients' medical files were exhaustively reviewed to eliminate a potential external source of HCV transmission. None of the health care personnel was known to be infected with HCV. No systematic screening of personnel was undertaken. No isolation policy was implemented in the unit. Multidose vials were not in use in the unit.

All patients who undergo regular hemodialysis are screened for anti-HCV antibodies every 3 months, in an effort to detect seroconversion. On 27 July 2004, a case of HCV seroconversion was detected through this screening. To determine whether this case was sporadic or part of an outbreak, all 52 patients with chronic renal failure who were undergoing regular hemodialysis in the unit were tested for anti-HCV antibodies and HCV RNA, as were all patients treated for acute renal failure in the unit during the at-risk period. Six (12%) of the 52 patients (patients 3–8) were known to be chronically infected with HCV, with HCV RNA levels ranging from 4.4 to >6.9 $\log_{10}$ IU/mL at the time of the study. All but 1 of these patients were to known to have been infected for several years (e.g., patient 3 has been infected since 2001). A second patient undergoing hemodialysis was found to be HCV RNA positive through culture of a blood sample obtained in July 2004 (tests for anti-HCV antibodies were negative), and an investigation was then launched.

Virological studies and phylogenetic analyses. Anti-HCV antibodies were detected with a third-generation EIA (Vitros ECi; Ortho-Clinical Diagnostics). We tested for HCV RNA in all patients' blood and in hemoglobin-positive surface swab eluates through use of a sensitive RT-PCR assay (Amplicor HCV, version 2.0; Roche Molecular Systems), with a detection limit of 50 IU/mL.

To estimate the genetic relatedness of HCV strains, 2 HCV genomic regions were PCR amplified and sequenced, including a 328–base pair portion of the nonstructural 5B (NS5B) coding region (nucleotide positions 8271–8597) and the 81–base pair region coding for hypervariable region 1 (HVR1) of the E2 envelope glycoprotein.[19]. HCV genotyping was based on phylogenetic analysis of NS5B sequences, which included prototype sequences of various subtypes of HCV genotypes 1–6. The genetic relatedness of HCV strains was studied by phylogenetic analysis of both the NS5B and HVR1 regions. Sequences were aligned with ClustalW software [20]. Phylogenetic relationships were deduced with the DNADIST-NEIGHBOR module of the Phylogeny Interference Package, version 3.5 [21]. For neighbor-joining analysis, a distance matrix was calculated using a Kimura 2-parameter distance matrix with a transition/transversion ratio of 4.0. Trees were drawn with TREVIEW or NJ-Plotprograms [22]. Their robustness was assessed by bootstrap analysis of 1000 replicates with the SEQBOOT module of the Phylogeny Interference Package program.

The index patient (patient 1) experienced HCV seroconversion in July 2004. The second case of HCV seroconversion during the study period (patient 2) was identified by systematic screening for HCV RNA. To determine whether chronically infected patients were the source of the new cases, the sequences of 2 HCV genomic regions, including a portion of the NS5B coding sequence and the sequence coding for HVR1, were compared among the 8 infected patients, relative to reference sequences. Phylogenetic analyses of the NS5B region (figure 1) and the HVR1 (figure 2) showed that newly infected patient 2 was infected with the same HCV genotype 1 strain as was chronically infected patient 3. In contrast, patient 1 was infected with an HCV genotype 3a strain that was unrelated to the strains infecting the other 6 chronically infected patients (all infected with genotype 1). Despite the proximity of the HCV strains from patients 4–8 in the NS5B phylogenetic tree (figure 1), HVR1 analysis showed that those patients were infected with unrelated strains (figure 2).

Thus, 2 patients were infected during the at-risk period, 1 of whom (patient 2) was infected with the same strain as was a chronically infected patient (patient 3). The other newly infected patient (patient 1) was infected with a genotype 3a strain, which could have been acquired either from a patient occasionally treated in the dialysis unit or from an external source.

Risk factors of HCV transmission. Potential risk factors of HCV transmission were hypothesized—namely, contamination of dialysis equipment (through machine sharing and inadequate

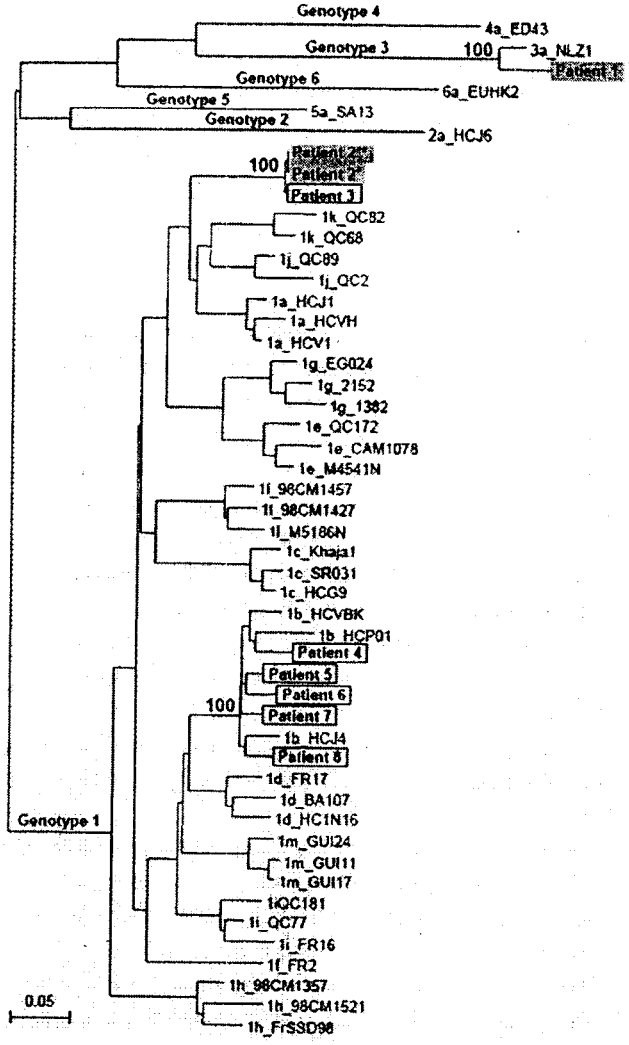

Figure 1. Phylogenetic tree plotted with nonstructural 5B sequences (nucleotide positions 8271–8597) from the 2 patients recently infected in our hemodialysis unit (patients 1 and 2; *shaded boxes*), the 6 patients known to be chronically infected with hepatitis C virus (HCV) and regularly treated in our hemodialysis unit (patients 3–8; *unshaded boxes*), and reference HCV strains of different genotypes (the type and subtype are indicated just before the strain identification letters and/or numbers). Two samples were available and were included in the analysis for patient 2, *July 2004; **September 2004. Nucleotide sequence of the nonstructural 5B gene of HCV-ED43 was used as an outgroup root.

environmental disinfection), noncompliance with standard precautions, and variation of the nurse-to-patient ratio in the hemodialysis unit.

The use and maintenance of dialysis equipment was reviewed by the local infection control team according to the written local procedures that are based on published data and recommendations. Dialyzers were not being reused, and dialysis machines (AK100; Gambro) were disinfected after each session, according to a written protocol combining chemical (peroxyacetic acid [Dialox]) and sodium hypochlorite) and heat disinfection.

Surfaces at risk of contamination with infected blood were defined as the most frequently manipulated surfaces—including dialysis machines, shared waste carts, patients' removable tables, and work benches. At-risk surfaces were swabbed during dialysis sessions (30 swabs per day on 25 consecutive days) on a surface area of ~100 cm^2, by using a cotton swab moistened with sterile distilled water that was then eluted in 1 mL of sterile distilled water. Hemoglobin was detected with reagent strips (Hemastix; Bayer HealthCare) with a detection limit of 150 μg Hb/L—that is, the equivalent of 5 erythrocytes per microliter. All hemoglobin-positive samples were tested for HCV RNA [23, 24].

Compliance with standard precautions (hand hygiene and glove use) was studied in the dialysis unit each day for three 30-min periods—during the morning, afternoon, and night shifts—for 7 weeks (2 weeks during September 2004 immediately after the first case alert and 5 weeks during June–July 2005). All staff categories were studied, in an open, unobtrusive manner, by 5 specially trained members of the infection control team, with use of a standardized questionnaire [25]. Hand hygiene opportunities tailored to the care activities in the hemodialysis unit were listed in the questionnaire (i.e., before and

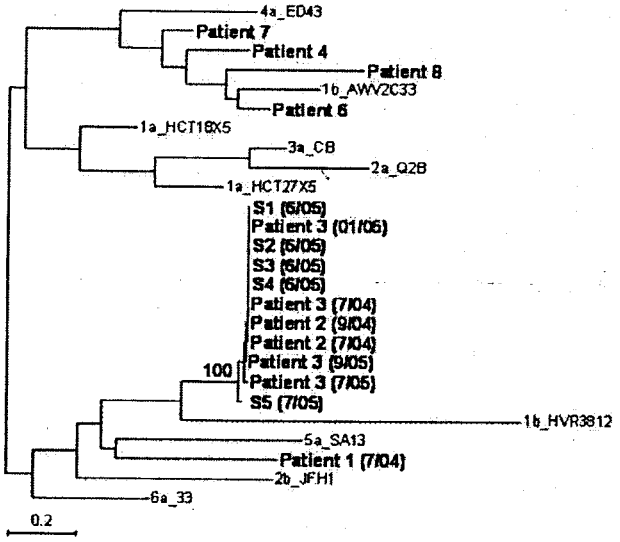

Figure 2. Phylogenetic tree plotted with hypervariable region (HVR) 1 sequences from the 6 patients known to be chronically infected with hepatitis C virus (HCV) and regularly treated in our hemodialysis unit (patients 3–8), including the patient who transmitted HCV to patient 2 (i.e., patient 3), the 2 patients infected in our hemodialysis unit (patients 1 and 2), the 5 environment surfaces that tested positive for HCV RNA and that could be PCR amplified in that region (S1–S5), and reference HCV strains of different genotypes (the type and subtype are indicated just before the strain identification letters and/or numbers). Dates of sampling are shown in parentheses.

after central venous catheter or fistula handling; preparation of material, connection, disconnection, dressing, and manipulation of lines and before and after direct contact with a patient; handling of other invasive devices, if present; measurement of temperature; measurement of arterial pressure; etc.). The handling of catheter and fistula were considered to be activities with high risk of HCV transmission. Overall, 2382 opportunities were observed during 197 shifts, with a total of 98 h of observation.

Glove use was observed during the same periods as was hand hygiene. For each care activity, the following variables were collected on the same standardized questionnaire as that used for hand hygiene: type of contact, wearing gloves during contact, and glove removal immediately after contact. Wearing gloves is recommended in the unit when exposure to body fluids is anticipated.

With consideration that the nurse-to-patient ratio (including nurses and nurse assistants) may influence the risk of HCV transmission, the ratio was recorded during each observation period, and the average nurse-to-patient ratio per shift (morning, afternoon, and night) was determined by calculating the median ratio for all the relevant observation periods. Hand hygiene compliance was also calculated for each of the 3 shifts.

Statistical analysis. Percentages and 95% CIs were calculated. The χ^2 test or Fisher's exact test was used, as appropriate, to compare proportions. The Mann-Whitney nonparametric test was used to compare continuous variables. Each potential risk factor for environmental hemoglobin contamination (i.e., nurse-to-patient ratio and hand hygiene compliance) was tested in a univariate model, and results were then entered in a logistic regression model. Variables were not dichotomized. To take into account the interdependence of observations made during the same shift, we used robust estimates of variance (generalized estimating equations) in which each shift observation was included as a cluster. Goodness of fit was assessed using the Hosmer-Lemeshow χ^2 test, and discrimination was determined from the area under the receiver operating characteristics curve. Accuracy was considered to be good when the area under the receiver operating characteristics curve had a range of 0.70–0.80 and was considered to be excellent when it was >0.80. The adjusted OR and 95% CI were calculated for each factor that was statistically significant in the logistic regression model. *P* values <.05 were considered to be statistically significant. All tests were 2 tailed. Statistical tests were performed using Intercooled Stata software, version 8.2 (Stata).

RESULTS

Virological study of environmental surfaces. A total of 740 surface samples were collected in the dialysis unit during June–August 2005, comprising 663 (90%) from dialysis machines and 77 (10%) from other surfaces (table 1). Hemoglobin was found in 82 samples (11%), including 71 (10%) from surfaces where blood was not evident. Among the 25 hemoglobin-positive samples collected from dialysis machines, 5 had been obtained after external disinfection of the machine. Six (7%) of the 82 hemoglobin-positive samples contained detectable levels of HCV RNA, comprising 4 samples taken from a dialysis machine and 2 from a shared waste cart (table 1). The HVR1-coding region could be PCR-amplified and sequenced in 5 of these 6 samples, designated S1–S5. These sequences were compared with HVR1 sequences recovered from patients 1–8 during the at-risk period (except for patient 5, in whom HVR1 could not be amplified) and also from patient 3 at the time of surface sampling (figure 2). As shown in figure 2, phylogenetic analysis revealed that all sequences found in environmental samples were closely related to those isolated from patient 2 when he was infected in 2004 and to those from patient 3, from whom samples were obtained both in 2004 and in 2005. Note also in figure 2 the very slow genetic evolution of the HVR1 in patient 3 (only 4 nucleotide substitutions accumulated in 14 months; data not shown), probably because of hemodialysis-associated immune suppression. Interestingly, the same HCV strain was isolated from 2 environmental samples taken at a 6-h interval from the same machine that had been used to treat 2 different patients.

Assessment of practices. Compliance with local precautions for machine use and internal disinfection was adequate. Multidose vials were never shared between patients. The finding that patients 2 and 3, who were infected with closely related HCV strains (figures 1 and 2), had always undergone dialysis during the same sessions but had never shared the same machine strongly suggested that patient 2 had been infected by patient 3 via the hands of a health care worker.

Compliance with standard precautions during the investigation is shown in figure 3. Overall, 2382 opportunities for hand hygiene were observed (2358 [99%] for nurses; 24 [1%]

Table 1. Environmental samples containing hemoglobin and/or hepatitis C virus (HCV) RNA.

Sample site	No. of samples	Positive samples, no. (%)	
		Hemoglobin	HCV RNA
Dialysis machine	663	36 (5)	4 (11)
Shared waste cart	27	24 (89)	2 (8)
Patients' removable table	9	6 (67)	0 (0)
Miscellaneous[a]	41	16 (39)	0 (0)
Total	740	82 (11)	6 (7)

NOTE. HCV RNA–positive findings are percentages of the number of hemoglobin-positive samples.

[a] Including nursing preparation area, wheelchairs, and patient file cart.

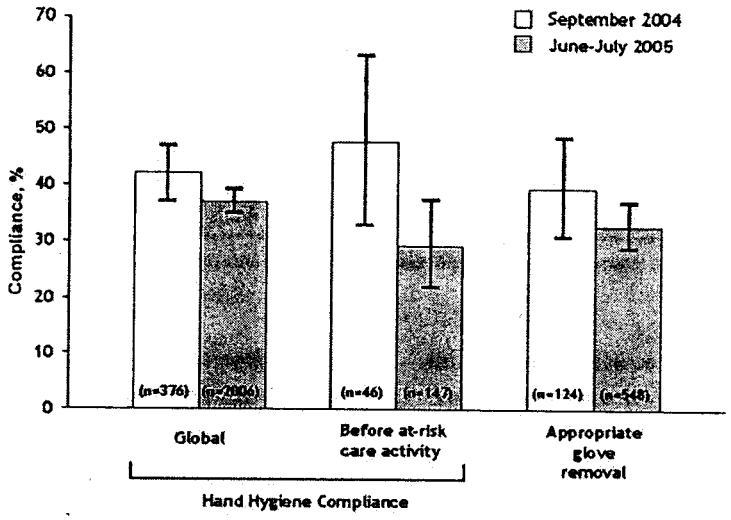

Figure 3. Compliance with guidelines for health care worker hand hygiene and appropriate glove use during dialysis. At-risk care activities consisted of handling dialysis catheters or fistulas. Whiskers, 95% CIs.

for nurse assistants). Immediately after the infection alert (September 2004), compliance with hand hygiene immediately before handling a dialysis catheter or fistula was significantly higher ($P < .001$) than it was several months later (figure 3). Globally, gloves were worn in 857 (36%) of observed contacts with a patient or the environment. When worn, gloves were removed immediately after a contact in only 672 (34.1%) of cases (95% CI, 30.5%–37.8%). There was no statistically significant difference between the findings of the 2 periods of observation. As shown in table 2, a low nurse-to-patient ratio and a poor rate of hand hygiene compliance were independently associated with the detection of hemoglobin on environmental surfaces.

DISCUSSION

Several reports of nosocomial HCV transmission in the hemodialysis setting have been published, but the investigations were incomplete and the routes of transmission remained unclear [13, 17, 18, 26]. Allander et al. [26] reported nosocomial HCV transmission in a series of patients who underwent dialysis at the same time but who did not share dialysis equipment. Those authors postulated, but did not show, that the environment was contaminated. Compliance with standard precautions was not studied.

To our knowledge, ours is the first study to demonstrate that a low nurse-to-patient ratio and poor compliance with guidelines for hand hygiene and glove use are independent predictors of environmental contamination by blood and HCV. By combining genetic and phylogenetic analyses of HCV recovered from patients' blood and the environment with measurements of compliance with standard precautions, we showed that: (1) 2 sporadic cases of HCV transmission occurred in the dialysis unit during the study period, 1 of which was unequivocally due to patient-to-patient transmission within the unit; (2) the dialysis environment was frequently contaminated by blood, including HCV-infected patients' blood, as shown by the detection of hemoglobin, sometimes associated with detectable levels of HCV RNA in a substantial proportion of swabs; and (3) compliance with guidelines for hand hygiene and glove use during patient care was poor, raising the possibility of HCV transmission via the hands of health care workers. Interestingly, all HCV-infected blood found in environmental samples belonged to the patient who indirectly infected another patient undergoing dialysis.

In our study, hemoglobin was found in 11% of environmental samples, and 7% of those positive samples contained detectable HCV RNA. Hepatitis B virus transmission has been linked to the presence of the virus on environmental surfaces—in the absence of visible blood [27]. Hepatitis B virus has been reported to remain viable on environmental surfaces for at least 7 days at room temperature [28, 29]. HCV RNA has been shown to be resistant for at least 48 h on inert surfaces at room temperature [24, 30, 31]. A robust cell culture system for HCV was recently developed, but it cannot be infected with viruses other than those produced after cell culture transfection of a specific HCV clone [32–34]. Cell culture systems that can be directly infected by HCV-infected patients' blood will be needed to determine how long HCV remains infective in the environment. Even in the absence of such data, our results strongly suggest that infectious HCV is present in the dialysis environment and that HCV can be transmitted by the hands of health care workers. We did not, however, sample health care workers'

Table 2. Factors independently associated with environmental blood contamination during nursing shifts.

Variable	Univariate analysis of environmental hemoglobin, by daily shifts		Multivariate analysis	
	Hemoglobin found (n = 28)	Hemoglobin not found (n = 14)	OR (95% CI)	P
Nurse-to-patient ratio, mean ± SD	0.55 ± 0.23	0.78 ± 0.50	0.03 (0.002–0.39)	.008
Hand hygiene compliance, mean % ± SD	39 ± 15	44 ± 17	0.93 (0.88–0.99)	.036

NOTE. Performance of the model, Hosmer-Lemeshow goodness-of-fit; P = .386; area under receiver operating characteristics curve, 0.768.

gloved or ungloved hands during care activities, because this would have hindered the assessment of compliance with standard precautions by increasing the Hawthorne effect.

The rate of compliance with standard precautions in our study was similar to that reported elsewhere about a similar setting [35, 36]. A recent survey of hand hygiene practices in 9 Spanish hemodialysis units showed poor compliance, both before and after contact with patients (14% and 36%, respectively) [36].

Permanent glove use can impair compliance with hand hygiene [37] and may thus lead to cross-transmission of infectious agents. This is the first time that glove use and removal have been studied in relation to the risk of environmental contamination. Gloves are worn mainly for health care worker self-protection, rather than to prevent patient cross-infection. The recommendation that gloves always be worn in the hemodialysis setting, whatever the type of contact (environment or patient) [38], therefore, may be confusing and may expose patients to HCV transmission if not followed properly, with systematic glove removal and hand hygiene between care procedures.

We found that a nurse-to-patient ratio <0.60 was independently associated with hemoglobin contamination of environmental surfaces. Understaffing is a recognized major risk factor for nosocomial infection [39–41]. Recently, a Brazilian study of 22 dialysis centers showed that the number of patients per health care worker was independently related to the risk of hepatitis B virus infection [16]. Petrosillo et al. [42] showed, in a prospective multicenter study in Italian hemodialysis units, that a low staff-to-patient ratio is an independent predictor of the risk of HCV nosocomial transmission. Therefore, to limit the spread of blood in the dialysis environment, we recommend that at-risk care procedures, such as connection and disconnection of equipment to the patient, be performed by a pair of nurses: one working with the patient and the other working with the machine.

In conclusion, blood-contaminated surfaces may represent a source of HCV transmission, via health care workers' hands or gloves. Environmental contamination is mainly a consequence of poor adherence to standard precautions in the hemodialysis setting. Strict adherence to guidelines for hand hygiene and glove use and strict organization of care procedures, with an adequate nurse-to-patient ratio, should help to reduce the risk of environmental contamination and, thus, HCV transmission in patients undergoing dialysis.

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Potential conflicts of interest. All authors: no conflicts.

