and horizontal axis – dilution folds of samples), the relative efficacies of concentration were 0·56–0·64. The reason for the low efficacy of HBsAg concentration compared to the efficacy of HBV DNA concentration (0·49–0·76) might be due to HBsAg (22 nm) being smaller than HBV (45 nm) and thus the efficacy of agglutination with poly-L-lysine being different.

In countries where NAT is not available or feasible, the use of a highly sensitive HBsAg assay is crucial in ensuring blood safety. Although individual NAT is the golden standard, at later stages of infection, low concentrations of infectious viruses, which may not be detectable by NAT, might be found in some HBsAg-positive blood donations [19,20]. HBsAg tests with high sensitivity are predicted to have a comparable yield to mini-pool NAT [21]. If the sensitivity of HBsAg detection would be increased by several times, NAT might not always be necessary in late-stage HBV infection. In our study, five samples with low-level HBsAg, detectable only after concentration, were not detected by conventional individual NAT (Table 4; lanes b, c). Twenty-seven of the 40 cases in which HBV DNA was detected were shown to have HBsAg after concentration. The remaining 13 cases (Table 4; lane I-d, e) could not be detected by HBsAg concentration, demonstrating the limitation of our method.

Although HBsAg-negative subjects may retain a low infectivity and have a low risk for progressive liver damage [17], HBV DNA testing or an HBsAg detection method with the highest sensitivity should be implemented to decrease the risk of post-transfusion HBV infection [26,27]. Our new HBV/HBsAg concentration method could contribute to increasing the sensitivity of HBV DNA/HBsAg detection. The concentration method could be combined with either Chemoluminescent Immunoassay (CLIA; PRISM, Abbott) or individual donation NAT to further increase the overall sensitivity of HBV detection. Alternatively, if a high-sensitivity method such as the CLIA was combined with our method, then it might be possible to undertake screening using pooled samples. Our concentration method would potentially be capable of replacing individual NAT by mini-pool NAT, although the present efficacy of concentration is not 1·0 but about 0·7 (Table 1).

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別紙様式第2-1

No. 40

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
				2008. 10. 17	該当なし	
一般的名称	人全血液		研究報告の公表状況	石田 高司、坂野 章吾、森 芙美子、伊藤 旭、李 政樹、稲垣 淳、楠本 茂、小松 弘和、神谷 忠、柚木 久雄、田中 靖人、溝上 雅史、飯田 真介、上田 龍三. 第70回 日本血液学会総会; 2008 Oct 10-12; 京都市.	公表国	
販売名（企業名）	人全血液-LR「日赤」（日本赤十字社） 照射人全血液-LR「日赤」（日本赤十字社）				日本	

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
○20プールNAT導入後、初めて確認された輸血によるHCV感染の一例 症例は新規に最重症再生不良性貧血と診断された54歳の女性で、2007年6月20日に初回輸血が実施され、初回輸血前感染症検査はHCV抗体陰性、HCVコア蛋白陰性であった。10月1日の輸血後感染症検査でHCVコア蛋白の陽性化［28,183.1 fmol/L（＜20.0）］が明らかとなったため、血液センターに連絡し遡及調査を開始した。初回輸血前感染症検査残余の保存血清でHCV-RNAが陰性であることを確認した（PCR）。患者には6月20日から10月1日の間に合計54本の赤血球濃厚液または濃厚血小板輸血があり、保管54検体についてHCV個別NAT（核酸増幅法）を施行したところ、2007年8月17日輸血の赤血球濃厚液からHCV-RNAを検出した。患者と献血者のHCV Core-E1-E2領域（1,279bp）の塩基配列をdirect sequence法で決定し、比較した結果両者は一致した。この結果、本症例は輸血によるHCV感染である可能性が極めて高いと結論した。 日本では1999年7月から献血血液の感染症検査に500プールNATを導入し、2000年には50プール、2004年には20プールとしてきた。世界で最も先進的かつ高感度システムといえる。20プールNAT陰性献血血液由来の血液製剤からのHCV感染の報告は本報告が初であり、NAT陰性献血血液からでもHCV感染が成立しうることが示された。 また、患者はHCV混入血の輸血から肺炎で死亡されるまでの約7ヵ月間、HCV抗体価が陽性になることはなく、10月24日以降HCVコア蛋白値は一貫して施設測定可能上限50,000.0以上であった。免疫抑制状態の患者に対するHCV感染については、輸血前後のスクリーニング検査としてHCVコア蛋白が必要である。	人全血液-LR「日赤」 照射人全血液-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 自発報告： 2007年10月19日付1-07000104