References

1. McHutchison JG, Bacon BR. Chronic hepatitis C: an age wave of disease burden. Am J Manag Care 2005; 11:S286-95.
2. Busch MP, Glynn SA, Stramer SL, et al. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005; 45:254–64.
3. Pillonel J, Laperche S. Trends in risk of transfusion-transmitted viral infections (HIV, HCV, HBV) in France between 1992 and 2003 and impact of nucleic acid testing (NAT). Euro Surveill 2005; 10:5–8.
4. Delarocque-Astagneau E, Pioche C, Desenclos JC. National surveillance of hepatitis C by voluntary hepatology reference centres, 2001–2004. Bull Epidemiol Hebdomadaire 2006; 51–52:414–8.
5. Allander T, Gruber A, Naghavi M, et al. Frequent patient-to-patient transmission of hepatitis C virus in a haematology ward. Lancet 1995; 345:603–6.
6. Bronowicki JP, Venard V, Bott, C, et al. Patient-to-patient transmission of hepatitis C virus during colonoscopy. N Engl J Med 1997; 337: 237–40.
7. Sartor C, Brunet P, Simon S, Tamalet C, Berland Y, Drancourt M. Transmission of hepatitis C virus between hemodialysis patients sharing the same machine. Infect Control Hosp Epidemiol 2004; 25:609–11.
8. Esteban JI, Gomez J, Martell M, et al. Transmission of hepatitits C virus by a cardiac surgeon. N Engl J Med 1996; 334:555–60.
9. Furusyo N, Kubo N, Nakashima H, Kashiwagi K, Etoh Y, Hayashi J. Confirmation of nosocomial hepatitis C virus infection in a hemodialysis unit. Infect Control Hosp Epidemiol 2004; 25:584–90.
10. Ross RS, Viazov S, Gross T. Transmission of hepatitis C virus from a patient to an anesthesiology assistant to five patients. N Engl J Med 2000; 343:1851–4.
11. Massari M, Petrosillo N, Ippolito G, et al. Transmission of hepatitis C virus in a gynecological surgery setting. J Clin Microbiol 2001; 39: 2860–3.
12. Schneeberger PM, Keur I, van Loon AM, et al. The prevalence and incidence of hepatitis C virus infections among dialysis patients in The Netherlands: a nationwide prospective study. J Infect Dis 2000; 182: 1291–9.
13. Halfon P, Roubicek C, Gerolami V, et al. Use of phylogenetic analysis of hepatitis C virus (HCV) hypervariable region 1 sequences to trace

an outbreak of HCV in an autodialysis unit. J Clin Microbiol 2002; 40:1541–5.
14. Kokubo S, Horii T, Yonekawa O, Ozawa N, Mukaide M. A phylogenetic-tree analysis elucidating nosocomial transmission of hepatitis C virus in a haemodialysis unit. J Viral Hepat 2002; 9:450–4.
15. Jadoul M, Poignet JL, Geddes C, et al. The changing epidemiology of hepatitis C virus (HCV) infection in haemodialysis: European multicentre study. Nephrol Dial Transplant 2004; 19:904–9.
16. Carrilho FJ, Moraes CR, Pinho JR, et al. Hepatitis B virus infection in haemodialysis centres from Santa Catarina State, Southern Brazil: predictive risk factors for infection and molecular epidemiology. BMC Pub Health 2004; 4:13.
17. Izopet J, Sandres-Saune K, Kamar N, et al. Incidence of HCV infection in French hemodialysis units: a prospective study. J Med Virol 2005; 77:70–6.
18. Lavillette D, Morice Y, Germanidis G, et al. Human serum facilitates hepatitis C virus infection, and neutralizing responses inversely correlate with viral replication kinetics at the acute phase of hepatitis C virus infection. J Virol 2005; 79:6023–34.
19. Pawlotsky JM, Pellerin M, Bouvier M, et al. Genetic complexity of the hypervariable region 1 (HVR1) of hepatitis C virus (HCV): influence on the characteristics of the infection and responses to interferon alfa therapy in patients with chronic hepatitis C. J Med Virol 1998; 54: 256–64.
20. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22:4673–80.
21. Felsenstein J. PHYLIP: phylogeny inference package, version 3.5c (distributed by the author). Department of Genetics, University of Washington, Seattle, 1993.
22. Page RD. TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 1996; 12:357–8.
23. Carducci A, Verani M, Casini B, et al. Detection and potential indicators of the presence of hepatitis C virus on surfaces in hospital settings. Lett Appl Microbiol 2002; 34:189–93.
24. Piazza M, Borgia G, Picciotto L, Nappa S, Cicciarello S, Orlando R. Detection of hepatitis C virus-RNA by polymerase chain reaction in dental surgeries. J Med Virol 1995; 45:40–2.
25. Girou E, Oppein F. Handwashing compliance in a French university hospital: new perspective with the introduction of hand-rubbing with a waterless alcohol-based solution. J Hosp Infect 2001; 48:S55-7.
26. Allander T, Medin C, Jacobson JA, Grillner L, Persson MAA. Hepatitis C transmission in a hemodialysis unit: molecular evidence for spread of virus among patients not sharing equipment. J Med Virol 1994; 43: 415–9.
27. Bond WW, Petersen NJ, Favero MS. Viral hepatitis B: aspects of environmental control. Health Lab Sci 1977; 14:235–52.
28. Favero MS, Maynard JE, Petersen NJ, et al. Letter: hepatitis-B antigen on environmental surfaces. Lancet 1973; 2:1455.
29. Bond WW, Favero MS, Petersen NJ, Gravelle CR, Ebert JW, Maynard JE. Survival of hepatitis B virus after drying and storage for one week. Lancet 1981; 7:550–1.
30. Piazza M, Borgia G, Picciotto L, Cicciarello S, Nappa S. HCV-RNA survival as detected by PCR in the environment. Boll Soc Ital Biol Sper 1994; 70:167–70.
31. Froio N, Nicastri E, Comandini UV, et al. Contamination by hepatitis B and C viruses in the dialysis setting. Am J Kidney Dis 2003; 42: 546–50.
32. Lindenbach BD, Evans MJ, Syder AJ, et al. Complete replication of hepatitis C virus in cell culture. Science 2005; 309:623–6.
33. Wakita T, Pietschmann T, Kato T, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 2005; 11:791–6.
34. Zhong J, Gastaminza P, Cheng G, et al. Robust hepatitis C virus infection in vitro. Proc Natl Acad Sci U S A 2005; 102:9294–9.
35. Arenas JMD, Sanchez-Paya J, Gonzales C, Rivera F, Antolin A, Arenas JD. Audit on the degree of application of universal precautions in a haemodialysis unit. Nephrol Dial Transplant 1999; 14:1001–3.
36. Arenas MD, Sanchez-Paya J, Barril G, et al. A multicentric survey of the practice of hand hygiene in haemodialysis units: factors affecting compliance. Nephrol Dial Transplant 2005; 20:1164–71.
37. Girou E, Chai SH, Oppein F, et al. Misuse of gloves: the foundation for poor compliance with hand hygiene and potential for microbial transmission? J Hosp Infect 2004; 57:162–9.
38. Centers for Disease Control and Prevention. Recommendations for preventing transmission of infections among chronic hemodialysis patients. MMWR Recomm Rep 2001; 50(RR-5):1–43.
39. Harbarth S, Sudre P, Dharan S, Cadenas M, Pittet D. Outbreak of *Enterobacter cloacae* related to understaffing, overcrowding, and poor hygiene practices. Infect Control Hosp Epidemiol 1999; 20:598–603.
40. Andersen BM, Lindemann R, Bergh K, et al. Spread of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive unit associated with understaffing, overcrowding and mixing of patients. J Hosp Infect 2002; 50:18–24.
41. Needleman J, Buerhaus P, Mattke S, Stewart M, Zelevinsky K. Nurse-staffing levels and the quality of care in hospitals. N Engl J Med 2002; 346:1715–22.
42. Petrosillo N, Gilli P, Serraino D, et al. Prevalence of infected patients and understaffing have a role in hepatitis C virus transmission in dialysis. Am J Kidney Dis 2001; 37:1004–10.

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研究報告の概要

○養豚業従事者のE型肝炎ウイルス(HEV)への職業的曝露
本研究の目的は、ブタ接触群(養豚業従事者)と非接触群のHEV陽性率とウイルス感染リスク因子を調べることであった。合計198名[非接触者97名(49%)、接触者101名(51%)]を対象にHEV感染の有無を調べた。抗HEV IgG抗体陽性率はブタ接触群が18.8%、非接触群が4.1%であった。ブタ接触者の抗HEV IgG抗体陽性リスクは5.4倍(P = 0.03)であった。IgG抗体陽性者10名(52.6%)は、未処理水の摂取およびブタへの接触という2つの汚染リスク因子を示した。以上のデータは、HEV感染を養豚従事者の職業病として扱うべきことを裏付けるものである。したがって、当該ウイルスへの曝露を予防するために、当該集団における包括的な衛生措置の適用が強く推奨される。

使用上の注意記載状況・その他参考事項等

合成血-LR「日赤」
照射合成血-LR「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
職業上のブタ接触群と非接触群のHEVの陽性率とウイルス感染リスク因子を調べたところ、抗HEV IgG抗体陽性率はブタ接触群が有意に高く、陽性者は未処理水の摂取およびブタへの接触という2つのリスクファクターを示したとの報告である。HEV感染については血液の安全対策上だけではなく、公衆衛生及び食品衛生上の問題でもある。	日本赤十字社では、輸血による肝炎ウイルス感染防止のため、血液中のALT値61IU/L以上の血液を排除している。また、厚生労働科学研究「E型肝炎の感染経路・宿主域・遺伝的多様性・感染防止・診断・治療に関する研究班」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。加えて、北海道における輸血後HEV感染報告を受け、試験的に北海道では研究的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。

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Short Report: Occupational Exposure to Hepatitis E Virus (HEV) in Swine Workers

Carolina Galiana, Salceda Fernández-Barredo, Angel García, María Teresa Gómez, and Maria Teresa Pérez-Gracia*

Departamento de Atención Sanitaria, Salud Pública y Sanidad Animal, Facultad de Ciencias Experimentales y de la Salud, Universidad CEU Cardenal Herrera, Moncada, Valencia, Spain

Abstract. The aim of this work was to study the prevalence of hepatitis E virus (HEV) and the risk factors for the acquisition of the virus in a population in contact with swine and unexposed to swine. A total of 198 individuals, 97 unexposed (49%) and 101 exposed (51%) to swine, were tested for the presence of HEV infection. The prevalence of anti-HEV IgG in the exposed group was 18.8% versus 4.1% in the unexposed to swine group. People exposed to swine were observed to be 5.4 times ($P = 0.03$) at risk of having anti-HEV IgG. Ten (52.6%) of the IgG-positive individuals showed two concomitant risk factors: untreated water consumption and exposure to swine. These data support that HEV infection should be treated as a vocational illness in swine workers. Therefore, systematic application of hygiene measures in this collective is highly recommended to avoid the exposition to this virus.

Hepatitis E virus (HEV) is the main causative agent of enterically transmitted non-A non-B hepatitis and self-limiting clinical presentation in humans.[1] It is a non-enveloped virus with a positive-sense, single-stranded RNA genome of ~7,200 nucleotides in length and contains three open reading frames (ORFs). Nowadays, HEV is classified into the family *Hepeviridae*, genus *Hepevirus*. Regarding the phylogeny, HEV has been divided into four genotypes,[2] although only one serotype of HEV is recognized.[3] Transmission of HEV infection primarily occurs through contaminated water, although person to person transmission and sexual transmission occur infrequently.

Hepatitis E has been considered an infectious endemic in developing areas such as India, Africa, and Southeast Asia, because of poor sanitary conditions in drinking water.[4] The mortality rate of hepatitis E in the normal population is generally < 1%, but it can be as high as 20–25% among pregnant women.

In industrialized countries, HEV has been found mainly in individuals who had traveled to endemic zones. Actually, the increasing number of autochthonous cases of hepatitis E[5] and the recent findings of HEV in domestic animals such as swine give rise to the suspicion that HEV is underdetected in idiopathic non-A non-B hepatitis. Therefore, the transmission pathways from animals to humans remain obscure. However, in developed countries, seroprevalence ranges varying from 1–18% have been reported. In the last years, several studies have been published describing differences in the prevalence of anti-HEV antibodies between people exposed and not exposed to swine,[6–12] but the risk factors for the acquisition of the virus have not been studied.

Accordingly, the aim of this work was to study the prevalence of HEV and the risk factors for the acquisition of the virus in healthy Spanish people distributed in exposed and unexposed to swine groups.

A retrospective study was carried out to determine the prevalence of HEV during the period from October 2004 to July 2007 in Spain.

A total number of 198 healthy individuals, 101 (51%) men and 97 (49%) women, were included in this study to detect the prevalence of HEV. Participants filled out an epidemiologic questionnaire including name, age, area of residence, travel abroad, exposure to swine, and consumption of raw vegetables, raw shellfish, and untreated water. Informed approval was obtained from all participants. Individuals were divided into two separate groups taking into consideration exposition to swine: 97 unexposed (NE; 27 men and 70 women) and 101 exposed (E; 74 men and 27 women). Individuals included in the E group were made up of swine farmers, pig handlers, and swine veterinarians, whereas the NE group was made up of volunteers with no contact with swine.

Blood samples were obtained from all the participants by venipuncture, and sera were obtained and frozen at –20°C until used. RNA was extracted from 140 μL of each serum using a commercial kit following the manufacturer's instructions (QIampViral RNA Kit; Qiagen, Valencia, CA). Two pairs of degenerate oligonucleotide primers[13] were used to amplify a 348-bp fragment of ORF-2 of HEV using a reverse transcriptase–nested polymerase chain reaction (PCR).[14] These primers were based on 18 human HEV sequences and the swine HEV prototype strain from the United States. A positive control from a naturally infected pig (GenBank accession number AY323506) was included in each procedure. Different stages of assay were performed in different places to avoid the possibility of cross-contamination. The PCR products were separated by electrophoresis in 2% agarose and were detected by staining with ethidium bromide.

Sera from all individuals were tested for the presence of HEV antibodies (anti-HEV IgG and IgM) using a commercial ELISA (Fortress Diagnostics, Antrim, UK) according to the manufacturer's instructions. This kit used polystyrene microwell strips precoated with recombinant HEV antigens (HEV-Ag) corresponding to structural proteins ORF2, derived from genotype 1. The sensitivity and specificity of the ELISA assay use in this study were determined by the manufacturer as 92% and 88%, respectively. Positive results obtained using this assay were confirmed by means of an HEV immunoblot test (Recomblot HEV IgG/IgM; Mikrogen, Martinsried, Germany). Antigens used in this kit were the N-terminal part of the capsid antigen (GST fusion protein O2N, 50 kd), the C-terminal part of the capsid antigen (triple band; O2C 38–41 kd), the middle part of the capsid antigen (O2M; 28 kd), and the ORF3 protein (O3; 15 kd) of genotypes 1 and 2.

* Address correspondence to M. T. Pérez-Gracia, Departamento de Atención Sanitaria, Salud Pública y Sanidad Animal, Facultad de Ciencias Experimentales y de la Salud, Universidad CEU Cardenal Herrera, Avenida Seminario s/n 46113, Moncada, Valencia, Spain. E-mail: teresa@uch.ceu.es

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.

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Authors' addresses: Carolina Galiana, Salceda Fernández-Barredo, Angel García, María Teresa Gómez, and Maria Teresa Pérez-Gracia, Area Microbiología, Facultad de Ciencias Experimentales y de la Salud, Universidad CEU Cardenal Herrera, Avenida Seminario s/n 46113, Moncada, Valencia, Spain.

REFERENCES

1. Perez-Gracia MT, Rodriguez-Iglesias M, 2003. [Hepatitis E virus: current status]. *Med Clin (Barc) 121:* 787–792.
2. Schlauder GG, Dawson GJ, Erker JC, Kwo PY, Knigge MF, Smalley DL, Rosenblatt JE, Desai SM, Mushahwar IK, 1998. The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. *J Gen Virol 79:* 447–456.
3. 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.
4. Aggarwal R, Krawczynski K, 2000. Hepatitis E: an overview and recent advances in clinical and laboratory research. *J Gastroenterol Hepatol 15:* 9–20.
5. Perez-Gracia MT, Garcia-Valdivia MS, Galan F, Rodriguez-Iglesias MA, 2004. Detection of hepatitis E virus in patients sera in southern Spain. *Acta Virol 48:* 197–200.
6. Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, Emerson SU, Purcell RH, 2002. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol 40:* 117–122.
7. Withers MR, Correa MT, Morrow M, Stebbins ME, Seriwatana J, Webster WD, Boak MB, Vaughn DW, 2002. Antibody levels to hepatitis E virus in North Carolina swine workers, non-swine workers, swine, and murids. *Am J Trop Med Hyg 66:* 384–388.
8. Bouwknegt M, Engel B, Herremans MM, Widdowson MA, Worm HC, Koopmans MP, Frankena K, Husman AM, De Jong MC, Der Poel WH, 2007. Bayesian estimation of hepatitis E virus seroprevalence for populations with different exposure levels to swine in The Netherlands. *Epidemiol Infect 136:* 1–10.
9. Drobeniuc J, Favorov MO, Shapiro CN, Bell BP, Mast EE, Dadu A, Culver D, Iarovoi P, Robertson BH, Margolis HS, 2001. Hepatitis E virus antibody prevalence among persons who work with swine. *J Infect Dis 184:* 1594–1597.
10. Hsieh SY, Meng XJ, Wu YH, Liu ST, Tam AW, Lin DY, Liaw YF, 1999. Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. *J Clin Microbiol 37:* 3828–3834.
11. Olsen B, Axelsson-Olsson D, Thelin A, Weiland O, 2006. Unexpected high prevalence of IgG-antibodies to hepatitis E virus in Swedish pig farmers and controls. *Scand J Infect Dis 38:* 55–58.
12. Vulcano A, Angelucci M, Candelori E, Martini V, Patti AM, 2007. HEV prevalence in the general population and among workers at zoonotic risk in Latium Region. *Ann Ig 19:* 181–186.
13. Huang FF, Haqshenas G, Guenette DK, Halbur PG, Schommer SK, Pierson FW, Toth TE, Meng XJ, 2002. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. *J Clin Microbiol 40:* 1326–1332.
14. Fernandez-Barredo S, Galiana C, Garcia A, Vega S, Gomez MT, Perez-Gracia MT, 2006. Detection of hepatitis E virus shedding in feces of pigs at different stages of production using reverse transcription-polymerase chain reaction. *J Vet Diagn Invest 18:* 462–465.
15. Amon JJ, Drobeniuc J, Bower WA, Magana JC, Escobedo MA, Williams IT, Bell BP, Armstrong GL, 2006. Locally acquired hepatitis E virus infection, El Paso, Texas. *J Med Virol 78:* 741–746.
16. Perez-Gracia MT, Mateos ML, Galiana C, Fernández-Barredo S, García A, Goméz MT, Moreira V, 2007. Autochthonous hepatitis E infection in a slaughterhouse worker. *Am J Trop Med Hyg 77:* 893–896.
17. Mizuo H, Yazaki Y, Sugawara K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H, 2005. Possible risk factors for the transmission of hepatitis E virus and for the severe form of hepatitis E acquired locally in Hokkaido, Japan. *J Med Virol 76:* 341–349.

18. Fernandez-Barredo S, Galiana C, Garcia A, Gomez-Munoz MT, Vega S, Rodriguez-Iglesias MA, Perez-Gracia MT, 2007. Prevalence and genetic characterization of hepatitis E virus in paired samples of feces and serum from naturally infected pigs. *Can J Vet Res 71:* 236–240.
19. Dalton HR, Thurairajah PH, Fellows HJ, Hussaini HS, Mitchell J, Bendall R, Banks M, Ijaz S, Teo CG, Levine DF, 2007. Autochthonous hepatitis E in southwest England. *J Viral Hepat 14:* 304–309.
20. Herremans M, Bakker J, Duizer E, Vennema H, Koopmans MP, 2007. Use of serological assays for diagnosis of hepatitis E virus genotype 1 and 3 infections in a setting of low endemicity. *Clin Vaccine Immunol 14:* 562–568.
21. Gessoni G, Manoni F, 1996. Hepatitis E virus infection in north-east Italy: serological study in the open population and groups at risk. *J Viral Hepat 3:* 197–202.
22. Boutrouille A, Bakkali-Kassimi L, Cruciere C, Pavio N, 2007. Prevalence of anti-hepatitis E virus antibodies in French blood donors. *J Clin Microbiol 45:* 2009–2010.
23. Dalton HR, Fellows HJ, Gane EJ, Wong P, Gerred S, Schroeder B, Croxson MC, Garkavenko O, 2007. Hepatitis E in New Zealand. *J Gastroenterol Hepatol 22:* 1236–1240.
24. Bortoliero AL, Bonametti AM, Morimoto HK, Matsuo T, Reiche EM, 2006. Seroprevalence for hepatitis E virus (HEV) infection among volunteer blood donors of the Regional Blood Bank of Londrina, State of Parana, Brazil. *Rev Inst Med Trop Sao Paulo 48:* 87–92.
25. Mateos ML, Camarero C, Lasa E, Teruel JL, Mir N, Baquero F, 1998. Hepatitis E virus: relevance in blood donors and other risk groups. *Vox Sang 75:* 267–269.
26. Buti M, Dominguez A, Plans P, Jardi R, Schaper M, Espunes J, Cardenosa N, Rodriguez-Frias F, Esteban R, Plasencia A, Salleras L, 2006. Community-based seroepidemiological survey of hepatitis E virus infection in Catalonia, Spain. *Clin Vaccine Immunol 13:* 1328–1332.
27. Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, Teo CG, 2006. Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. *Transfusion Med 16:* 79–83.

別紙様式第２－１

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	乾血小板		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

Keiji Matsubayashi, Jong-Hon Kang, Hidekatsu Sakata, Kazuaki Takahashi, Motohiro Shindo, Masaru Kato, Shinichiro Sato, Toshiaki Kato, Hiroyuki Nishimori, Kunihiko Tsuji, Hiroyuki Maguchi, Jun-ichi Yoshida, Hiroshi Maekubo, Shunji Mishiro, and Hisami Ikeda

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.

From the Hokkaido Red Cross Blood Center, Sapporo; the Center for Gastroenterology, Teine Keijinkai Hospital, Sapporo; the Department of Medical Sciences, Toshiba General Hospital, Tokyo; the Third Department of Internal Medicine, Asahikawa Medical College, Asahikawa; the Department of Internal Medicine, Kitami Red Cross Hospital, Kitami; and the Department of Internal Medicine, Teine Keijinkai Hospital, Sapporo, Japan.

Address reprint requests to: Keiji Matsubayashi, Hokkaido Red Cross Blood Center, 2-2 Yamanote, Nishi-ku, Sapporo 063-0002, Japan; e-mail: kmatsu@hokkaido.bc.jrc.or.jp.

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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

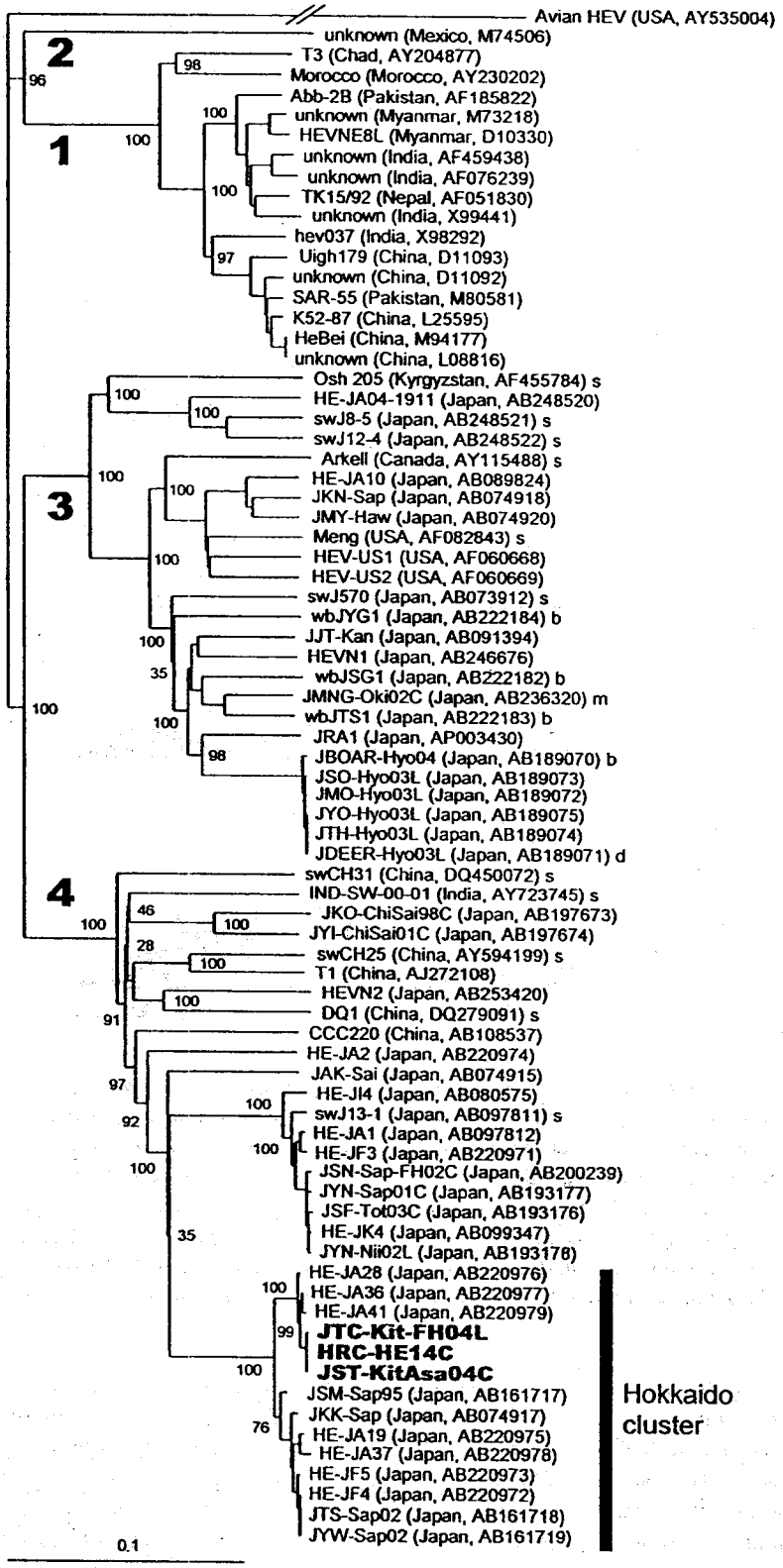

Fig. 1. Phylogenetic tree constructed by the neighbor-joining method based on the entire or nearly entire sequences of HEV genome of 77 isolates using an avian HEV (AY535004) as an outgroup. After the isolate name, the name of the country where the strain was isolated and accession numbers are shown in parentheses. The numbers 1, 2, 3, and 4 in bold indicated HEV genotypes. The 3 isolates HRC-HE14C from the causative donor, JST-KitAsa04C from the patient, and JTC-Kit-FH04L from the donor's father are indicated in bold. The letters "s," "b," "d," and "m" after parentheses denote HEV isolates from farmed pig, wild bore, wild deer, and mongoose, respectively. A vertical bar represents a cluster consisting of strains indigenous to Hokkaido, Japan. Bootstrap values are indicated for the major nodes as a percentage of the data set obtained from 1000 resamplings.

September 6 was released because it showed normal ALT and passed all the current blood screening tests. Transfusion was carried out 3 days after the blood donation, and the total amount of HEV in the PLT concentrate was estimated to be approximately 5.4 log copies. He was asymptomatic and did not feel tired or febrile in the periods near the two occasions of blood donation.

A minioutbreak of HEV infection in family members of the causative donor

Besides the causative donor, HEV RNA was detected in the blood of his 69-year-old father, who developed acute hepatitis on September 14, 2004, and finally died of fulminant hepatitis on October 14. Retrospective analysis of the father's blood sample taken on September 24, 41 days after the dining, revealed that the HEV strain, JTC-Kit-FH04L, was genotype 4. HEV RNA sequence analysis of the HEV isolates from the causative donor and his father showed only 9-nucleotide differences of 6588 nucleotides, suggesting that the two strains were extremely close but not identical (Fig. 1).

By retroactive interviewing, it was revealed that the causative donor and his 12 relatives gathered to enjoy grilled meats

including pig liver and intestines at a barbecue restaurant on August 14, 2004.[21] Blood samples from the relatives were tested for HEV markers with informed consent. Seven of the family members who ate grilled pig liver and/or intestines had IgM- and/or IgG-class anti-HEV in the blood samples taken 37 to 92 days after the barbecue party. Retrospectively, in the previous 6 months or more, dining out at that restaurant was the only occasion all the 13 relatives had eaten together.

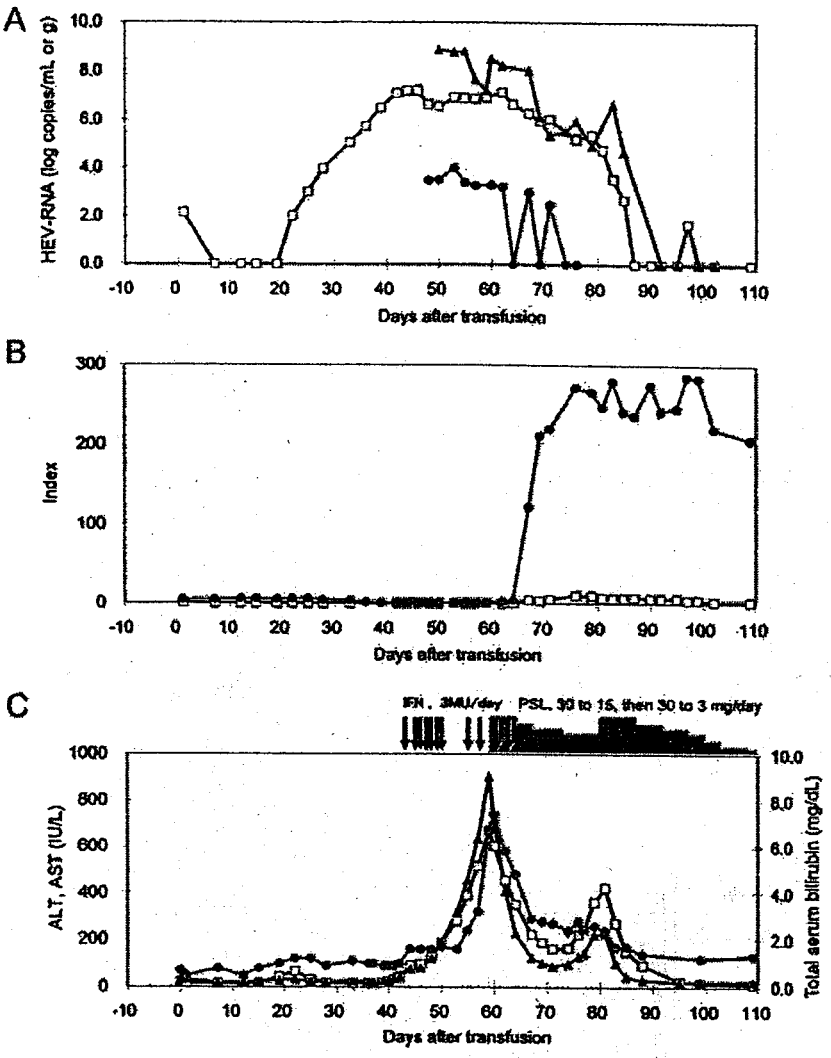

Fig. 2. Clinical course of transfusion-transmitted hepatitis E with kinetics of (A) HEV RNA, (B) serologic, and (C) biochemical markers after transfusion. The patient had transfusion of PLT concentrates contaminated with HEV on Day 0. (A) HEV RNA load was represented as log copies per mL of serum (□) or saliva (●) or per g of feces (▲). There were no data between Day 0 and Day 44 in feces and saliva. (B) Cutoff values of anti-HEV IgM (□) and IgG (●) antibodies are 30 and 13, respectively. (C) Medications were administered with IFN-α from Day 43 through Day 62 and with PSL from Day 59 through Day 112. (□) ALT; (▲) AST; (●) total serum bilirubin.

Clinical course of the patient

It was confirmed that the PLT concentrate (approx. 200 mL) contaminated with HEV was transfused to a 64-year-old Japanese male patient with non-Hodgkin's lymphoma on September 9, 2004, as shown Day 0 in Fig. 2. The patient had been treated with autologous peripheral blood stem cell transplantation accompanied with heavy chemotherapy since July 30, 2004. In the first 3 weeks after the transfusion, liver function tests sustained to be normal. On Day 22, the ALT level increased transiently at 67 IU per L, and HEV was detected in serum. While the ALT level returned to normal, the viral load in serum showed an exponential increase. Levels of aspartase aminotransferase (AST) and ALT took an upward turn on Day 41. There was no evidence for acute infection of hepatitis A virus, HBV, HCV, cytomegalovirus, or Epstein-Barr virus. He was diagnosed as acute hepatitis E. On Day 45, he was referred to the liver unit of Teine Keijinkai Hospital to treat presumed developing acute hepatitis E. Despite antiviral therapy with interferon (IFN) from Day 45, 2′,5′-oligoisoadenylate synthetase in serum never showed apparent increase and no obvious decrement of viral load had obtained (Fig. 2A). Levels of AST and ALT indicated creeping increase to reach highest levels of 903 and 673 IU per L on Day 59, respectively (Fig. 2C). The treatment was switched from IFN to predonisolone (PSL) in expectation of its anti-inflammatory effect. From Day 59 after induction of PSL treatment, AST and ALT showed rapid decrease and improvement of prothrombin time was observed (data not shown). Dosage of PSL was tapered gradually and discontinued on Day 113. Soon after anti-HEV IgG emerged on Day 67, HEV load in the serum sample had declined rapidly, although anti-HEV IgM in the serum sample remained negative (Figs. 2A and 2B). The levels in aminotransferases were normalized after Day 95 (Fig. 2C). The HEV strain JST-KitAsa04C detected in the patient was genotype 4 and its entire sequence analysis showed only a 1-nucleotide difference of 7255 nucleotides, suggesting the two isolates were identical (Fig. 1).

Serial quantitative changes of HEV load in serum, saliva, and feces of the patient

HEV RNA and anti-HEV were measured for every serum sample before and after the transfusion. In addition, HEV loads were also assessed prospectively for feces and saliva after his transference to the liver unit on Day 45. Any marker for HEV was not detected in serum sampled 37 days before the transfusion. A small amount of HEV RNA was transiently detected in his serum on Day 1, the next day of the transfusion. After the reappearance on Day 22, HEV RNA showed exponential increment with doubling every 29 hours and reached the peak level of 7.2 log copies per mL on Day 44. Beyond its plateau phase lasting 3 weeks, viral load revealed gradual decline over 2 weeks and thereafter decreased promptly. HEV viremia had been finally sustained for 63 days. HEV RNA remained detectable up to Day 97 in serum, Day 71 in saliva, and Day 85 in feces. Peak levels of HEV RNA were found on Day 53 in saliva at 4.0 log copies per mL and on Day 50 in feces at 8.9 log copies per g, respectively. HEV RNA was no longer detectable after Day 99 (Fig. 2A).

DISCUSSION

In Japan, a nonendemic country for hepatitis E, HEV infection is occurring more frequently than previously recognized. The prevalence of anti-HEV IgG in healthy Japanese persons ranged from 1.9 to 14.1 percent, depending on the geographic area,[20] and the prevalence of HEV RNA among Japanese blood donors with ALT level of at least 201 IU per L was 2.8 percent.[21] The risks of transfusion transmission of HEV might be low; however, five molecularly confirmed cases of transfusion-transmitted HEV infection have been reported in nonendemic countries so far.[12-16] In none of them, HEV infection routes of the causative donors are known. In this report, we have described the first case that the infection route of donor is clarified as zoonotic food-borne. The conclusion is based mainly on two observations.

First, by the epidemiologic study, the donor was determined to be infected in a minioutbreak of HEV infection in the context of food-borne transmission. Six of the 13 relatives who dined out together were positive for the presence of HEV RNA and/or IgM anti-HEV in their serum samples obtained 37 to 92 days after dining at the restaurant (Appendix 1). As for 4 relatives who were positive for the presence of IgM anti-HEV, HEV viremia might have transiently occurred without any symptom and had subsided by the time when blood samples were taken. Since IgM anti-HEV are regarded as the markers of acute HEV infection besides HEV RNA,[10] these facts strongly suggest that family members had recently become infected with HEV probably at the same time and remained asymptomatic. The party at the barbecue restaurant was the only opportunity all the 13 members had eaten together in the estimated period of HEV infection, 2 to 10 weeks.[22,23] Although it was difficult to identify the source of infection because no meat was left, they ingested various kinds of pig meats including liver and intestines, according to the replies to the questionnaire from the family members.[24] From this retrospective research, it is strongly suspected that the family members shared the motive of infection with HEV by ingestion of pig liver and intestines. In Japan, HEV has been isolated from farmed pigs,[9,25] wild deer,[8,26,27] and wild boar[10,11,26,27] as well as humans and recent studies also indicated that HEV is moderately resistant to heat inactivation.[28,29] Some reports suggest that a number of hepatitis E cases in Japan may be via a zoonotic food-borne route.[8-11,25-27,30]

Second, a single transmission route of HEV in this minioutbreak is corroborated by molecularly confirmed facts. From full-length sequence analysis, HEV RNAs detected in the donor and recipient were identical and closely related to that in his father. Among the strains of genotype 4 indigenous to Hokkaido, Japan, these three strains were segregated into a distinct cluster with a bootstrap value of 99 percent in a phylogenetic tree based on the entire or nearly entire sequences of HEV genome. Moreover, when comparing 412-nucleotide sequences (nucleotides 5985-6396 of HRC-HE14C) of ORF2 region, where many sequences of Japanese swine HEV are retrievable in DDBJ/EMBL/GenBank nucleotide sequence databases, high similarity (409/412 nucleotides, 99.3%) was observed between the HEV sequences derived from the causative donor and his father and strain swJL145 (AB105902),[9] which was detected in pig liver sold at a drug store in Hokkaido, Japan.

To date, in acute hepatitis E including transfusion transmission cases, dynamic relationships between infection markers for HEV and disease progression throughout the course from HEV transmission to convalescence of disease have not been demonstrated. This is the first case of acute hepatitis E, in which HEV kinetics in serum as well as in feces and saliva were described by using quantitative RT-PCR for HEV RNA from transfusion up to the end of viremia accompanied by disease progression, and the emergence and increase of anti-HEVs. In the current case, HEV viremia had lasted for 9 weeks or more and viral load reached its peak 15 days before the peak of aminotransferase level and died out promptly right after the appearance of anti-HEV IgG on Day 67. The results led us to understand the chronologic relationship between preceding viremia and after emergence and increase of anti-HEV.

Besides serum, the kinetics of HEV load in feces and saliva were concomitantly observed for the first time in hepatitis E in humans. After the transmission, HEV RNA remained detectable until Day 71 in saliva and Day 85 in feces. Among sera, saliva, and feces, every time point at peak viral loads resembled each other, 50 to 60 days after transmission. These facts may indicate that viral loads in

saliva and feces would also reflect viremia state. In addition, the results for saliva suggest that besides fecal-oral route, oral-oral transmission manner can be another route of human-to-human infection of HEV.

Soon after the transference to liver unit in the hospital, IFN-α therapy was started against HEV infection, indicating the exponential increase of viral load in sera. The levels in 2′,5′-oligoadenylate synthetase, however, induced by IFN and regarded as a predictive marker for favorable IFN efficacy,[31] did not show sufficient increase in serum (data not shown), and HEV load monitored concomitantly indicated no actual decrement during treatment. Thereafter, single-nucleotide polymorphisms in markers predicting the therapeutic efficacy of IFN, such as mannose-binding lectin,[32] MxA,[33] LMP7,[34] and osteopontin,[35] were examined, and all of them did not show the phenotype associated with favorable efficacy of IFN (data not shown).

Throughout his clinical course, no distinct positive result for IgM anti-HEV was observed. It is possible that the concentration of IgM anti-HEV was too low to be detected by the method we used. In fact, some of his samples showed equivocal reaction. Furthermore, underlying disease and the preceding treatment including autologous peripheral blood stem cell transplantation and large dosage chemotherapy might have led the patient to an immunocompromised state that responds inadequately for HEV infection. In fact, both serum levels in IgG and IgM had been indicated consistently less than lower limitation of normal ranges in the entire course (data not shown).

We should note that the present case was not revealed if the two practices had not been introduced, which are not widespread outside Japan. They are ALT screening and donor blood sample repository system. As a safety measure, the Japanese Red Cross Blood Center introduced ALT testing for a surrogate marker for non-A, non-B hepatitis virus infection. Because ALT testing contributes little for HCV infection after HCV antibody testing started, ALT screening has been discontinued in the United States and some other countries. Although the cutoff value may need to be reevaluated, the current case suggests that ALT testing may contribute to excluding blood with the presence of HEV. On the other hand, the Japanese Red Cross has established storing repository samples of all donations since 1996. Blood samples are collected from each donation and stored for 10 years at –30°C to investigate for lookback study such as the suspected cases of transfusion-transmitted infection and alloantibodies for TRALI. This system plays a very important role in the hemovigilance system in Japan.[36,37]

In the present case of transfusion-transmitted acute hepatitis E, the infection route in the blood donor was, for the first time, clarified to be zoonotic food-borne manner. In addition, the entire course including incubation period and disease progression in acute HEV infection was followed by serologic and virologic markers, and the patient was treated by monitoring them. To our knowledge, this is the first report for acute HEV infection in humans, in which various infection markers were prospectively monitored simultaneously with disease progression, excepting experimental hepatitis E in a volunteer.[38]

Our data suggest that hepatitis E is likely caused by consumption of contaminated pig meat, and there is a risk of transfusion transmission of HEV in Japan. The most effective preventive measure to reduce the risk of blood-borne transmission is to screen the blood supply for HEV or to implement pathogen inactivation. The epidemiology and the transfusion-related risks for HEV infection have not been fully understood in industrialized countries including Japan. We are undertaking epidemiologic studies of HEV infection in Japanese blood donors and a feasibility study of NAT screening for HEV in Hokkaido, Japan.