報告企業の意見	今後の対応
日本において、プールNAT導入後3例の輸血によるHCV感染症例があるが、本症例は20プールNAT導入後初めて確認された輸血によるHCV感染の報告である。	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。

③

MedDRA/J Ver.11.0J

OS-1-40 血液疾患患者における末梢血細菌・真菌PCR検査の有用性の検討
PCR analysis of blood for diagnosis of bacterial and fungal infection in hematological patients

○杉本 由香[1]、中村 明子[2]、大石 晃嗣[3]、宮田 恵里[1]、門間 文彦[1]、田丸 智巳[1]、藤枝 敦史[1]、山口 素子[1]、西井 一浩[1]、桝屋 正浩[1]、中瀬 一則[1]、松島 佳子[3]、和田 英夫[2]、登 勉[1]、片山 直之[1]（三重大学 血液腫瘍内科[1]、三重大学医学部附属病院 中央検査部[2]、三重大学医学部附属病院 輸血部[3]、三重大学 がんセンター[4]）

【目的】血液疾患の感染症治療における末梢血の細菌・真菌PCR検査の有効性につき前向きに検討した。【方法】2007年4月より当院で化学療法あるいは造血幹細胞移植を受けた白血病患者のうち、同意が得られた延べ8人に対して、定期的（1週間毎）にまたは発熱時に末梢血の細菌・真菌PCR検査と血液培養を施行した。PCR結果は原則的に非開示とした。【結果】全例経過中に発熱がみられた。PCR検査は延べ14回陽性（細菌13回、真菌1回）、血液培養は延べ6回陽性で（すべて細菌）、そのうち3回で両方陽性となった。なお、連続陽性は1回とカウントした。培養でのみ陽性となった3回すべてで検出されたのは皮膚常在菌であり臨床的にもcontaminationと考えられた。培養とPCRの両方で細菌が検出された3回のうち、1回は同時期の血液で、2回は培養陽性となる2、9日前の血液ですでにPCR陽性であった。細菌PCRのみ陽性であった10回のうち8回は臨床経過から感染の原因菌と考えられたが、経験的抗生剤治療により多くは解熱が得られていた。しかし、Stenotrophomonas maltophiliaが同定された1回では全身状態が増悪したため結果を開示し、抗生剤の変更により改善がみられた。真菌PCRのみ陽性の1回では、臨床的に侵襲性肺アスペルギルス症と診断される20日前からAspergillus fumigatusが検出されていた。【結論】細菌感染の多くは、血液培養の結果あるいは経験的抗生剤投与により治療可能であった。しかし、血液培養が陽性となる前からPCR陽性となっていたケースや、血液培養では検出されずPCRでのみ陽性のケースもみられ、細菌PCRの結果を参考に、より早期から確実に原因菌を想定した抗生剤治療が開始できていた可能性がある。また、真菌感染症においても、血液PCRの結果が臨床経過の改善に有用な症例があることが示唆された。今後さらに多くの症例で、細菌・真菌PCR検査の臨床的有効性を前向きに検討することが必要であると考えられた。

OS-1-41 LevofloxacinとPolymyxin Bを消化管殺菌として好中球減少期に投与された血液悪性疾患119例での感染症合併
Infections in neutropenic patients who received prophylactic Levofloxacin or Polymyxin B