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REFERENCES

1. Purcell RH, Emerson SU. Hepatitis E virus. In: Knipe GM, Howley PM, editors. Fields virology. 4th ed. Philadelphia (PA): Lippincott, Williams & Wilkins; 2001. p. 3051-61.
2. Schlauder GG, Dawson GJ, Erker JC, Kwo PY, Knigge MF, Smalley DL, Rosenblatt JE, Desai SM, Mushahwar IK. The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. J Gen Virol 1998;79:447-56.
3. Worm HC, Wurzer H, Frosner G. Sporadic hepatitis E in Austria. N Engl J Med 1998;339:1554-5.
4. Takahashi K, Iwata K, Watanabe N, Hatahara T, Ohta Y, Baba K, Mishiro S. Full-genome nucleotide sequence of a hepatitis E virus strain that may be indigenous to Japan. Virology 2001;287:9-12.
5. Mizuo H, Suzuki K, Takikawa Y, Sugai Y, Tokita H, Akahane Y, Itoh K, Gotanda Y, Takahashi M, Nishizawa T, Okamoto H. Polyphyletic strains of hepatitis E virus are responsible for sporadic cases of acute hepatitis in Japan. J Clin Microbiol 2002;40:3209-18.
6. Mansuy JM, Peron JM, Abravanel F, Poirson H, Dubois M, Miedouge. M, Vischi F, Alric L, Vinel JP, Izopet J. Hepatitis E in the south west of France in individuals who have never visited an endemic area. J Med Virol 2004;74:419-24.
7. Ijaz S, Arnold E, Banks M, Bendall RP, Cramp ME, Cunningham R, Dalton HR, Harrison TJ, Hill SF, Macfarlane L,

Meigh RE, Shafi S, Sheppard MJ, Smithson J, Wilson MP, Teo CG. Non-travel-associated hepatitis E in England and Wales: demographic, clinical, and molecular epidemiological characteristics. J Infect Dis 2005;192:1166-72.
8. Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003;362:371-3.
9. Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, Okamoto H. 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 2003;84:2351-7.
10. Tamada Y, Yano K, Yatsuhashi H, Inoue O, Mawatari F, Ishibashi H. Consumption of wild boar linked to cases of hepatitis E. J Hepatol 2004;40:869-70.
11. Li TC, Chijiwa K, Sera N, Ishibashi T, Etoh Y, Shinohara Y, Ishida M, Sakamoto S, Takeda. N Miyamura T. Hepatitis E virus transmission from wild boar meat. Emerg Infect Dis 2005;11:1958-60.
12. Matsubayashi K, Nagaoka Y, Sakata H, Sato S, Fukai K, Kato T, Takahashi K, Mishiro S, Imai M, Takeda N, Ikeda H. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 2004;44:934-40.
13. Mitsui T, Tsukamoto Y, Yamazaki C, Masuko K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. J Med Virol 2004;74:563-72.
14. Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, Teo CG. Transfusion-transmitted hepatitis E in a "nonhyperendemic" country. Transfus Med 2006;16:79-83.
15. Tamura A, Shimizu YK, Tanaka T, Kuroda K, Arakawa Y, Takahashi K, Mishiro M, Shimizu K, Moriyama M. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. Hepatol Res 2007;37:113-20.
16. Colson P, Coze C, Gallian P, Henry M, De Micco P, Tamalet C. Transfusion-associated hepatitis E, France. Emerg Infect Dis 2007;13:648-9.
17. Thompson JD, Higgins DG, Gibson TJ, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673-80.
18. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406-25.
19. Page RD. TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 1996;12: 357-8.
20. Li TC, Zhang J, Shinzawa H, Ishibashi M, Sata M, Mast EE, Kim K, Miyamura T, Takeda N. Empty virus-like particle-based enzyme-linked immunosorbent assay for antibodies to hepatitis E virus. J Med Virol 2000;62:327-33.
21. Gotanda Y, Iwata A, Ohnuma H, Yoshikawa A, Mizoguchi H, Endo K, Takahahsi M, Okamoto H. Ongoing subclinical infection of hepatitis E virus among blood donors with an elevated alanine aminotransferase level in Japan. J Med Virol 2007;79:734-42.
22. Khuroo MS. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. Am J Med 1980; 68:818-24.
23. Balayan MS, Andjapardze AG, Savinskaya SS, Ketiladze ES, Braginsky DM, Savinov AP, Poleschuk VF. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. Intervirology 1983;20:23-31.
24. Kato M, Taneichi K, Matsubayashi K. A mini-outbreak of HEV infection in those who enjoyed *Yakiniku* party: one died of fulminant hepatitis. Kanzo 2004;45:688.
25. Takahashi M, Nishizawa T, Miyajima H, Gotanda Y, Iita T, Tsuda F, Okamoto H. Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. J Gen Virol 2003;84:851-62.
26. Takahashi K, Kitajima N, Abe N, Mishiro S. Complete or near-complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. Virology 2004;330:501-5.
27. Sonoda H, Abe M, Sugimoto T, Sato Y, Bando M, Fukui E, Mizuo H, Takahashi M, Nishizawa T, Okamoto H. Prevalence of hepatitis E virus (HEV) infection in wild boars and deer and genetic identification of a genotype 3 HEV from a boar in Japan. J Clin Microbiol 2004;42:5371-4.
28. Emerson SU, Arankalle VA, Purcell RH. Thermal stability of hepatitis E virus. J Infect Dis 2005;192:930-3.
29. Tanaka T, Takahashi M, Kusano E, Okamoto H. Development and evaluation of an efficient cell-culture system for hepatitis E virus. J Gen Virol 2007;88:903-11.
30. Abe T, Aikawa T, Akahane Y, Arai M, Asahina Y, Atarashi Y, Chayama K, Harada H, Hashimoto N, Hori A. Demographic, epidemiological, and virological characteristics of hepatitis E virus infections in Japan based on 254 human cases collected nationwide. Kanzo 2006;47:384-91.
31. Schattner A, Merlin G, Wallach D, Rosenberg H, Bino T, Hahn T, Levin S, Revel M. Monitoring of interferon therapy by assay of 2′-5′ oligo-isoadenylate synthetase in human peripheral white blood cells. J Interferon Res 1981;1:587-94.
32. Matsushita M, Hijikata M, Ohta Y, Iwata K, Matsumoto M, Nakao K, Kanai K, Yoshida N, Baba K, Mishiro S. Hepatitis C virus infection and mutations of mannose-binding lectin gene MBL. Arch Virol 1998;143:645-51.
33. Mochida S, Hashimoto M, Matsui A, Naito M, Inao M, Nagoshi S, Nagano M, Egashira T, Mishiro S, Fujiwara K. Genetic polymorphisms in promoter region of osteopontin gene may be a marker reflecting hepatitis activity in chronic hepatitis C patients. Biochem Biophys Res Commun 2004;23:1079-85.

34. Hijikata M, Ohta Y, Mishiro S. Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt -88) correlated with the response of hepatitis C patients to interferon. Intervirology 2000;43:124-7.
35. Sugimoto Y, Kuzushita N, Takehara T, Kanto T, Tatsumi T, Miyagi T, Jinushi M, Ohkawa K, Horimoto M, Kasahara A, Hori M, Sasaki Y, Hayashi N. A single nucleotide polymorphism of the low molecular mass polypeptide 7 gene influences the interferon response in patients with chronic hepatitis C. J Viral Hepat 2002;9:377-84.
36. Satake M. Japanese repositories. Transfusion 2007;47:1105.
37. Okazaki H. The benefits of the Japanese haemovigilance system for better patient care. ISBT Sci Ser 2007;2:104-9.
38. Chauhan A, Jameel S, Dilawari JB, Chawla YK, Kaur U, Ganguly NK. Hepatitis E virus transmission to a volunteer. Lancet 1993;341:149-50.

APPENDIX 1

HEV infection markers in the 13 family members who participated in the dinner on August 14, 2004

Number*	Age (years)	Sex	Days after Aug 14, 2004	ALT (IU/L)	HEV markers		
					RNA (10^6/mL)	IgM† (index)	IgG‡ (index)
1	39	Male	23	27	+(3.1)	–(3.4)	–(2.0)
			37	236	+(4.8)	+(60.4)	+(14.2)
			49	70	+(2.1)	+(269.5)	+(154.7)
			53	44	–	+(257.8)	+(150.5)
			77	20	–	+(174.6)	+(163.0)
2	69	Male	41	1511	+(2.6)	+(187.2)	+(271.4)
3	43	Male	92	34	–	+(174.7)	+(297.7)
4	68	Male	79	15	–	+(51.7)	+(283.3)
5	37	Female	79	13	–	+(110.9)	+(90.3)
6	15	Male	90	17	–	+(63.3)	+(250.6)
7	58	Female	79	25	–	–(4.0)	+(25.9)
8	67	Female	79	15	–	–(1.4)	–(12.9)
9	38	Female	89	12	–	–(6.1)	–(1.1)
10	15	Male	77	19	–	–(0.3)	–(0.5)
11	14	Male	77	19	–	–(7.5)	–(0.3)
12	46	Male	90	15	–	–(2.2)	–(0.4)
13	6	Female	90	15	–	–(26.6)	–(1.1)

Data shown were originally reported by Kato et al.[24] without describing quantitative test results of antibodies and viral RNA and follow-up data of the causative donor.
* Number 1 is the causative donor; Number 2 is the donor's father and died of hepatitis E; others are their relatives.
† Positive ≥30 index.
‡ Positive ≥13 index.

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研究報告の概要

＜目的＞
中国の4つの都市における血液ドナー中のHEV陽性率を評価し、HEV感染を排除するためのALT測定の価値を評価すること。
＜方法＞
ルーチンのスクリーニング検査（HCV抗体、HIV 1/2抗体、HBsAg、梅毒およびALT）で陰性と判定されたドナー検体とALT値が高いだけの検体を、中国の4つの都市（北京、ウルムチ、昆明、広州）の4つの血液センターから2005年に収集し、-40℃で冷凍した。全部で6,665の血液ドナーの検体について、2007年にHEV IgG抗体、HEV IgM抗体、HEV Agの測定を行った。
＜結果＞
検査を実施した6,665の血液ドナーのうち、HEV IgG抗体、HEV IgM抗体、HEV Agの各々の陽性率は、24.23%(1,615/6,665)、1.08%(72/6,665)、0.03%（2/6,665）であった。ALTのみが高かった487のドナーのHEV IgG抗体、HEV IgM抗体、HEV Agの陽性率（30.80%、2.05%、0.21%）はすべて、ルーチンスクリーニングで陰性であった6,178のドナーの陽性率（23.71%、1.00%、0.02%）よりも高かった（P<0.05）。2名のHEV Ag陽性ドナーのうち、1名はルーチンのスクリーニングで陰性で、HEV Ag ELISA S/COの平均値が3.4、HEV IgG抗体が陰性、HEV IgM抗体が陰性であった。他方の1名はALTのみが高く、HEV Ag ELISA S/COの平均値が18.0、HEV IgG抗体が陽性でS/COの平均値が10.8、HEV IgM抗体が陰性であった。
＜結論＞
HEVは中国における風土病である。中国におけるルーチンのスクリーニングで陰性と判定された血液ドナーの中で、1%がHEV IgM抗体陽性またはHEV Ag陽性であり、HEVに感染性がある可能性がある。ALTスクリーニングは、中国ではHEV感染血液の排除に一定の役割を有している可能性がある。

使用上の注意記載状況・その他参考事項等

代表として静注用ヘブスブリン－IHの記載を示す。
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ろ過膜処理（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。

報告企業の意見	今後の対応
中国における血液ドナーの約1%は、抗HEV IgM陽性又はHEV抗原陽性であり、HEV感染の可能性があるとの報告である。 静注用ヘブスブリン-IHについては、万一、原料血漿にHEVが混入したとしても、EMCおよびCPV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。 ヘブスブリンについては、EMCおよびCPV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では本剤の製造工程において十分なLRVが得られないため、製造工程における不活化・除去が十分であるとは説明困難である。そのため、ヘブスブリン用の原料血漿については、弊社にてHEVについてのミニプールNATを試行的に導入した。	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。

easy to use, FDA approved test to confirm repeat reactives or to resolve discrepant results is lacking.
Aims: To develop a supplemental test for confirming the presence of antibodies to *T. cruzi* in repeatedly reactive blood or plasma units identified by a screening assay.
Methods: The immunoblot assay is based on four different recombinant antigens (rAgs) FP3, FP6, FP10, and TcF, for the detection of antibodies to *T. cruzi*. Each rAg was constructed with multiple antigenic domains of *T. cruzi* including repetitive sequences and non-repetitive sequences. The rAgs are fitted as discrete lines onto the strip. Antibody responses were visually assessed against two internal calibrators (low and high) also applied to the immunostrip as discrete lines. The immunoblot assay sensitivity was evaluated with 688 RIPA confirmed chagasic specimens. The specificity was evaluated with 821 unscreened specimens from random U.S. blood donors and 531 specimens of 30 different unrelated medical conditions, including leishmaniasis, malaria, and autoimmune diseases, or potentially interfering substances. The interpretation of results was as follows: (a) no bands or a single test band = NEGATIVE; (b) two or more test bands with a least on band having intensity of 1+ or higher = POSITIVE; and (c) multiple faint test bands (±) = INDETERMINATE. All samples were initially tested in the PRISM Chagas screening assay; and reactive samples were also tested in two different ELISA and in a radio-immunoprecipitation assay (RIPA).
Results: All 688 chagasic samples showed two to four rAg test bands and were interpreted as positive in the immunoblot assay; sensitivity of 100% (688/688). Among 821 unscreened specimens of random donors, 819 showed none or a single test band, and one gave two faint test bands. One specimen was repeatedly reactive in PRISM Chagas assay, two reference ELISAs, and confirmed in RIPA as positive; while another specimen was non-reactive in these reference tests. Of the 531 specimens with disease states or potentially interfering substances, 525 tested negative, two confirmed positive, 1 false-positive, and three indeterminate.
Conclusions: The sensitivity of the immunoblot assay in the geographically-diverse group of chagasic specimens was 100% (688/688). The resolved specificity of random donor specimens was also 99.88% (819/820). The recombinant antigen based immunoblot assay, in multiple lots and run by multiple technicians, has demonstrated great potential as a supplemental test to confirm the presence of antibodies to *T. cruzi* in blood specimens. Design verification and validation of this assay are ongoing.

P-615

HEPATITIS B VIRUS DETECTION AMONG VOLUNTARY BLOOD DONORS IN THE MUNICIPALITY OF STRUMICA

Timova TT[1], Kocovska EK[1], Maninska LM[1], Momirovska TM[2], Stambolieva DS[1], Gorgevska VG[1], Kostovska SK[1]
[1]General Hospital, Strumica, Macedonia [2]General Hospital -Prilep, Prilep, Macedonia [3]National Institute for Transfusion Medicine, Skopje, Macedonia

In spite of the progress in the development of diagnostic, therapeutic and prophylactic methods, virus hepatitis still present a serious global health problem. The possibility of transmission of these infections through transfusion of blood and blood derivates implies obligatory control of the donated blood.
Aim: To show the prevalence of Hepatitis B (HBsAg) in volunteer blood donors for the period from 2001 till 2006.
Materials: The presence of virus markers was analyzed in the serum of 9166 blood donors who donated blood at the Department of transfuziology, General Hospital-Strumica, in the period from 2001 till 2006.
Methods: The samples were tested for the presence of viral markers (HBsAg), using tests for HBsAg (Abbott Auxyme Monoclonal EIA).
Results: The presence of markers for Hepatitis B (HBsAg) were found in 89 (0.97%) blood donors. In 2001 the presence of HBsAg was found in 12 blood donors, 2002 – in 20 blood donors, 2003 in 14 blood donors, 2004 in 17 blood donors, 2005 in 14 blood donors, 2006 in 12 blood donors. With A blood group were 42 (47.2%) blood donors, with 0 blood group were 28 (31.4%) blood donors, with B blood group were 10 (11.2%) blood donors and with AB blood group were nine (10.2%) blood donors.
Conclusion: The obligatory testing of the donors blood is of exceptional importance to prevent the transmission of diseases. Moreover, a significant ring in the chain for ensuring safe blood is the selection of a qualitative donor, that is a donor who donates blood volontarily, freely, anonymously and periodically.

P-616

OCCULT HEPATITIS B VIRUS INFECTION IN BLOOD DONORS FROM CENTRAL PORTUGAL

Teixeira MJ, Henggeler F, Diniz C, Caldeira M, Mateus F, Freitas C, Gonçalves H
Instituto Português di Sangue, Coimbra, Portugal

Background: The detection of HBV DNA in serum without HBsAg and with/without the presence of antibodies (anti-HBc/anti-HBs), defines the state of the occult hepatitis B virus infection. The prevalence in endemic areas varies from 7% to 19%, while in the west countries varies from 0% to 9%, being greater in people with anti-HBc and/or anti-HBs. Low serum HBV DNA titers, in the range of 100–1000 copies/mL, are typical in occult HBV infection. A high prevalence of occult HBV has been reported in hepatocellular carcinoma (HCC).
Aims: The appearance of the nucleic acid testing (NAT) with great sensibility allows us to identify a population with HBsAg negative but with low levels of HBV DNA in serum. In our Centre all donors are screened for HBV DNA, HIV RNA and HCV RNA.
Methods: In the screening of the hepatitis B serologic markers we have used ELISA and quimioluminiscence tests. In the screening of the HBV DNA we have used the Transcription Mediated Amplification (TMA) technology, in single testing, with predicted HBV detection rate of 50% and 95% of 3.1 and 7.4 IU/mL, respectively.In the screening of HBV viral load we have used PCR technology, with detection limit of 60 IU/mL.
Results: The Regional Blood Centre (Coimbra) started the screening of the HBV DNA to all donors in October 2006. Until November 2007, we have studied 20.881 donors. We found three cases of occult hepatitis B virus infection.
Conclusions: Some aspects need to be investigated, especially the relationship between the occult hepatitis B virus infection and the infectivity of the different blood components. The sensibility of the NAT is very important in the precocious detection of the HBV DNA in blood donors.

P-617

PREVALENCE OF HEPATITIS E VIRUS INFECTION IN BLOOD DONORS IN DIFFERENT CITIES OF CHINA

Ren FR[1], Wang ZY[1], Gong XY[1], Song ML[1], Lv QS[1], Tiemuer MHL[2], Yao FZ[3], Zheng YR[4], Wang YC[5], Zhuang H[6]
[1]Beijing Red Cross Blood Center, Beijing, China [2]Urumchi Blood Center of Xinjiang Uygur Autonomous Region, Urumchi, China [3]Yunnan Kunming Blood Center, Kunming, China [4]Guangzhou Blood Center, Guangzhou, China [5]National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China [6]Peking University Health Science Center, Beijing, China

Background: Hepatitis E virus (HEV) is a single strand and non-enveloped RNA virus. HEV infection is normally transmitted via the faeco-oral route. However HEV recently emerged as a transfusion-transmitted pathogen. Several transfusion-transmitted HEV infections have been reported in

HEV-hyperendemic or nonhyperendemic countries. In China, neither HEV antibodies nor HEV RNA are systematically tested in blood donors. Alanine aminotransferase (ALT) in serum/plasma has been tested in all blood donors since 1960s in China, before hepatitis B surface antigen screening. With the introduction of specific anti-HCV and viral nucleic acid testing (NAT), ALT test is no longer used in routine donor screening in many countries. However, ALT measurement is still retained as a screening tool for blood donors in China, in consideration that viral hepatitis is endemic in China, although ALT has low specificity for detecting individuals with transfusion-transmitted virus infection risk and its value is controversial.

Aims: To evaluate the prevalence of HEV infection among blood donors in four cities of China and to evaluate the value of ALT measurement for eliminating HEV infectious blood in blood donors.

Methods: Donor samples with negative results in routine screening (anti-HCV, anti-HIV1/2, HBsAg, syphilis and ALT) and samples with ALT elevated alone were collected from four blood centers in four Chinese cities, Beijing (North), Urumchi (Northwest), Kunming (Southwest), and Guangzhou (South) in 2005 and were frozen at -40?. A total of 6665 blood donor samples were tested for anti-HEV IgG, anti-HEV IgM and HEV Antigen (Ag) by enzyme-linked immunoassays (WANTAI Biological Enterprise Co. Ltd, Beijing, China) in 2007. Repeated positive results defined as a positive result. The Person Chi-Squared test or Fisher's exact test were used for the statistical analysis.

Results: Of the 6665 blood donors tested, the prevalence of anti-HEV IgG, anti-HEV IgM and HEV Ag were 24.23% (1615/6665), 1.08% (72/6665) and 0.03% (2/6665) respectively. The prevalence of anti-HEV IgG, anti-HEV IgM and HEV Ag were all higher in 487 donors with elevated ALT alone (30.80%, 2.05% and 0.21%, respectively) than in 6178 donors with negative results in routine screening (23.71%, 1.00% and 0.02%)

Table HEV Seroprevalence in blood donors

Samples	Cities	Numbers Tested	Anti-HEV IgG : %	Anti-HEV IgM %	HEV Ag (%)
Samples with negative results in routine screening	Beijing	2378	458 (19.26%)	30 (1.26%)	0 (0.00%)
	Urumchi	1910	341 (17.85%)	14 (0.73%)	1 (0.05%)
	Kunming	1170	431 (36.84%)	11 (0.94%)	0 (0.00%)
	Guangzhou	720	235 (32.64%)	7 (0.97%)	0 (0.00%)
	Total	6178	1465 (23.71%)	62 (1.00%)	1 (0.02%)
Samples with elevated ALT alone	Beijing	72	16 (22.22%)	2 (2.78%)	0 (0.00%)
	Urumchi	247	45 (18.22%)	1 (0.40%)	0 (0.00%)
	Kunming	152	84 (55.26%)	6 (3.95%)	0 (0.00%)
	Guangzhou	16	5 (31.25%)	1 (6.25%)	1 (6.25%)
	Total	487	150 (30.80%)	10 (2.05%)	1 (0.21%)
Total		6665	1615 (24.23%)	72 (1.08%)	2 (0.03%)

Data were shown as "numbers of positive samples (positive rate)"

($P < 0.05$). Of the two HEV Ag positive donors, one had negative results in routine screening and had average HEV Ag ELISA S/CO ratio of 3.4, anti-HEV IgG (-), anti-IgM (-); the other had elevated ALT alone and had average HEV Ag ELISA S/CO ratio of 18.0, anti-HEV IgG (+) with average S/CO ratio of 10.8, anti-HEV IgM (-). The following table shows the more detailed results.