○後藤 秀樹[1,2]、西尾 充史[1,2]、遠藤 知之[1,2]、山本 聡[1,2]、小原 雅人[1,2]、山口 圭介[1,2]、武田 紫[1,2]、笠原 郁美[1,2]、佐藤 典宏[1,3]、小池 隆夫[2]（北海道大学病院造血細胞治療センター[1]、北海道大学病院 第2内科[2]、北海道大学病院 高度先進医療支援センター[3]）

【背景】Giampaoloらは血液悪性疾患を含む担癌患者への化学療法において、プラセボと比較してLevofloxacin（LVFX）が細菌感染予防に有用である、と報告した。（NEJM, 2006）このような報告を受け、血液悪性疾患治療における好中球減少期の消化管殺菌として、非吸収性のPolymyxin B（PMB）に代わり、LVFXが用いられることが多くなったが、この二剤の感染予防効果の差については不明な点が多い。当科では消化管殺菌として、1999年4月から2005年6月まではPMBを、その後現在まではLVFXを使用してきた。この二剤投与下での感染症などについて比較検討した。【患者と方法】対象は当科で血液悪性疾患に対する治療を受けた119例で、PMB群66例、LVFX群53例。年齢・性別に差はなく、疾患はPMB群がNHL46例、MM13例、HL3例、その他4例、LVFX群がAML15例、ALL12例、NHL12例、MDS5例、MM3例、その他6例。治療はPMB群が自家移植64例、同種移植2例、LVFX群が化学療法21例、自家移植11例、同種移植21例。移植前疾患状態はPMB群がCRまたはPR61例、その他5例、LVFX群がCRまたはPR35例、その他18例。好中球減少期に38度以上の発熱が生じた際には各種培養を行うと共に、発熱性好中球減少症のガイドラインに基づいて点滴抗生剤や抗真菌剤の投与を行った。【結果】好中球1000/μl以下の期間はPMB群11±4日、LVFX群18±12日と有意にLVFX群で長かった。血液培養陽性はPMB群7例（グラム陽性菌3例、陰性菌4例）、LVFX群5例（グラム陽性菌4例、陰性菌1例）。感染によりPMB群でのみ2例が死亡した。38度以上の発熱期間、点滴抗生剤の使用、最大CRP値などには二群間で差を認めなかった。【考察】今回の検討は患者背景も異なり、直接の比較ではないが、LVFX群で感染に不利と思われる因子が多いにも関わらず、検討したパラメータでは少なくとも同等ないしは勝っており、LVFXの血液悪性疾患における消化管殺菌としての有用性が示唆された。

OS-1-42 20プールNAT導入後、初めて確認された輸血によるHCV感染の一例
The first case of transfusion-transmitted HCV infection slipping through the 20-member-pool NAT

○石田 高司[1]、坂野 章吾[1]、森 芙美子[1]、伊藤 旭[1]、李 政樹[1]、稲垣 淳[1]、楠本 茂[1]、小松 弘和[1]、神谷 忠[2]、柚木 久雄[3]、田中 靖人[5]、溝上 雅史[5]、飯田 真介[1]、上田 龍三[1]（名古屋市立大学 腫瘍免疫内科学[1]、名古屋市立大学 輸血部[2]、愛知県赤十字血液センター[3]、日本赤十字社中央血液研究所[4]、名古屋市立大学 臨床分子情報医学[5]）