Conclusions: Hepatitis E virus is endemic in China. Among blood donors with negative results in routine screening in China, about 1% are anti-HEV IgM (+) or HEV Ag (+) and may be HEV infectious. ALT screening may have some role in eliminating HEV infectious blood in China.

Acknowledgements: This work was supported by the '863' project (grant No. 2006AA02Z453) from Chinese Ministry of Science and Technology in 2006.

P-618

~~Abstract withdrawn.~~

P-619

POLYMORPHISM OF HLA-DRB1 OF THE UYGHURS IN CHRONIC HEPATISIS B IN KHOTAN AREA XINJIANG CHINA

Kurexijiang KT[1], Wupuer H[2], Yunusi K[2], Zhang Z[1], Shawuer R[1]
[1]*Uyghur Traditional Medicine Hospital of Khotan Area, Xinjiang, Khotan, China* [2]*Xinjiang Medical University, Urumqi, China* [3]*HLA Laboratory, Beijing Red Cross Blood Center, Beijing, China*

This abstract is read by title only.

P-620

IMPACT OF PHOTOCHEMICAL TREATMENT OF PLATELET COMPONENTS (INTERCEPT™) ON PLATELET AND RBC COMPONENT USE BY HEMATOLOGY PATIENTS DURING 3 YEARS OF ROUTINE PRACTICE

Osselaer JC[1], Doyen C[1], Defoin L[1], Debry C[1], Goffaux M[1], Messe N[1], Van Hooydonk M[2], Bosly A[2], Lin JS[3], Corash LM[3]
[1]*Cliniques Universitaires de Mont Godinne, Yvoir, Belgium* [2]*Hematology Svc, Cliniques Universitaire de Mont Godinne, Yvoir, Belgium* [3]*Cerus Corporation, Concord, CA, USA*

Background: In 2003 the Blood Transfusion Center (BTC), Cliniques Universitaires Mont Godinne (CUMG) initiated universal use of pathogen inactivated INTERCEPT Platelets (I-P, Cerus Europe BV, Amersfoort, Netherlands) for transfusion (txn) support of thrombocytopenia. Hematology patients require intensive txn support.

Aims: To examine the impact of I-P adoption on platelet (PLT) and red blood cell concentrate (RBC) use by hematology patients, the duration of support, the number of PLT txn per patient, total PLT dose per patient, and total RBC units per patient were compared for 3 years before I-P adoption, when only conventional PLT (C-P) were used, and for 3 years after adoption of I-P. RBC use served as a surrogate for hemostasis efficacy of PLT txn and was evaluated during periods of PLT support and periods without PLT txn support.

Methods: In both periods, PLT were collected by apheresis in reduced plasma concentration with process leukocyte reduction. For C-P, T-Sol (Fenwal, La Chatre, France) with a ratio to plasma of 70:30% was used. For I-P, Intersol (Cerus) with a ratio to plasma of 65:35% was used. I-P components (2.5–6.0–E11 PLT) were treated with amotosalen (150 μmol/L) plus UVA (3 J/cm sq) to inactivate pathogens and leukocytes. I-P replaced gamma irradiation, bacteria detection, and CMV serology. I-P and C-P were available for issue the day after collection. Days of txn support were calculated from the first PLT txn until 5 days after the last PLT txn. An

Effect of I-P Adoption on Platelet and RBC Use

Parameter	CP	IP	P
Platelet Use (mean/median)			
Patients supported	272	276	
Days of PLT support	31.6/15	33.1/15	0.70
PLT txn/pt	20.8/10	24.2/11	0.17
Total PLT dose (10^{11})/pt	87.3/41	100.8/43	0.19
RBC Use During Platelet Support (mean/median)			
Patients transfused	222	244	
Total RBC units/pt	16.4/8.0	17.6/7.0	0.64
RBC Use Outside of Platelet Support (mean/median)			
Patients transfused	237	235	
Total RBC units/pt	12.7/8.0	12.7/8.0	0.99

別紙様式第2-1

No. 30

医薬品 研究報告 調査報告書

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一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		ABC Newsletter, No. 26. 2008 Jul 4.	米国

研究報告の概要

○米国医師会がゲイ男性の供血5年延期を「容認できる」との考え
米国医師会(AMA)は、男性同性愛行為を行った供血者の供血延期期間を生涯から5年間に変更するとした連邦の方針を支持するという声明を採択した。この声明は2008年のAMA年次総会で採択され、「AMAは、現在の科学的エビデンスとリスク分析モデルに基づき、MSMに対する5年間の供血延期は容認できる(supportable)と認める」と述べている。AMAによると、「容認できる」という言葉は、基本的に、FDAに対して新しい方針を通知し「実施に協力する」ことを意味している。また、AMAは今回の変更に対して反対を主張しない。
FDAは1977年以降、採血事業者に対し、MSMの供血を生涯延期とすることを求めてきた。AMAの声明は、血液事業者団体が主張する1年間の供血延期により近いものとなっている。血液事業者は、供血延期は金銭や薬物と引き替えのセックスなどハイリスク行為に対して実施すべきであると主張してきた。また、最近ではゲイ・グループによる反対運動、政府機関や大学での議論も行われ、一部の大学では構内での移動採血を中止しようとする動きが出ていた。

報告企業の意見	今後の対応
米国医師会は、男性同性愛行為を行った供血者の供血延期期間を生涯から5年間に変更するとした連邦の方針を支持するという声明を採択したとの報告である。MSMのHIV等ウイルス感染率は高く、日本においても1年間の献血延期の他、検査目的の献血禁止などの対策を引き続き行っていく必要がある。	日本赤十字社は、輸血感染症対策として、男性と性的接触を持った男性は1年間献血不適としている。今後も引き続き情報の収集に努める。

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9

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2008 #26 July 4, 2008

INSIDE:

AMA Deems Five-Year Blood Donor Deferral for Gay Men "Supportable"

The American Medical Association (AMA) has adopted a statement indicating it may support changing the federal policy imposing a lifetime deferral for potential blood donors who have had sex with men to a five-year deferral.

The statement, adopted by the AMA House of Delegates at the 2008 AMA Annual Meeting June 14-18 in Chicago, reads: "*The AMA recognizes that based on existing scientific evidence and risk assessment models, a shift to a five-year deferral policy for blood donation from men who have sex with men (MSM) is supportable.*"

According to the AMA, the word "supportable" basically means the organization will notify the Food and Drug Administration of its new policy and "will be open to work with groups to advance the policy." In addition, the AMA will not speak up against efforts to examine changing the federal deferral requirement.

The FDA requires blood collectors to permanently defer men who have had sex with men (MSM) since 1977 from blood donation. The AMA statement, recommended by its Council on Science and Public Health, hews closer to the one-year deferral for MSM called for in a joint recommendation by America's Blood Centers, AABB, and the American Red Cross. The organizations said such a policy is more consistent with deferrals for other high-risk activities, such as receiving money or drugs for sex. They have argued that public education and the development of sensitive nucleic acid amplification tests have significantly reduced the residual risk of sexually transmitted diseases entering the blood supply.

In recent years, the controversial federal policy has sparked a number of protests by gay groups, who say it was inspired by and promotes unfair stereotypes, and arguments among government officials and academics, who say it is violates non-discrimination policies. This year alone, California's San Jose State University decided to ban blood drives on its 30,000-student campus over discrimination concerns. At Sonoma State University in Santa Rosa, a professor suggested ending blood drives there because the lifetime deferral violates the university's non-discrimination policy, though after a protracted debate involving faculty and students the university decided to allow blood collection to continue. The Santa Clara County Board of Supervisors in February voted unanimously to oppose the federal policy and encourage federal lobbyists to work to overturn the ban.

(continued on page 2)

AMA Statement (continued from page 1)

The AMA statement is expected help in those efforts because it underlines the problems of the mathematical models being used to assess risk.

"Any policy decision on blood donation deferral of the MSM population must be governed by the best available scientific evidence, but there are inherent weaknesses in mathematical models used in the risk assessments on this issue that continue to generate some uncertainty. With respect to the MSM population, it appears that a policy change from a permanent lifetime deferral to a five-year deferral following the last MSM contact may be supportable, but societal and ethical consequences must be analyzed should this decision be advanced," according to the statement.

The AMA considers current risk models weak because they rely on an insufficient number of studies and study groups that aren't large enough to provide predictive results, the organization said. AMA also found that, depending on the inputs, modeling studies reflect different risk assessments, creating uncertainty in the data.

The residual risk that an HIV-infected unit of blood will enter the blood supply is estimated at about 1 infected donation for every 2.1 million donations. Given that there are about 14.5 million blood donations annually, the residual risk is about 7 infected units every year. However, the AMA said, it is clear that 7 HIV-infected units do not enter the US blood supply annually undetected. Since the implementation of NAT in 1999, there have been four incidences where HIV has been transmitted via a blood transfusion, the last in 2002. In all four cases, the donors denied engaging in risky behavior at screening. So, out of more than 112 million whole blood units transfused, only 4 resulted in HIV transmission – far lower than predicted by the risk models.

In suggesting that a five-year deferral might be warranted, the AMA pointed to a study that found, compared to blood donors who did not report MSM contact, blood donors who reported the behavior within five years had five times the number of reactive test results. However, those who had not practiced male-to-male sex in at least five years had no significant difference in reactive tests than those who did not report MSM contact at all. The organization reasoned then that data suggest men who practice five-year abstinence from homosexual sex "essentially present no greater risk than the general population."

(continued on page 3)

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tel: (202) 393-5725
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Finnish Parliament Finds Red Cross MSM Policy Justified

The Finnish Red Cross Blood Service policy imposing a lifetime ban on blood donation on men who have had sex with men cannot be considered unlawful, Finland's parliamentary ombudsman said in a statement Monday (6/30/08).

The ombudsman, Riitta-Leena Paunio, said in the statement that the decision was based on "appropriately reasoned epidemiological information to the effect that sex between men clearly increases the risk of contracting serious blood-transmitted diseases, such as HIV and hepatitis B and C, and thereby increases the safety risk in blood transfusion ... The ombudsman emphasizes that the ban is not due to sexual orientation, which enjoys constitutional protection against discrimination, but rather to sexual behavior."

The ombudsman pointed out that in addition to gay men, the Finnish Red Cross does not accept blood from anyone over 65 years of age or people who had visited Britain during the bovine spongiform encephalopathy outbreak. The ombudsman was responding to two complaints that alleged the Blood Service was violating the constitutional prohibition of discrimination in considering sex between men to be a permanent obstacle to blood donation.

According to the ombudsman's opinion, the measures undertaken by the Blood Service are not discriminatory and, hence, not in contravention of the Constitution. "The ombudsman considers that there is appropriate justification for regarding sex between men as a permanent obstacle to blood donation. ...At present, sex between men still carries an elevated risk of HIV infection. Statistics from the National Public Health Institute of Finland indicate that 330 men contracted HIV through sex between men and 247 men through heterosexual intercourse in Finland during the period 2000-2007.

"It is estimated that some 5 percent of all men have had sexual contacts with other men, which makes the risk of recent HIV infection through sex between men about 25-fold compared with that in heterosexual relationships. The selection of blood donors is largely based on assessment of risks in various donor groups and less so on individual risk behaviour." (Sources: NewsRoom Finland, 6/30/08; Ombudsman Statement, 6/30/08; Finnish Red Cross release, 6/30/08)

AMA Statement (continued from page 2)

As for a one-year deferral, the AMA said "while the increased risk with a one-year abstinence from blood donation from the last MSM contact would be very small, it is not zero. This small but scientifically real increase in risk represents a clear violation of ethical principles and therefore is not tolerable. If a 5- or 10-year deferral policy is considered, risk management calculations would yield risks at a level that many might consider acceptable."

The AMA had considered other language pointing out the weaknesses of current risk assessment models and a recommendation to ask the AMA Ethical and Judicial Council to examine the societal and ethical impacts of moving to a five-year deferral.

But the organization concluded that the data and explanations offered in the report itself, combined with the discussion at the hearing, supported a decision to remove the wording relating to the weakness of the models. The House of Delegates also removed the second recommendation of the report because the issue at hand was a risk- and science-based decision and further ethical scrutiny by the Council was deemed unnecessary. The Council's examination of any issue is always science-based, while any consideration of the ethical impact of a change in policy for MSM would be based, at least in part, on societal values, the AMA said. The AMA statement can be found at www.ama-assn.org/ama/pub/category/18644.html ♦

医薬品 研究報告 調査報告書

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研究報告の概要

○米国における供血者の*Tripanosoma Cruzi*(*T. cruzi*)スクリーニング10ヶ月間の経験:検出頻度、リスク要因、費用対効果

背景:供血者の*T. Cruzi*スクリーニングは血液の安全性を高めるが、財政的な負担と潜在的な供血者損失の原因ともなり得る。ここでは、米国の全供血者を対象に*T. cruzi*検査が導入された2007年1月30日以降、10ヶ月間の経験を報告する。

方法:供血者は、供血前の問診の際に、出生国と*T. cruzi*流行地の中南米で過ごした期間についての質問に回答した。ELISA法で*T. cruzi*繰り返し陽性(RR)となった供血者は通知を受け、シャーガス病のリスク要因と症状についてのインタビューに回答した。ELISA RRの供血はRIPAで確認試験を行った。また、費用対効果分析によって全供血者対象の*T. cruzi*スクリーニングの医療経済的な面を検討した。

結果:約652,000名の供血適格者のうち、リピートドナーの2.1%、初回ドナーの4.8%が、問診で中南米に3ヵ月以上の滞在歴があると回答した。期間中に93名(うち3名は自己血ドナー)が*T. cruzi* RRとなった。適合血供血のRR発生率は0.0138% (90/651,471; 1:7,239)だった。RRの供血のうちRIPA陽性は34% (28/82)、陽性確認率は特異度99.99%で0.0043% (1:23,267)だった。リスク要因としては、中南米の農村部居住歴、わらぶき屋根や泥の壁の家の居住歴、母方の家族が中南米出身、などが報告された。シャーガス病関連の症状を報告した人の割合は、RIPA陽性及び陰性供血者で同程度だったが、無症候のドナーはそれよりも多く、ELISA RRの供血者でも20%では症状が報告されなかった。予備的費用対効果分析では、スクリーニングはスクリーニング未実施と比較して$10,000,000/QALYを超える費用効果であることが示された。

結論:*T. cruzi*感染のリスク要因発現率は、検査前の予想と同程度だった。RR供血の大半はRIPAで陰性だったが、ELISAの特異度は、供血者損失と比較して良好だった。RIPA陽性の供血者は地理的な暴露リスクを報告したが、シャーガス病関連の症状を報告した人は少数だった。症状に関連した質問は、別の疾患で同じ症状を発症する場合があるため、地理的なリスク要因の質問よりも有益ではないと考えられた。全供血のスクリーニングは費用対効果が低く、出生地と初回供血者に対象を絞った対策の検討が示唆された。

報告企業の意見	今後の対応
米国の供血者を対象にT. cruzi検査が導入された後10ヶ月間で、陽性確認率は0.0043%だった。症状に関連した質問は地理的なリスク要因の質問よりも有益ではないと考えられること、全供血のスクリーニングは費用対効果が低く、出生地と初回供血者に対象を絞ったスクリーニング戦略の検討が示唆されたとの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血-LR「日赤」
照射合成血-LR「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

S19 - Emerging Infections

3C-S19-01

10-MONTH EXPERIENCE SCREENING USA BLOOD DONORS FOR *TRYPANOSOMA CRUZI*: YIELD, RISK FACTORS, AND COST EFFECTIVENESS

Custer S[1], Kamel HT[2], Tomasulo PA[2], Murphy EL[3], Busch MP[1]
[1]Blood Systems Research Institute, San Francisco, USA [2]Blood Systems Inc., Scottsdale, AZ, USA [3]University of California, San Franscisco, CA, USA

Background: Screening blood donors for the parasite *Trypanosoma cruzi*, the cause of Chagas disease, can improve transfusion safety but may come at a high price financially and potentially in donors lost. Since January 30, 2007 all donors have been tested for *T. cruzi* by an USA FDA-approved ELISA. Here we report our experience during the first 10 months of testing and interviewing donors.

Methods: Donors complete a pre-donation health questionnaire that includes questions on country of birth and time spent in Mexico, Central and South America, areas endemic for *T. cruzi*. Donors who test ELISA repeat reactive (RR) for *T. cruzi* are informed by telephone and asked to complete an interview to assess risk factors for and symptoms of Chagas disease. ELISA RR donations are tested by radioimmunoprecipitation assay (RIPA) to discriminate confirmed- from false-positive results. We also conducted a cost-effectiveness analysis to assess the health economics of universal donor screening for *T. cruzi* in the USA using an updated version of a published model [].

Results: Of nearly 652,000 eligible allogeneic donors, 2.1% of repeat donors and 4.8% of first-time donors report having spent 3 months or more in Latin America based on pre-donation questions. 93 donors (including 3 autologous donors) tested T cruzi RR in the first 10 months of testing. The RR rate for allogeneic donations was 0.0138% (90/651,471; 1:7239). Only 34% (28 of 82 tested to date) RR donations tested RIPA-positive, for a confirmed yield of 0.0043% (1:23,267) with a specificity of 99.99%. The yield of RIPA-positive donations according to region of birth is provided in the table.

Reported risk factors include previously living in rural areas of Latin America, living in housing with thatched roofs and/or mud walls, and maternal family history in Latin America. RIPA-positive and negative donors reported similar frequencies of symptoms that could indicate Chagas disease, yet no symptom was reported by more that 20% of ELISA RR donors. Preliminary cost effectiveness analysis comparing no screening to screening using ELISA and supplemental RIPA indicated a cost-effectiveness of >$10,000,000/QALY.

Birth country or region	RIPA positive prevalence
USA	1:108,207
Mexico	1:1800
Central or South America	1:154
All other countries	1:13,410
Missing/Unknown	1:82,485

Conclusion: The prevalence of and risk factors for *T. cruzi* infection are consistent with pre-testing expectations. Although the majority of RR donations did not test RIPA-positive, the specificity of the ELISA was good with substantial donor loss not evident. RIPA-reactive donors have reported geographical exposure risks and a small number have indicated symptoms consistent with Chagas disease. Symptom-related questions appear less valuable for targeting screening than geographic risk factor questions due to the potential for other health conditions to cause the same symptoms. The cost-effectiveness of screening all donations is poor and may represent an extremely inefficient use of resources, indicating that targeted screening strategies focused on country of birth and first-time donor-status should be considered.

Reference: Wilson LS, Strosberg AM, Barrio K. Cost-effectiveness of Chagas disease interventions in latin America and the Caribbean: Markov models. Am J Trop Med Hyg 2005; 73: 901–910.

3C-S19-02

EVALUATING THE EFFECTIVENESS OF MALARIA DEFERRALS THROUGH ANTIBODY TESTING

Leiby D, Nguyen L, Goff T, Gibble J
American Red Cross, Rockville, MD, USA

Background: For decades US blood collection organizations have used risk-factor questions to defer donors deemed to be at-risk for infection with *Plasmodium* spp., the etiologic agents of malaria. Risk factors are broadly classified as travel to or residence in a Plasmodium-endemic country, or past history of malaria. Affirmative responses to any one these risk-factor questions results in deferral from donating blood for 1–3 years. In recent years it has become clear that this approach has a negative impact on blood availability. Despite < 5 cases of transfusion-transmitted malaria in the US since 1998, over 100,000 potential donors are lost to malaria related deferrals each year. Thus, malaria can now viewed primarily as a blood availability issue, as opposed to a blood safety issue.

Aim: Assess the effectiveness of current malaria risk-factor questions by testing groups of deferred and non-deferred donors.

Methods: Blood donors previously deferred for malaria risk, defined as travel to or residence in *Plasmodium* spp. endemic areas or a prior history of malaria, were recruited and enrolled in the present study following administration of consent. Each study subject provided 10 ml of blood (EDTA) and completed a detailed questionnaire regarding risk factors for exposure to *Plasmodium* spp. Blood samples were tested by EIA (NewMarket Laboratories, UK) for *Plasmodium* spp. antibodies as per the manufacturers' instructions. Those samples found to be repeat reactive by EIA were considered positive and tested by real-time PCR for the presence of parasite DNA, and subsequent speciation. In addition, a group of randomly selected, non-deferred donors was selected and tested to determine assay specificity.

Results: A total of 1473 deferred donors enrolled in the study and provided a blood sample for EIA testing. Among those tested, 21 (1.43%) were initially reactive and 20 (1.36%) were repeat reactive. All samples tested by real-time PCR were negative for parasite DNA. The distribution of the 20 repeat reactive donors among the deferral categories was as follows: 14 for travel, 5 for residency and 1 for malaria history. The results of the risk factor questionnaire revealed that most seropositive donors had multiple risk factors including 17 (85%) with either residence in an endemic country or a past history of malaria. A group of non-deferred donors (n = 3229) was also tested by EIA and 21 (0.65%) were initially reactive and 11 (0.34%) were repeat reactive. Four of these 11 had a past history of malaria and three others had spent extensive time in *Plasmodium*-endemic countries.

Conclusions: Blood donors seropositive for *Plasmodium* spp. were detected among non-deferred and deferred donors. The relationship between long-term antibody titers and the risk for transmitting infection remains unclear, but semi-immune donors have been implicated in transfusion cases previously. The current approach to donor deferral is inconsistent, failing to defer donors with residence in endemic areas and/or a past history of malaria, two factors shown to be associated with transfusion transmission. In contrast, excessive donor deferral for travel to Latin America produces unnecessary donor loss, despite minimal risk for transmitting infection.

3C-S19-03

GENETIC VARIABILITY OF WEST NILE VIRUS (WNV) IN CLINICAL ISOLATES FROM US

Rios M, Grinev A, Chancey C, Daniel S, Rios M
Food and Drug Administration, Bethesda, MD, USA

Background: WNV is endemic in the US and has caused 1.5–3.5 million human infections since 1999, with >1000 cases of neurological diseases and ≥100 deaths yearly since 2002. WNV is transmissible by transfusion

別紙様式第2-1

No. 33

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	機構処理欄
				2008. 6. 23	該当なし	

一般的名称	（製造販売承認書に記載なし）	研究報告の公表状況		公表国
販売名（企業名）	合成血-LR「日赤」（日本赤十字社） 照射合成血-LR「日赤」（日本赤十字社）		Walderhaug M, Menis M. XXIIXth Congress of the International Society of Blood Transfusion; 2008 Jun 7-12; Macao.	米国

研究報告の概要

○パンデミックインフルエンザが米国の血液供給に与える影響のシミュレーション

米国におけるパンデミックインフルエンザ発生に備えて、パンデミックによる供血の減少と製造担当職員の不足により、供血数と職員数が通常程度に回復する前に在庫がなくなる可能性を分析した。米国では、年間約1450万製剤分の供血が行われ、約530万件の輸血が行われている。パンデミック中に起こりうるシナリオを検証するために、米国の血液供給量、1日当たりの供血数、1日当たりの需要について、個々にコンピュータシミュレーションを行った。シミュレーションは、製剤に関しては「先入れ先出し」法で行い、各製剤の供血後の日数の経過を追った。1日のシミュレーションで保存期間が42日を超えた製剤は供給から排除された。1日当たりの供血数については、供血記録から得られた通常の供給量と標準的逸脱数に基づく確率的シミュレーションを行った。1日当たりの需要のデータは、米国メディケア＆メディケイドサービス由来の、65歳以上の入院患者の1日当たりの輸血実施数に関するデータと同様の方法で算定した。1日当たりの供血数と血液需要に関する分析は、1週間のうち日曜日の供血と需要が最も少なく、週半ばが最も多いというパターンを示した。1日の血液供給のシミュレーションを複数年分続けた場合では、血液供給量の見積もりは夏に減少し冬に回復するパターンを示した。パンデミックインフルエンザの影響を検証するため、3ヶ月間の供血量が50%減少したとしてシミュレーションを行ったところ、血液需要に何も制限がない場合は、血液供給量のほとんどを使い尽くした。しかし、血液の使用を必要最低限に制限した場合は、3ヶ月間供血が減少した場合でも血液在庫がなくなることはなかった。このシミュレーションモデルは、実際の血液供給量に関して適切であり、パンデミックインフルエンザ中に考えられるシナリオの範囲を策定する際に有用と考えられる結果を導き出した。

報告企業の意見	今後の対応
米国におけるパンデミックインフルエンザのシミュレーションで、3ヶ月間の血液供血量が50%減少した場合、血液需要に制限がない場合は血液在庫のほとんどを使い尽くしたが、血液の使用を必要最低限に制限した場合は血液在庫がなくなることはなかったとの報告である。日本赤十字社では家禽に高病原性トリインフルエンザの流行が認められた場合、当該飼養農場の関係者や防疫作業従事者の献血制限を行っている。	日本においてもパンデミックインフルエンザの発生が予期されることから、安全な血液の安定供給を確保し血液事業を継続するための対応計画を検討する必要がある。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血-LR「日赤」
照射合成血-LR「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

and since 2003 blood donations are screened for WNV RNA. Investigation of WNV genetic variation is important since persistent reoccurrence suggests viral adaptation through mutations that can potentially interfere with diagnostic and screening assays, pathogenesis and therapeutic approaches. This study reports the genomic variation of WNV observed in 67 clinical isolates obtained in the continental US during 6 consecutive years (2002–2007).

Methods: RNA extracts were prepared from WNV and subjected to RT-PCR and sequencing. Sequences were compared to the prototype WN-NY99 and other isolates previously studied using NTI Vector. We also developed and validated a multiplex RT-PCR assay to investigate if the newly identified deletion found in ID was also observed in other states. All specimens were tested for WNV 3'UTR deletion using this assay.

Results: Sequence results from 16 complete genomic sequences revealed 20–48 nucleotide (nt) mutations compared to the prototype WN-NY99. We observed an increase of a nucleotide divergence in the full WNV genomes from 0.18% in 2002 to 0.48% in 2006. It should be noted that 80% of the nt changes in structural regions are transitions (U ' C) and 75% are silent mutations. Twelve new mutations identified in 2005, became fixed in 2006. The 2006 and 2007 isolates shared three amino acid substitutions (Val449Ala, Ala2209Thr and Lys2842Arg), but most nt changes are silent transitions (U ' C, A'G). A 13-nt deletion in the 3'NCR (10414–10426) was identified in isolates from Idaho (ID-Δ13). Further investigation of 47 isolates from 2006 and 2007 for ID-Δ13, showed geographical localization of this variant as observed in 12/25 (48%) of isolates from ID, and in one 2006 isolate from ND. The new ID-Δ13 variant of WNV became fixed in 2007.

Conclusion: In this study we report the emergence of a new genetic variant of WNV carrying a 13-nt deletion at the 3'NCR (WNV-ID-Δ13), found in Idaho. The 3'NCR is known to be critical for WNV replication, however WNV-ID-Δ13 grows well in Vero cell cultures, but preliminary study showed steady replication efficiency and normal plaque in Vero cells. The impact of ID-Δ13 in viral pathogenesis is under investigation. Nucleotide sequence alignments indicate that, most new mutations are not fixed, but WNV has continued to diverge and the number of fixed mutations as well as overall genetic divergence has significantly increased. Surveillance for genetic variation is essential to assure public health since emergence of mutants could potentially decrease sensitivity of screening and diagnostic assays, affect viral pathogenesis, and negatively impact the efficacy of vaccines and the development of specific therapies.

3C-S19-04

SCREENING OF BLOOD DONORS FOR CHIKUNGUNYA VIRUS – DEVELOPMENT AND EVALUATION OF MINIPOOL-NAT AND ANTIBODY TESTS

Schmidt M[1], Hourfar MK[1], Drosten C[2], Seifried E[1], Panning M[2]

[1]*German Red Cross, Institute Frankfurt, Frankfurt am Main, Germany*

[2]*Institute of Virology, University of Bonn Medical Centre, Bonn, Germany*

Background: The outbreak of Chikungunya fever in the southeastern islands of the Indian Ocean has drawn the attention of the transfusion community to Chikungunya virus. The virus has now spread to India and wide parts of Southeast Asia. Additionally many infections in European travellers returning from these regions to their home countries have been reported. Chikungunya virus can cause a wide spectrum of disease which may range from no or mild symptoms to death. It is known to be spread by blood in symptomatic cases and likely it could be spread by transfusion and transplantation of organs from people with pre-symptomatic or asymptomatic disease. Adequate screening procedures to identify viremic donations, however, were not available until now.

Methods: A real-time minipool NAT assay for the current epidemic strain of Chikungunya virus was used on a total of 29,568 blood donor samples, tested in minipools of up to 96 donations. To validate the sensitivity of the assay, routine donor minipools were spiked with inactivated virus and were used as positive controls. Additional to NAT-testing 9600 blood donations were screened for IgG-antibodies against Chikungunya virus to determine the prevalence of the infection in our blood donor population. Plasma samples from symptomatic Chikungunya virus infected travellers were analyzed for virus-load and antibody status.

Results: By testing 9600 blood donations for Chikungunya-specific IgG-antibodies no reactive donation was detected. Likewise, no viremic donation was identified by screening 29,568 clinically asymptomatic blood donors by minipool-NAT. The minipool-NAT assay provided sufficient sensitivity to detect plasma samples from symptomatic patients infected with the pathogen. It can be expected that the assay is also capable to detect viremic donations from pre-symptomatic or asymptomatic donors. This is because it was found that virus load in Chikungunya virus infected travellers was highest with onset of symptoms (day 0). After day 7 after onset of symptoms no Chikungunya virus RNA was found in symptomatic travellers. Specificity of the assay was 100% because none of the tested blood donors were found to be positive for the reemerged Alphavirus.

Discussion: Although no donation infected with Chikungunya virus has been identified among the donors subject to our study it is accepted that the reemerged pathogen poses a risk for recipients of blood products - in particular for immunocompromized patients. A recent outbreak of Chikungunya virus in Italy has shown that this virus also poses a risk to countries of the western hemisphere if competent vectors are prevalent. With the assay described for the first time highly sensitive screening of blood-donations on a routine basis is feasible. Since as no approved inactivation procedures exist for red blood cells exist, screening for viremic donations may be the method of choice in order to guarantee safe blood products in countries affected by the Chikungunya epidemic.

3C-S19-05

SIMULATING THE IMPACT OF PANDEMIC INFLUENZA ON THE US BLOOD SUPPLY

Walderhaug M, Menis M

US Food & Drug Administration, Rockville, MD, USA

In order to prepare for a possible pandemic influenza event in the US, we investigated the potential for reduced donations and blood-processing staff shortages due to an influenza pandemic to exhaust blood stocks before normal donations and staff levels are restored. Approximately 14.5 million units of blood are collected annually in the US and approximately 5.3 million receive blood transfusions per year. To examine a range of potential scenarios that might occur during a pandemic, we developed a discrete event computer simulation of the estimated aggregate US blood supply, daily blood donations, and daily demand. The simulation used a 'first in, first out' rule with respect to blood units, and kept track of the number of days post collection of each simulated blood unit. During a day's simulation any units older than 42 days were eliminated from the aggregate supply. Daily blood donations were probabilistically simulated based on a normal distribution of means and standard deviations obtained from donation records. Daily blood demand data were estimated in a similar manner based on multiple years of U.S. Centers for Medicare & Medicaid Services (CMS) MedPAR derived data on the daily number of inpatient blood transfusion procedures recorded for elderly patients 65 years old and over. An analysis of daily donations and blood demand showed similar patterns through the week with the least amount of donations and demand on Sunday with peak donations and demand at mid-week. Simulating the daily blood supply for multiple years in simulation showed the estimated aggregate blood supply behavior was similar to observed patterns of blood supply levels in the US specifically, showing a decline in overall levels during the summer followed by a recovery of levels in the winter. To examine the impact of pandemic influenza, a 50% decline in blood donations for 3 months was simulated, and the effect was a depletion most of the aggregate blood supply, if no limitation of blood demand was applied; however, if blood demand is limited to essential uses, then a three month period of reduced donations can be endured despite a significant depletion of aggregate blood stocks. The simulation model provided results that appear to be reasonable with respect to observed estimates of aggregate blood supply and to be useful in exploring a range of possible scenarios expected during pandemic influenza.

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-6

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008 年 6月 4日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	New arenavirus discovered in Bolivia Lancet Infect Dis 2008; 8: 355	公表国 米国	
販売名（企業名）						

研究報告の概要

ボリビア，ペルー及び米国疾病予防管理センター（CDC）の国際チーム（アトランタ，ジョージア州，米国）はボリビアの出血熱の死亡症例において新型のアレナウイルスを発見した。完全ゲノム解析でアレナウイルス属の新型ウイルスであることが認められ，アンデス山脈の山麓にちなんで Chapare virus と名付けられた。当該ウイルスは，系統発生学的には南米で出血熱を自然発生させる他のアレナウイルス，特にサビアウイルスに近いウイルスであった。疾病管理予防センター研究調査員の Stuart Nichol は，「アレナウイルスに関連した出血熱は，アルゼンチン（フニンウイルス），ボリビア（マチュポウイルス及び，現在は Chapare virus），ベネズエラ（グアナリトウイルス）及びブラジル（サビアウイルス）で報告されている。年間の症例数は地域を合わせて数十件から数百件前後まで大きなばらつきがある」とし，また，「Chapere virus がげっ歯類を宿主として長期間存在してきた可能性は非常に高いが，人類への波及はおそらくまれであったと思われる」とも述べている。ハーバード大学医学部（ボストン，マサチューセッツ州，米国）の Michael Farzan 氏は，「南米の野生のげっ歯類において複製するウイルスが人類への感染能を獲得し，重篤な疾患を引き起こすことは容易に起こり得る。これらのげっ歯類の生息環境は様々な形で人類によって破壊されてきていることからも，この点が心配される」と述べている。ウガンダでは，赤オナガザルにおける血清学的検査で新型ポックスウイルスの可能性があるウイルスが発見された。イリノイ大学（Urbana，イリノイ州，米国）主席研究員の Tony Goldberg 氏はこのウイルスは既知のオルソポックスウイルスに類似しているが全く同じものではないとし，さらに「近い将来にこの新型のウイルスが人類に感染する可能性はおそらく低く，また当該研究分野においてポックスウイルスがヒトに感染したエビデンスはない。我々の試験が主に示唆することは，環境において新型であり，また実体の明らかでないポックスウイルスが存在するということである」と述べた。しかしながら，Goldberg は，ポックスウイルスは種のバリアを乗り越えることで悪評が高いことも指摘している。双方の新型ウイルスで懸念されるのは，新たに出現した感染が過去 50 年で約 4 倍に増加しており，野生動物の疾患がこうした疾患の大半を占めているということである。

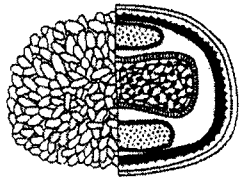
使用上の注意記載状況・その他参考事項等

BYL-2008-0336

PLoS Pathog 2008; 4: e1000047; DOI:10.1371/journal.

Emerg Infect Dis 2008; 14: http://www.cdc.gov/eid/content/14/5/801.htm

Arenavirus



報告企業の意見	今後の対応
2 種類の新規ウイルス病原体はどちらもエンベロープウイルスであり，血漿分画製剤の製造工程におけるウイルス除去・不活化工程により除去・不活化されるウイルスである。また，本報告では新たに出現した感染が過去 50 年で約 4 倍に増加していることを強調している。血漿分画製剤の製造工程におけるウイルス除去・不活化工程は，新たに出現するエンベロープウイルスに対しては効果的であるが，非エンベロープウイルスに対しては未だ完全であるとは考えられない。	今後も，新規ウイルス病原体の出現に関する情報収集に努める。

Highlights from the 18th ECCMID

First European Infection Day

The launch of the first European Day of Fighting Infection took place at the 18th annual European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) in Barcelona, Spain (April 19–23). "We need to make people more aware of infections, and to highlight to the general public in particular that everyone can play a part—for example, in the correct use of antibiotics", Giuseppe Cornaglia (University of Verona, Italy) told *TLID*. "The day will also serve to reinforce collaborations between all players in the field of infectious diseases in Europe and to improve knowledge", he added. The day has been created to mark the 25th anniversary of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). "An important aim for us now is to work towards fostering greater collaboration between eastern and western Europe, through professional exchange and improving our support to young scientists."

ECCMID research highlights

A key focus of the meeting was around antibiotic resistance in Europe and how best to ensure more rational use of antibiotics by clinicians. In a press conference, Fernando Baquero (Hospital Ramón y Cajal, Madrid, Spain) said clinicians are particularly concerned about resistance to antibiotics commonly used in children. He said: "Innovative antibiotics are not being developed, and industrial research facilities on antimicrobial agents are increasingly being shut down...we therefore cannot use all the antibiotics commonly available for use in adults for the treatment of children".

Sore throats are common in children, yet only 15–30% of them are caused by pathogenic bacteria, most frequently group A streptococci. In an expert session, Paul Little (University of Southampton, UK) warned clinicians against prescribing antibiotics immediately. "There are several alternatives: if rapid streptococcal tests are available it takes just 5 min to exclude or confirm infection. If a rapid test is not available, it's safe to wait 3 days before using antibiotics", he said. Antibiotic therapy should be started after 3–4 days if necessary, "in the meantime you can give anti-inflammatory drugs to control the symptoms".

E Tacconelli and colleagues (Catholic University, Rome, Italy) did a 1-year cohort study to analyse the risk factors for infections by antibiotic-resistant bacteria in hospital admissions. Infections caused by antibiotic-resistant bacteria were diagnosed in 398 patients (seven cases per 1000 admissions). They report an increased risk associated with colonisation in patients aged >60 years with urinary catheters and clinical signs of bacterial infections at admission and in patients previously treated with antibiotics, and conclude that greater recognition of these risk factors may influence the selection of empirical treatment.

Sally Hargreaves

The printed journal includes an image merely for illustration

For more on ESCMID see http://www.escmid.org

New arenavirus discovered in Bolivia

An international team from Bolivia, Peru, and the US Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) has discovered a new arenavirus in a fatal case of haemorrhagic fever in Bolivia. Complete genome analysis revealed a distinct member of the arenavirus family; named Chapare virus, after a river in the foothills of the Andes. The virus is phylogenetically related to other arenaviruses that naturally cause haemorrhagic fever in South America, particularly Sabia virus.

Study investigator Stuart Nichol (CDC) said that "arenavirus-associated haemorrhagic fever has been described in Argentina (Junin virus), Bolivia (Machupo and now Chapare virus), Venezuela (Guanarito virus), and Brazil (Sabia virus). The number of cases per year varies substantially, from around a few hundred cases down to double digits for the whole region". Nichol added: "It is highly likely that Chapere virus has been present in a rodent reservoir for a long time, although spill-over to human beings is probably infrequent". Michael Farzan (Harvard Medical School, Boston, MA, USA) said: "The discovery underscores the ease with which viruses replicating in South American wild rodents can acquire the ability to infect human beings and cause serious disease. This is especially a concern, since the natural habitats of these rodents are being disrupted in a variety of ways".

A possible new poxvirus has been discovered following serological tests in red colobus monkeys in Uganda. Lead investigator Tony Goldberg (University of Illinois, Urbana, IL, USA) said that the virus is similar, but not identical, to known orthopoxviruses, which includes smallpox virus.

Goldberg added: "The likelihood of the new virus infecting human beings in the near future is probably low; there was no evidence of human poxvirus infection in the study area. One of the main implications of our study is that there are new, as yet unidentified poxviruses in the environment". Nevertheless, Goldberg pointed out that poxviruses are notorious for crossing species barriers.

The concern with both new viruses is that emerging infections have roughly quadrupled over the past 50 years, and that wildlife zoonoses account for the majority of such diseases.

Cathel Kerr

For more on **Chapare virus** see *PLoS Pathog* 2008; **4**: e1000047; DOI:10.1371/journal.ppat.1000047

For more on the **novel poxvirus in colobus monkeys in Uganda** see *Emerg Infect Dis* 2008; **14**: http://www.cdc.gov/eid/content/14/5/801.htm

For more on **emerging infectious diseases and wildlife zoonoses** see Newsdesk *Lancet Infect Dis* 2008; **8**: 218–19

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 2008年9月16日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	Proc Natl Acad Sci U S A. 2008;105:14124-14129	公表国 米国	
販売名（企業名）	別紙のとおり					

研究報告の概要

問題点：齧歯類の重症疾患の原因ウイルスとされていた cardiovirus がヒトにおいても存在することが遺伝子学的手法により確認された。

齧歯類の重症疾患の原因となる picornavirus 科に属する cardiovirus は、その罹患率、多様性、ヒトでの症状等についてはあまり知られていない。発熱のある乳児の便検体から 1981 年に培養された Saffold virus は、cardiovirus に分類されている。今回、患者検体から直接ヒト cardiovirus をクローニングしたことについて報告する。これはインフルエンザ様の症状を示した子供の呼吸分泌物から pan-viral microarray 法を用いて発見した最初の報告である。ほぼ全長のウイルスゲノム(7961 bp)の系統樹解析で、ウイルスは cardiovirus のサブグループである Theiler's murine encephalomyelitis virus(TMEV)に属し、Saffold virus と最も密接に関係があった。719 の呼吸器サンプル（急性呼吸器症状を示した患者からは 637 検体(89%)）と神経系疾患患者（無菌性髄膜炎、脳炎及び多発性硬化症）からの髄液検体 400 の RT-PCR によるスクリーニングでは、cardiovirus 感染の痕跡は認められなかった。しかし、胃腸炎患者 498 人の排泄物 751 検体のスクリーニングの結果、6 検体より cardiovirus（1.2%）が検出された。これら Saffold virus を含む 8 つのヒト cardiovirus は、系統樹解析によりすべて同じところにクラスターされたが、VP1 遺伝子にかなりの多様性が認められた(アミノ酸の相同性は 66.9%–100%)。これらの結果は、これまでほとんど確認されていなかったが、現在は主に消化管において確認され、無症候で排出され、そして腸内外の疾患に関連している可能性がある新しいヒト TMEV 様の cardiovirus の多様な集団が存在することを示唆している。

報告企業の意見	今後の対応
別紙のとおり	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

使用上の注意記載状況・その他参考事項等

記載なし

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン 20 “化血研”、②献血アルブミン 25 “化血研”、③人血清アルブミン “化血研” *、④ “化血研” ガンマーグロブリン、⑤献血静注グロブリン “化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン “化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500 注射用
報告企業の意見	cardiovirus は、picornavirus 科に分類される属名の一つである。cardiovirus 属のウイルスはエンベロープを持たず、直径約 30nm で正 20 面体のカプシッドを持ち、核酸は一本のプラス鎖 RNA である。cardiovirus 属には次の 2 つのサブグループがある；脳心筋炎ウイルス (encephalomyocarditis virus ; EMCV)、タイラーのマウス脳脊髄炎ウイルス (Theiler's murine encephalomyelitis virus ; TMEV)。これらのウイルスは、げっ歯類に感染し消化器官で増殖した後、糞便経口ルートで伝播する。ウイルスが腸管感染しても大抵は軽度か無症状であるが、腸管外に拡がると全身性の疾患を惹き起こす。EMCV 系統のウイルスは脳炎及び心筋炎を惹き起こし、TMEV 系統のウイルスは中枢神経系感染に関連している。ヒトから分離されたとされる cardiovirus 属のウイルスも報告されているが、ヒトから直接クローニングされたことはなく、その罹患率、多様性、ヒトでの症状等についてはあまり知られていない。 本剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン (医薬発第 1047 号、平成 11 年 8 月 30 日)」に従い、ウシウイルス性下痢ウイルス (BVDV)、仮性狂犬病ウイルス (PRV)、ブタパルボウイルス (PPV)、A 型肝炎ウイルス (HAV) または脳心筋炎ウイルス (EMCV) をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告した cardiovirus 属には、モデルウイルスとして使用している EMCV そのものが属しており、上記バリデーションの結果から、本剤の製造工程が EMCV の除去・不活化効果を有することを確認している。また、これまでに本剤による cardiovirus 感染の報告例は無い。 以上の点から、本剤は cardiovirus に対する安全性を確保していると考える。

*現在製造を行っていない

Identification of cardioviruses related to Theiler's murine encephalomyelitis virus in human infections

Charles Y. Chiu[†‡], Alexander L. Greninger[†], Kimberly Kanada[†], Thomas Kwok[†], Kael F. Fischer[†], Charles Runckel[†], Janice K. Louie[§], Carol A. Glaser[‡§], Shigeo Yagi[§], David P. Schnurr[§], Tom D. Haggerty[¶], Julie Parsonnet[¶], Don Ganem[‡∥††], and Joseph L. DeRisi[†,†††‡‡]

[†]Department of Biochemistry and Biophysics, [‡]Department of Microbiology, [‡]Division of Infectious Diseases, Department of Medicine, and [††]Howard Hughes Medical Institute, University of California, 1700 4th Street, Box 2542, San Francisco, CA 94143; [§]Viral and Rickettsial Disease Laboratory, California Department of Health Services, 850 Marina Bay Parkway, Richmond, CA 94804; and [¶]Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, 300 Pasteur Drive, S-169, Stanford, CA 94305

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Cardioviruses comprise a genus of picornaviruses that cause severe illnesses in rodents, but little is known about the prevalence, diversity, or spectrum of disease of such agents among humans. A single cardiovirus isolate, Saffold virus, was cultured in 1981 in stool from an infant with fever. Here, we describe the identification of a group of human cardioviruses that have been cloned directly from patient specimens, the first of which was detected using a pan-viral microarray in respiratory secretions from a child with influenza-like illness. Phylogenetic analysis of the nearly complete viral genome (7961 bp) revealed that this virus belongs to the Theiler's murine encephalomyelitis virus (TMEV) subgroup of cardioviruses and is most closely related to Saffold virus. Subsequent screening by RT-PCR of 719 additional respiratory specimens [637 (89%) from patients with acute respiratory illness] and 400 cerebrospinal fluid specimens from patients with neurological disease (aseptic meningitis, encephalitis, and multiple sclerosis) revealed no evidence of cardiovirus infection. However, screening of 751 stool specimens from 498 individuals in a gastroenteritis cohort resulted in the detection of 6 additional cardioviruses (1.2%). Although all 8 human cardioviruses (including Saffold virus) clustered together by phylogenetic analysis, significant sequence diversity was observed in the VP1 gene (66.9%–100% pairwise amino acid identities). These findings suggest that there exists a diverse group of novel human Theiler's murine encephalomyelitis virus-like cardioviruses that hitherto have gone largely undetected, are found primarily in the gastrointestinal tract, can be shed asymptomatically, and have potential links to enteric and extraintestinal disease.

DNA microarrays | gastroenteritis | influenza-like illness | picornavirus | virus discovery

Picornaviruses are positive single-stranded RNA viruses that cause a variety of important disease states in humans and animals. Several genera of picornaviruses are recognized, based on genomic sequence and virus biology. The *Cardiovirus* genus of the family Picornaviridae consists of two subgroups: Theiler's murine encephalomyelitis virus (TMEV) and related viruses (Theiler-like virus NGS910 of rats, Vilyuisk virus) (1–3), and encephalomyocarditis virus (EMCV) and related viruses (EMCV, Mengovirus, Columbia SK virus, Maus–Elberfeld virus) (4). All these viruses infect rodents, replicate in the gastrointestinal (GI) tract and are transmitted by the fecal-oral route. Although enteric infection by these viruses is often mild or asymptomatic, extraintestinal spread of these viruses can occur and can lead to systemic disease (1). As their name implies, the EMCV-like agents cause encephalitis and myocarditis, whereas the TMEV family is linked to CNS infection. In experimental settings, intracerebral inoculation of mice with TMEV can produce acute encephalomyelitis and/or a chronic demyelinating disease resembling human multiple sclerosis (MS), depending upon the strain of TMEV used (5). Oral inoculation with TMEV may also result in encephalomyelitis, especially when large inocula are delivered to neonatal mice (6).

Whether authentic human cardioviruses exist has long been debated. The first candidate human cardiovirus was Vilyuisk virus, which was linked to Vilyuisk encephalitis, an unusual neurodegenerative disease found among the Yakuts people of Siberia in the 1950s and still endemic to the region (7, 8). The Vilyuisk virus was initially isolated from the cerebrospinal fluid (CSF) of an affected patient and underwent 41 serial passages in mice before sequencing and characterization as a TMEV-like picornavirus (3, 9). Given its sequence similarity to TMEV and its extensive passage history in mice, questions have arisen as to whether the virus may in fact be of murine origin. In 1981, another TMEV-related cardiovirus was cultured from the stool of an infant who presented with a febrile illness (10). Although early passages appeared to show that the virus was transmissible, long-term continuous propagation of the isolate has been problematic. The nearly complete genomic sequence of this isolate (provisionally called Saffold virus) was recovered from frozen stocks by cloning in 2007 and was found to be much more divergent from TMEV than Vilyuisk virus (10). However, neither Vilyuisk nor Saffold virus was cloned directly from primary clinical specimens, and the diversity, prevalence, and potential clinical manifestations of human cardiovirus infection have remained largely unexplored.

We have previously developed a pan-viral DNA microarray (Virochip; University of California, San Francisco) designed to detect known and novel viruses in clinical specimens on the basis of homology to conserved regions of known viral sequences (11). The current study uses microarrays from the third and fourth generations of this platform (Viro3, Viro4). The Viro3 platform has 19,841 viral oligonucleotides derived from all publicly available viral sequence as of June 2004 (12, 13). The Viro4 platform is a streamlined update of the Viro3 platform consisting of 14,740 viral oligonucleotides derived from all publicly available viral sequence as of June 2006. The Virochip has been used to detect novel pathogens such as the severe acute respiratory syndrome coronavirus (14) and XMRV, a retrovirus identified in prostate tissue of men with germ-line mutations in RNase L (15). The platform has also been successfully used to detect

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The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

‡‡To whom correspondence should be addressed. E-mail: joe@derisilab.ucsf.edu.

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www.pnas.org/cgi/doi/10.1073/pnas.0805968105

known and divergent viruses in acute respiratory tract infections in several recently published studies (12, 13, 16, 17).

In this study, we used the Virochip to screen respiratory secretions from patients with influenza-like illness who lacked a diagnosis despite extensive microbiological testing. In one such patient, we detected and fully sequenced a cardiovirus in the Saffold group. Related cardioviruses were subsequently found in stool specimens from an additional six individuals collected as part of a study examining household transmission of gastroenteritis (18). We report here the existence and overall phylogeny of a diverse group of human cardioviruses and discuss their potential association with human disease.

Results

Detection of a Cardiovirus in a Patient with Influenza-Like Illness. A total of 460 respiratory secretions from patients meeting a case definition of influenza-like illness were screened for respiratory viruses by culture. In 108 culture-negative specimens selected from elderly and pediatric patients, 16 specimens remained negative after subsequent RT-PCR testing for respiratory syncytial virus (RSV), influenza A/B (Flu A/B), rhinovirus (RV), and enterovirus (EV). These 16 specimens were assayed for the presence of viruses using the Virochip (Viro3), with microarray analysis carried out using E-Predict and ranked z score analysis, as previously described (12, 19).

Four of the 16 specimens yielded a positive microarray hybridization signature suggestive of a virus. Two of the signatures corresponded to metapneumovirus, one signature corresponded to adenovirus, and one signature indicated the presence of a cardiovirus related to TMEV. From the microarray containing the cardiovirus signature, the highest intensity oligonucleotides mapped to the 5′-untranslated region (5′-UTR) and 2C gene of the TMEV genome, the most conserved regions among cardioviruses and picornaviruses in general (Fig. 1*A*, "ARRAY"). To recover viral sequence, we designed primers based on the highest intensity array features and alignment of well conserved sequences from four cardioviruses (TMEV-DA, TMEV-GDVII, Theiler-like NGS910 virus, and EMCV). One set of primers successfully amplified a 224-bp fragment from the viral 5′-UTR. The fragment shared 90% nucleotide identity with the 5′-UTR region of Theiler-like NGS910 virus. This finding established that the virus in question was indeed a cardiovirus and a relative of the TMEV group of viruses. We designated this initial cardiovirus strain UC1.

Complete Genome Sequencing and Analysis of UC1. To clone and sequence the remainder of the UC1 genome, additional short fragments were first obtained from conserved regions in the 2C (helicase) and 3D (polymerase) genes by use of consensus PCR primers derived from alignment of the four cardioviruses mentioned previously. Long-range RT-PCR using specific primers was then used to bridge the gaps. This resulted in PCR amplification of two long overlapping fragments (≈5.3 and 3.7 kB in size) jointly spanning nearly the entire length of the virus genome (Fig. 1*A*, "RT-PCR"). Cloned ends of the genome were recovered and sequenced using a RACE amplification protocol (20, 21).

The nearly complete sequence of UC1 is 7961 nt in length and forms a distinct branch in the *Cardiovirus* genus with Saffold virus (Fig. 1*B*). The overall nucleotide identity to Saffold virus is >90% in the 5′-UTR and the region coding for the nonstructural proteins but only 70% in the region coding for the capsid proteins (Fig. 1*A*, "Saffold"). There is much less overall nucleotide sequence identity to other members of the TMEV subgroup (70–80%) and EMCV (50–55%). A poly(C) tract that has been reported in EMCV but not in TMEV strains is not present in the 5′-UTR of UC1. Similar to other cardioviruses, the ORF of UC1 is predicted to code for a single 2296-amino acid polyprotein that is subsequently cleaved into the L protein, the

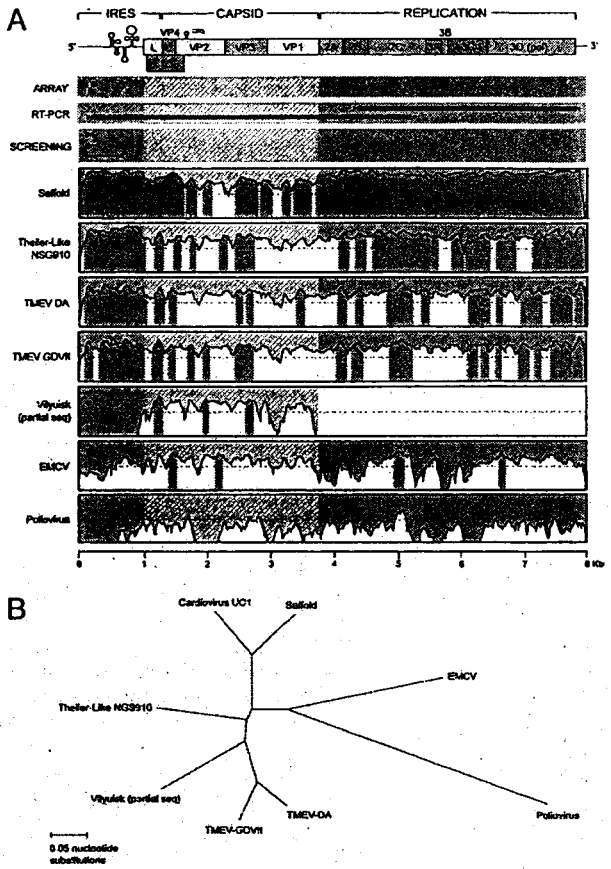

Fig. 1. Genome sequence of UC1. (*A*) Genome sequence similarity plots compare UC1 with Saffold virus, Theiler-Like NGS910 virus, TMEV-DA, TMEV-GDVII, Vilyuisk virus (partial sequence only), EMCV, and poliovirus. The y axis scale for each plot represents percentage of nucleotide identities from 0% to 100%. Regions of the genome with percentage of nucleotide identities of >70% are highlighted in pink. The Virochip oligonucleotides used to detect UC1 ("ARRAY"), the fragments generated by long-range RT-PCR and used to sequence most of the virus ("RT-PCR"), and the cardiovirus primers and resulting PCR fragments used for screening of stool, CSF, and respiratory secretions ("SCREENING") are also shown mapped onto the UC1 genome. The sequences of these primers are provided in Table S1. (*B*) Radial tree depicts the phylogenetic relationships between the genomes of UC1 and the seven aforementioned cardioviruses.

capsid proteins (VP1, VP2, VP3, and VP4), and nonstructural proteins involved in viral replication (2A, 2B, 2C, 3A, 3B, 3C, and 3D) (Fig. 1*A*). Like Saffold virus, UC1 encodes an L protein containing a zinc finger, an acidic domain, and a partially deleted Ser/Thr-rich domain (22, 23) and potentially encodes a severely truncated L* protein that begins with an ACG codon rather than AUG (22, 23) [supporting information (SI) Fig. S1*A*].

In cardioviruses, the surface loops CD of VP1 and EF of VP2 are exposed on the capsid surface and are thought to be involved in host cell tropism and viral pathogenesis (24). These loops are the regions of greatest divergence between UC1 and the other cardioviruses, including Saffold virus (Fig. S1*B*). Between UC1 and Saffold virus, there is 52% and 61% amino acid identity in the exposed surface loops CD and EF, respectively. The corresponding identities (29% and 24%) are much lower between UC1 and the rodent cardioviruses.

Comparison of UC1 Amino Acid Sequence with Other Cardioviruses. The level of divergence between the sequence of UC1 and other cardioviruses is maintained at the amino acid level. Between UC1

Table 1. Amino acid identity of predicted UC1 proteins

Gene	Predicted size, aa	Percent amino acid identity to					
		Saffold	NGS910	Vilyuisk	TMEV-DA	EMCV	Polio
L protein	71	77	61	60	60	26	0
VP4	72	99	72	72	68	62	19
VP2	269	83	69	67	71	64	30
VP3	231	85	80	76	75	68	28
VP1	275	77	56	55	59	48	14
Nonstructural	1389	98	91		83	40	22
Polyprotein	2296	91	76		71	52	22

and Saffold virus, the capsid proteins VP1, VP2, and VP3 are only 77–85% identical, whereas the nonstructural proteins are highly conserved (98% overall identity) (Table 1). The amino acid identities between UC1 and its closest rodent relatives (NGS910 virus and TMEV) are much lower, 56–80% for the capsid proteins and 83–91% for the nonstructural proteins. These comparisons confirm that UC1 is most closely related to Saffold virus, although there is significant sequence divergence in the capsid proteins containing the putative receptor binding sites.

Prevalence of Cardioviruses in Clinical Specimens. To investigate the prevalence of cardiovirus infection in acute human illnesses, we designed PCR primers targeting the 5′-UTR to amplify cardioviruses by real-time one-step RT-PCR. In our initial screen, we ran two RT-PCRs using conserved primers designed to amplify 102-bp and 224-bp fragments from the 5′-UTR of UC1, Saffold virus, or all mouse strains of TMEV. By probit analysis (i.e., the concentration of the target sequence testing positive in 95% of cases) using *in vitro* transcribed UC1 mRNA, the sensitivity of the RT-PCR assay for detection of cardioviruses was 600 copies. Standard curves generated using pooled cardiovirus-negative specimens spiked with UC1 mRNA were linear from 10^4 to 10^{11} copies/ml ($R^2 = 0.9831$–0.9944, Fig. S2). The presence of PCR inhibitors was estimated to be <3% by yeast RNA spiking experiments on randomly selected stool specimens (only 2 of 95 RT-PCRs failed to amplify the yeast positive control). All positives in the initial screen were sequenced and then further confirmed by another RT-PCR using primers designed to amplify an overlapping 608-bp fragment (Fig. 1*A*, "SCREENING").

Since UC1 was first identified in respiratory secretions, we screened 719 respiratory specimens from two large groups of patients: 278 nasopharyngeal aspirates from pediatric patients at a single hospital (190 specimens from patients with an acute respiratory illness) (13) and 441 pooled oropharyngeal and nasopharyngeal swabs from individuals in California with influenza-like illness (25). None of the 719 total respiratory specimens tested was positive for cardioviruses.

We next conducted screening of CSF specimens from patients with aseptic meningitis ($n = 60$), patients with encephalitis ($n = 300$), and patients with MS ($n = 40$) for cardioviruses by RT-PCR. None of the 400 CSF specimens tested was found to be positive.

Given the prominent association of picornaviruses with enteric infection and the known fecal-oral route of transmission, we then sought to assess the prevalence of human cardioviruses in stool. We examined 751 stool specimens from 498 individuals collected as part of a cohort study of household transmission of *Helicobacter pylori* and gastroenteritis (18). The vast majority of subjects were children, with 443 (89%) children younger than 5 years, 30 (6%) children between 5 and 18 years, and 25 (5%) adults. Specimens from 6 children (1.2% of the 498 individuals) were positive for cardioviruses (strains UC2–UC7). All cardiovirus-positive stool specimens were from children <2 years old and from different households. Symptoms in the 6 children included diarrhea and vomiting in 3 (50%) and diarrhea only in 1 (17%); the remaining 2 children were asymptomatic. Of note, from 2 of the symptomatic children, one with diarrhea and vomiting and the other with diarrhea, a cardiovirus was identified not during acute illness but in a specimen obtained months after each child had recovered.

To investigate the possibility of coinfection with additional viruses, we used the Virochip (Viro4) to analyze the nine available specimens collected from the six cardiovirus-positive cases (Table 2). As expected, all six cardiovirus-positive cases were positive for a cardiovirus by Virochip. In three of the cardiovirus-positive stool specimens, there was evidence of coinfection: in two specimens by caliciviruses (norovirus and sapovirus) and in one specimen by a rotavirus. In the other three individuals, viruses other than cardioviruses were detected in the stool at the time of the first visit (adenovirus, norovirus and parechovirus, norovirus and enterovirus), but only cardiovirus

Table 2. Patients with stool positive for cardioviruses

ID	Age at first visit, months	Number ill in household	Days between visits 1 and 2	Clinical symptoms		Virochip/PCR results	
				10 days prior to visit 1	Between visits 1 and 2	Visit 1	Visit 2
UC2	8.4	4/10	—	Diarrhea/ vomiting	—	Cardiovirus, rotavirus	—
UC3	6.1	1/5	139	None	none	—	Cardiovirus, norovirus
UC4	21.4	1/9	91	None	none	Adenovirus	Cardiovirus
UC5	16.3	6/6	95	Diarrhea/ vomiting	none	Norovirus, parechovirus	Cardiovirus
UC6	14.0	1/5	—	Diarrhea/ vomiting	—	Cardiovirus, sapovirus	—
UC7	18.6	3/7	94	Diarrhea	none	Norovirus, enterovirus	Cardiovirus

Dashes indicate entries for which data and/or specimens were not available.

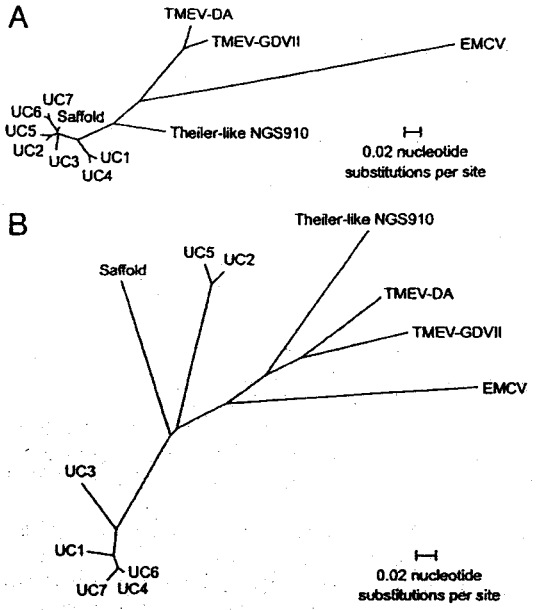

Fig. 2. Strain variation of human cardioviruses. (*A*) Radial tree of a 608-bp region within the 5′-UTR. (*B*) Radial tree of an 819-bp region corresponding to the VP1 gene. Strain designations UC2 to UC7 correspond to patients as listed in Table 2.

was detected in the stool by the second visit. All Virochip results were subsequently confirmed by PCR and sequencing using virus-specific primers.

To assess the sequence variation within different cardiovirus strains, we analyzed a 608-bp region from the 5′-UTR and an 819-bp region corresponding to the VP1 gene for the six positive cardiovirus cases (Fig. 2). The sequence variations within the 608-bp region from the 5′-UTR (2.0–9.1%) and within the 819-bp region corresponding to the VP1 gene (0.3–36.7%) were consistent with infection by independently acquired cardiovirus strains. The amino acid sequence identities in the VP1 gene were lowest between UC2/UC5 and the other cardioviruses (66.9% for Saffold virus, 71.0–72.8% for the other UC strains).

Discussion

Using a pan-viral microarray, we analyzed 16 respiratory specimens from patients with influenza-like illness who still lacked a diagnosis after extensive tests for respiratory viruses. In one specimen, we found a signature for a cardiovirus. Sequence recovery of the genome and phylogenetic analysis revealed that this virus (UC1) is divergent from the rodent cardioviruses and clusters with the Saffold agent. Like Saffold virus, UC1 may code for a truncated L* protein (Fig. S1*A*) that has been implicated in viral persistence and chronic infection of the CNS in TMEV (26). However, because the L* protein of UC1 begins with an ACG codon rather than AUG, it is unclear whether any functional protein is actually expressed, although small amounts of L* protein have been detected in TMEV strains carrying the ACG codon (27).

The binding of sialic acid to TMEV is strongly associated with persistence and neurovirulence, and three amino acids in the VP2 protein are directly involved in this interaction (28, 29). In both UC1 and Saffold virus, there is a substitution or deletion at each of these three positions (Fig. S1*B*), suggesting that sialic acid is unlikely to serve as a receptor for these viruses. Although the cellular receptor is presently unknown, the sequences of UC1 and Saffold virus are most divergent in the capsid region, sharing only 77% and 83% amino acid identity in the VP1 and VP2 proteins, respectively, and 52% and 61% identity in the exposed surface loops CD and EF, respectively. These differences may reflect the use of distinct cellular receptors or may be the result of immune selection during virus evolution (or both); further studies will be required to shed light on these issues.

Cardioviruses were detected in six children out of a total of 498 individuals (1.2%) enrolled in a large gastroenteritis study. Although the initial specimen that was used to culture Saffold virus was collected >25 years ago, cardioviruses UC1 through UC7 were collected from 2000 to 2006, indicating that human cardioviruses continue to circulate in the population. Despite the use of screening RT-PCR assays able to detect all strains of TMEV, cardioviruses detected in human clinical specimens clustered together and were phylogenetically distinct from the rodent cardioviruses (Fig. 2).

Further studies will be required to define the pathogenic role of cardiovirus infection in the intestine fully. Although we did recover a cardiovirus from a number of cases with symptomatic enteritis, other potential GI pathogens were also detected in these cases. Thus, it is presently unclear how frequently enteric cardioviral infection produces clinical illness. Moreover, we detected cardioviruses in stool from subjects without enteritis, suggesting that asymptomatic shedding of cardioviruses in the GI tract can and does occur. In this respect, cardiovirus infection in humans may mimic that of murine TMEV, which is often shed asymptomatically in naturally acquired infections (30).

Cardiovirus infection outside the GI tract is sometimes associated with severe disease in rodents, including encephalomyelitis, demyelinating disease, and myocarditis (1), although only a small percentage of mice naturally infected with TMEV develop systemic disease (1, 5). Our wider screening for cardioviruses indicates that cardiovirus infection is uncommon in the setting of acute respiratory or neurological disease (e.g., aseptic meningitis, encephalitis, MS). However, while this manuscript was under review, Abed and Boivin (31) reported detection of Saffold-like cardioviruses in three children with acute respiratory illness. Moreover, in a case of influenza-like illness reported here, a cardiovirus was the sole agent identified despite comprehensive testing with culture, PCR, and a pan-viral microarray, suggesting that cardioviruses may be pathogenic outside the GI tract in at least some instances.

One remarkable finding from this study was the diversity of the human cardioviruses that have been identified. For the family Picornaviridae, the definition of a new species in a genus is having <70% amino acid identity in the coding regions of either VP1, 2C, 3C, or 3D (32). By this strict definition, cardioviruses UC2 and UC5 would classify as a novel species distinct from Saffold virus, with 66.9% amino acid identity in the VP1 gene. However, since cardioviruses UC1 through UC7 and Saffold virus as a whole clearly define a separate group within the *Cardiovirus* genus by phylogenetic analysis (Figs. 1*B* and 2), we propose a systematic nomenclature for the human cardioviruses, designating all members of this group HTCV, for human TMEV-like cardiovirus, and referring to the strains in this group by a brief suffix (e.g., Saffold agent would be designated HTCV-Saf, UC1 would be designated HTCV-UC1).

Several lines of evidence support the inference that HTCVs are bona fide human viruses and not the products of sporadic viral cross-over events from rodents to humans: (*i*) all seven cardioviruses from humans in this study are strains of HTCV, with no mouse TMEV sequences detected in 1870 total clinical specimens despite screening using two consensus PCR primer sets designed to amplify UC1, Saffold virus, or mouse TMEV; (*ii*) sequence variations within HTCV UC1–7 are most consistent with independent acquisition of different virus strains by patients; and (*iii*) HTCV is substantially diverged from the rodent cardioviruses, especially in the capsid region containing

the putative receptor-binding sites. Taken together, our findings indicate that HTCVs are novel human picornaviruses in the *Cardiovirus* genus that are found primarily in the GI tract, can be shed asymptomatically, and have potential links to self-limited enteric disease and, rarely, to influenza-like illness. Although the full spectrum of clinical diseases linked to HTCV and the mechanisms underlying viral replication remain to be elucidated, the studies reported here now open all these questions to direct experimental scrutiny.

Materials and Methods

Clinical Specimens. ***Respiratory secretions from the California Influenza Surveillance Program study.*** A total of 943 respiratory specimens were sent to the California Department of Health Services (DHS) during the 2005–2006 season (25). Among these 943 specimens, 460 were pooled nasopharyngeal and oropharyngeal swabs collected as part of the California Influenza Surveillance Program (CISP) study under protocols approved by the DHS. Patients enrolled in the CISP study fulfilled a clinical case definition of influenza-like illness (temperature of 37.8°C or greater and a cough and/or sore throat in the absence of a known cause other than influenza). Sixty percent, or 280 specimens, were positive for a virus by culture. Among the remaining 180 culture-negative specimens, a subset of 108 specimens selected from elderly and pediatric patients was then subjected to further screening by RT-PCR to exclude cases of RSV, Flu A/B, RV, and EV (33). Sixteen specimens negative by culture and RT-PCR were then examined using the Virochip. We subsequently screened 441 CISP specimens with remaining available specimen material (96% of the 460 total collected specimens) for cardioviruses by RT-PCR.

Respiratory secretions from the UCSF pediatric respiratory infections study. This collection consisted of 278 consecutive nasopharyngeal aspirates from pediatric patients seen at UCSF from December 2003 to June 2004 (13). All specimens were collected under protocols approved by the UCSF Institutional Review Board. In this group, 190 of the patients (68%) had a respiratory illness, defined as an upper respiratory infection, bronchiolitis, croup, asthma exacerbation, or pneumonia. The remaining 88 patients (32%) were asymptomatic.

Stool from the Stanford Infection and Family Transmission cohort. The Stanford Infection and Family Transmission (SIFT) cohort of 4333 individuals was initiated in 1999 to evaluate the association between *H. pylori* infection and gastroenteritis transmission prospectively (18). Among the 3063 subjects who consented to further use of biological specimens, 774 stool specimens were obtained from 514 individuals; of those, 751 specimens from 498 subjects were available for study. Additional details on the 751 specimens screened for cardioviruses by RT-PCR are described in *SI Text*.

CSF specimens from patients with aseptic meningitis, encephalitis, and MS. A total of 60 CSF specimens from patients with clinically diagnosed aseptic meningitis, 300 CSF specimens from patients with encephalitis (who lacked a diagnosis despite comprehensive testing) (34), and 40 CSF specimens from patients with MS were screened for cardioviruses by RT-PCR. Specimens were collected under protocols approved by the California DHS (encephalitis specimens) or the UCSF Institutional Review Board (aseptic meningitis and MS specimens).

Specimen Preparation and Diagnostic Testing. In the CISP study, routine tube culture or shell vial culture of pooled nasopharyngeal and oropharyngeal swab specimens followed by specific monoclonal antibody testing for viral identification was performed as previously described (33, 35). Total nucleic acid was then extracted from the specimens using the MasterPure Complete DNA and RNA Purification Kit (Epicentre). Real-time one-step RT-PCR assays for RSV, FluA/B, and picornavirus (inclusive of RV and EV) were then performed as previously described (25, 33, 36). In the UCSF pediatric respiratory infections study, 200-μl aliquots of nasopharyngeal lavage were used to extract RNA using the RNeasy Mini Kit (Qiagen Corporation), including on-column DNase digestion. In the SIFT cohort, stool was suspended in 2 ml of PBS at 10% weight per volume and the PureLink96 Viral RNA/DNA Kit (Invitrogen) was used to extract RNA for RT-PCR and Virochip analysis. Cerebrospinal fluid specimens were processed using either a Zymo MiniRNA Isolation Kit (Zymo Research) or the MasterPure Complete DNA and RNA Purification Kit.

Virochip analysis of CISP and SIFT specimens was carried out as previously described (14). Extracted nucleic acid specimens were amplified and labeled using a Round A/B protocol and were hybridized to the Virochip. Microarrays (National Center for Biotechnology Information GEO platforms GPL3429 for Viro3 and GPL6862 for Viro4) were scanned with an Axon 4000B scanner (Axon Instruments). Virochip results were analyzed using cluster analysis, E-Predict, and *z* score analysis as previously described (12, 19, 37). All Virochip microarrays have been submitted to the GEO database (National Center for Biotechnology Information GEO series number GSE11569, accession numbers GSM291246–GSM291254).

Complete Genome Cloning and Sequencing (UC1 strain). Conserved primers from the 5′UTR of cardioviruses were designed based on the highest intensity microarray oligonucleotides and alignment of well conserved sequences from four cardioviruses for which full genome sequences were available: TMEV-DA, TMEV-GDVII, Theiler-like NGS910 virus, and EMCV. After short viral fragments were obtained, six sets of specific primers derived from sequenced fragments and conserved primers were then used to sequence the genome by long-range RT-PCR and 5′/3′ RACE (rapid amplification of cDNA ends). Amplicons for sequencing were cloned into plasmid vectors using the TOPO TA Cloning System (Invitrogen) and sequenced on an ABI3130 Genetic Analyzer (Applied Biosystems) using standard Big Dye terminator (version 3.1) sequencing chemistry. The completed genome sequence of UC1 has been deposited into GenBank (GenBank accession number EU376394).

Phylogenetic Analysis (UC1 strain). Nucleotide and protein sequences associated with the following reference virus genomes were obtained from GenBank: Saffold virus (NC_009448), TMEV-DA (M20301), TMEV-GDVII (NC_001366), Theiler-like NGS910 virus (AB090161), EMCV (NC_001479), poliovirus (NC_002048), and the partially sequenced genome of Vilyuisk virus (M94868). For amino acid analysis, ORFs predicted using ORF Finder (National Center for Biotechnology Information) were used. Multiple sequence alignment was performed using ClustalX (version 1.83). Neighbor-joining trees using the Kimura two-parameter distance correction were generated using 1000 bootstrap replicates and displayed using MEGA (version 3.1). Sequence identities were calculated using BioEdit (version 7.0.9.0).

RT-PCR Screening for Cardioviruses. Real-time quantitative RT-PCR (qRT-PCR) screening for cardioviruses with SYBR Green I (Invitrogen) was performed using conserved PCR primer sets CardioUTR-1F/CardioUTR-2R-A and CardioUTR-1F/CardioUTR-2R-B (Table S1) on a DNA Engine Opticon System (Bio-Rad). To determine limits of sensitivity of the qRT-PCR assay, probit analysis of results from 10 qRT-PCR replicates of eight serial half-log dilutions of *in vitro* transcribed UC1 mRNA (from a starting concentration of $\sim 10^5$ copies/ml) was performed using StatsDirect (StatsDirect Ltd.). Standard curves of the qRT-PCR assay were calculated from 3 qRT-PCR replicates of seven serial log dilutions of RNA extracted from pooled respiratory secretions, stool suspensions, and PBS spiked with UC1 RNA (10 specimens per pool). To assess for the presence of PCR inhibitors, RT-PCR for yeast was carried out on 95 randomly selected stool samples, each spiked with 1 ng of *in vitro* transcribed *Saccharomyces cerevisiae* intergenic RNA as a positive control (38).

Positive bands corresponding to the expected 102-bp and 224-bp amplicons were cloned and sequenced in both directions using vector primers M13F and M13R. Secondary confirmation of all positive reactions was performed using RT-PCR with primers CardioUTR-1F and CardioUTR-3R (Table S1), which generated a larger 608-bp amplicon, also in the 5′-UTR. To obtain the full sequences of the VP1 gene in strains UC2 through UC7, RT-PCRs were performed using conserved primers flanking the VP1 region of UC1 and Saffold virus (Table S1). The sequences of the 5′-UTR and VP1 amplicons corresponding to cardiovirus strains UC2 through UC7 have been deposited in GenBank (accession numbers EU604739–EU604750).

PCR Confirmation for Virochip-Positive Stool Specimens. All nine specimens collected from the six positive cardiovirus cases were analyzed using the Virochip as previously described (11, 12). Confirmatory PCR for calicivirus, adenovirus, and parechovirus was carried out using conserved primers as previously reported (39–41). Amplified PCR bands of the expected size were gel extracted and sequenced using standard BigDye chemistry on an ABI3130 (Applied Biosystems).

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1. Brahic M, Bureau JF, Michiels T (2005) The genetics of the persistent infection and demyelinating disease caused by Theiler's virus. *Annu Rev Microbiol* 59:279–298.
2. Ohsawa K, Watanabe Y, Miyata H, Sato H (2003) Genetic analysis of a Theiler-like virus isolated from rats. *Comp Med* 53:191–196.
3. Pritchard AE, Strom T, Lipton HL (1992) Nucleotide sequence identifies Vilyuisk virus as a divergent Theiler's virus. *Virology* 191:469–472.
4. Duke GM, Hoffman MA, Palmenberg AC (1992) Sequence and structural elements that contribute to efficient encephalomyocarditis virus RNA translation. *J Virol* 66:1602–1609.
5. Oleszak EL, Chang JR, Friedman H, Katsetos CD, Platsoucas CD (2004) Theiler's virus infection: A model for multiple sclerosis. *Clin Microbiol Rev* 17:174–207.
6. Ha-Lee YM, *et al.* (1995) Mode of spread to and within the central nervous system after oral infection of neonatal mice with the DA strain of Theiler's murine encephalomyelitis virus. *J Virol* 69:7354–7361.
7. Petrov PA (1970) V. Vilyuisk encephalitis in the Yakut Republic (U.S.S.R). *Am J Trop Med Hyg* 19:146–150.
8. Vladimirtsev VA, *et al.* (2007) Family clustering of Viliuisk encephalomyelitis in traditional and new geographic regions. *Emerg Infect Dis* 13:1321–1326.
9. Lipton HL, Friedmann A, Sethi P, Crowther JR (1983) Characterization of Vilyuisk virus as a picornavirus. *J Med Virol* 12:195–203.
10. Jones MS, Lukashov VV, Ganac RD, Schnurr DP (2007) Discovery of a novel human picornavirus in a stool sample from a pediatric patient presenting with fever of unknown origin. *J Clin Microbiol* 45:2144–2150.
11. Wang D, *et al.* (2002) Microarray-based detection and genotyping of viral pathogens. *Proc Natl Acad Sci USA* 99:15687–15692.
12. Chiu CY, *et al.* (2006) Microarray detection of human parainfluenzavirus 4 infection associated with respiratory failure in an immunocompetent adult. *Clin Infect Dis* 43:e71–e76.
13. Chiu, CY, *et al.* (2008) Utility of DNA microarrays for detection of viruses in pediatric acute respiratory infections. *J Pediatr* 153:76–83.
14. Wang D, *et al.* (2003) Viral discovery and sequence recovery using DNA microarrays. *PLoS Biol* 1:E2.
15. Urisman A, *et al.* (2006) Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. *PLoS Pathog* 2:E25.
16. Chiu CY, *et al.* (2007) Diagnosis of a critical respiratory illness caused by human metapneumovirus by use of a pan-virus microarray. *J Clin Microbiol* 45:2340–2343.
17. Kistler A, *et al.* (2007) Pan-viral screening of respiratory tract infections in adults with and without asthma reveals unexpected human coronavirus and human rhinovirus diversity. *J Infect Dis* 196:817–825.
18. Perry S, de la Luz Sanchez M, Hurst PK, Parsonnet J (2005) Household transmission of gastroenteritis. *Emerg Infect Dis* 11:1093–1096.
19. Urisman A, *et al.* (2005) E-Predict: A computational strategy for species identification based on observed DNA microarray hybridization patterns. *Genome Biol* 6:R78.
20. Scotto-Lavino E, Du G, Frohman MA (2006) 3' end cDNA amplification using classic RACE. *Nat Protoc* 1:2742–2745.
21. Scotto-Lavino E, Du G, Frohman MA (2006) 5' end cDNA amplification using classic RACE. *Nat Protoc* 1:2555–2562.
22. Kong WP, Ghadge GD, Roos RP (1994) Involvement of cardiovirus leader in host cell-restricted virus expression. *Proc Natl Acad Sci USA* 91:1796–1800.
23. Kong WP, Roos RP (1991) Alternative translation initiation site in the DA strain of Theiler's murine encephalomyelitis virus. *J Virol* 65:3395–3399.
24. Jnaoui K, Michiels T (1998) Adaptation of Theiler's virus to L929 cells: Mutations in the putative receptor binding site on the capsid map to neutralization sites and modulate viral persistence. *Virology* 244:397–404.
25. Louie JK, *et al.* (2007) Creating a model program for influenza surveillance in California: Results from the 2005–2006 influenza season. *Am J Prev Med* 33:353–357.
26. Ghadge GD, Ma L, Sato S, Kim J, Roos RP (1998) A protein critical for a Theiler's virus-induced immune system-mediated demyelinating disease has a cell type-specific antiapoptotic effect and a key role in virus persistence. *J Virol* 72:8605–8612.
27. van Eyll O, Michiels T (2002) Non-AUG-initiated internal translation of the L* protein of Theiler's virus and importance of this protein for viral persistence. *J Virol* 76:10665–10673.
28. Kumar AS, Kallio P, Luo M, Lipton HL (2003) Amino acid substitutions in VP2 residues contacting sialic acid in low-neurovirulence BeAn virus dramatically reduce viral binding and spread of infection. *J Virol* 77:2709–2716.
29. Zhou L, Luo Y, Wu Y, Tsao J, Luo M (2000) Sialylation of the host receptor may modulate entry of demyelinating persistent Theiler's virus. *J Virol* 74:1477–1485.
30. Brownstein D, Bhatt P, Ardito R, Paturzo F, Johnson E (1989) Duration and patterns of transmission of Theiler's mouse encephalomyelitis virus infection. *Lab Anim Sci* 39:299–301.
31. Abed Y, Boivin G (2008) New saffold cardioviruses in 3 children, Canada. *Emerg Infect Dis* 14:834–836.
32. Fauquet, CM, Mayo MA, Maniloff J (2005) *Virus Taxonomy, Classification, and Nomenclature of Viruses* (Elsevier Academic, San Diego, CA).
33. Louie JK, *et al.* (2005) Characterization of viral agents causing acute respiratory infection in a San Francisco University Medical Center Clinic during the influenza season. *Clin Infect Dis* 41:822–828.
34. Glaser CA, *et al.* (2006) Beyond viruses: Clinical profiles and etiologies associated with encephalitis. *Clin Infect Dis* 43:1565–1577.
35. Louie JK, *et al.* (2005) Rhinovirus outbreak in a long term care facility for elderly persons associated with unusually high mortality. *Clin Infect Dis* 41:262–265.
36. Kares S, *et al.* (2004) Real-time PCR for rapid diagnosis of entero- and rhinovirus infections using LightCycler. *J Clin Virol* 29:99–104.
37. Eisen MB, Spellman PT, Brown PO, Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 95:14863–14868.
38. Shock JL, Fischer KF, DeRisi JL (2007) Whole-genome analysis of mRNA decay in Plasmodium falciparum reveals a global lengthening of mRNA half-life during the intra-erythrocytic development cycle. *Genome Biol* 8:R134.
39. Echavarria M, Forman M, Ticehurst J, Dumler JS, Charache P (1998) PCR method for detection of adenovirus in urine of healthy and human immunodeficiency virus-infected individuals. *J Clin Microbiol* 36:3323–3326.
40. Farkas T, *et al.* (2004) Genetic diversity among sapoviruses. *Arch Virol* 149:1309–1323.
41. Legay V, Chomel JJ, Lina B (2002) Specific RT-PCR procedure for the detection of human parechovirus type 1 genome in clinical samples. *J Virol Methods* 102:157–160.

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No. 23

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研究報告の概要

○ヘンドラウイルス感染、ヒト、ウマ―オーストラリア
オーストラリア、ブリスベーンの動物病院スタッフがヘンドラウイルスに感染した。看護師1名と獣医師1名が、感染したウマを治療した後でウイルスに感染したと診断された。患者は2名とも重症である。潜伏期間は最大14日間で、スタッフの間から新たな患者が出ないか監視が続けられている。ウマは感染した3頭中1頭が死亡、1頭を安楽死させたが、1頭は回復に向かっている。
ヘンドラウイルスが以前に流行したのは1994年で、ウマ14頭と調教師1名が死亡した。ヒト-ヒト感染の証拠はなく、広範囲に流行する危険性はない。

報告企業の意見	今後の対応
オーストラリア、ブリスベーンの動物病院スタッフがヘンドラウイルスに感染し、重症となったとの報告である。ヘンドラウイルスはニパウイルスに近縁のウイルスで、現在のところオーストラリア以外での発生はない。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き、新たなウイルス等による感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血-LR「日赤」
照射合成血-LR「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