症例は新規に最重症再生不良性貧血と診断された54歳女性。初回輸血前感染症検査でHCV抗体陰性、HCVコア蛋白陰性。6月20日初回輸血。2007年10月1日の輸血後感染症検査でHCVコア蛋白の陽性化［28183.1 fmol/L（＜20.0）］が明らかとなった。直ちに血液センターに報告し遡及調査を開始。はじめに患者の初回輸血前感染症検査残余の保存血清でHCV-RNAが陰性であることを確認した（PCR）。初回輸血から10月1日の間に合計54本のRCCまたはPC輸血があった。それら対象の保管54検体についてそれぞれHCV個別NAT（核酸増幅法）を施行、うち1検体（2007年8月17日輸血RCC）からHCV-RNAを検出した。患者HCVと献血者のHCV Core-E1-E2領域（1279bp）の塩基配列をdirect sequence法で決定し、比較した結果両者は一致した。この結果、本症例は輸血によるHCV感染である可能性が極めて高いと結論した。日本では1999年7月から献血血液の感染症検査に500プールNATを導入し、2000年には50プールに、2004年からは20プールNATとし、そのスクリーニング感度を上げてきた。世界で最も先進的かつ高感度システムといえる。20プールNAT陰性献血血液由来の血液製剤からのHCV感染の報告は本報告が初である。本発表の第1のメッセージは【NAT陰性献血血液由来の血液製剤からでもHCV感染が成立しうる】ことである。また、本症例は2007年10月17日に同種骨髄移植を施行し、2008年3月30日に肺炎のため死亡された。HCV混入血の輸血から約7ヶ月の全経過でHCV抗体価が陽性になることはなく、10月24日からはHCVコア蛋白値は一貫して施設測定可能上限50000.0以上であった。すなわち、免疫抑制状態の患者に対するHCV感染についてはHCV抗体検査のみでは不十分であることを意味する。これらの事実から、第2のメッセージは【輸血前後のスクリーニング検査としてHCVコア蛋白が必要である】ことである。本症例をふまえ、発表当日は【血液製剤の安全性】について議論したい。

別紙様式第2-1

No. 37

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
				2008. 9. 18	該当なし	
一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	Aaron S, McMahon JM, Milano D, Torres L, Clatts M, Tortu S, Mildvan D, Simm M. Clin Infect Dis. 2008 Oct 1;47(7):931-4.	公表国 米国	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)					

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
○C型肝炎ウイルスの鼻腔内伝播:ウイルス学的および臨床的エビデンス 汚染した薬物吸引器具によるC型肝炎ウイルス(HCV)の鼻腔内伝播の可能性が考えられてはいるが、ウイルス感染源として確定されていない。ニューヨーク市のコミュニティ・クリニックから18歳以上で血液中のHCV PCR陽性の吸引用麻薬常用者38名をリクルーティングした。鼻汁検体を採取したほか、被験者が通常薬物を使用する時のようにストローを使用し、このストローを回収して、血液及びHCV RNAの存在を調べた。鼻汁検体28(74%)、ストロー3(8%)で血液が検出された。HCV RNAは鼻汁検体5(13%)、ストロー2(5%)で検出された。被験者のうち11名では、鼻中隔穿孔など慢性的薬物吸引と関連する鼻の異常が見られた。鼻汁検体と薬物吸引器具に血液とHCV RNAが存在することから、HCV鼻腔内伝播のウイルス学的妥当性が示された。	解凍赤血球濃厚液「日赤」 照射解凍赤血球濃厚液「日赤」 解凍赤血球-LR「日赤」 照射解凍赤血球-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
汚染した薬物吸引器具によるC型肝炎ウイルスの鼻腔内伝播のウイルス学的妥当性を示したとの報告である。	HCV感染の新たな伝播ルート等について、今後も情報の収集に努める。

(4)

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Intranasal Transmission of Hepatitis C Virus: Virological and Clinical Evidence

Sagar Aaron,[1] James M. McMahon,[1,2] Danielle Milano,[1] Lillani Torres,[1] Michael Clatts,[3] Stephanie Tortu,[3] Donna Mildvan,[4] and Malgorzata Simm[1]

[1]Molecular Virology Division, St. Luke's–Roosevelt Institute for Health Sciences/Columbia University, [2]National Development and Research Institutes, [3]Bonken Neighborhood Health Center, and [4]Division of Infectious Diseases, Beth Israel Medical Center, New York, and [5]School of Nursing, University of Rochester Medical Center, Rochester, New York; [6]School of Public Health, Louisiana State University, New Orleans; and [7]School of Public Health, Center for Global Health Research, University of Puerto Rico, San Juan

Intranasal transmission of hepatitis C virus (HCV) via contaminated drug-sniffing implements is a potential but unconfirmed source of viral infection. We demonstrate the virological plausibility of intranasal transmission by confirming that blood and HCV RNA are present in the nasal secretions and drug-sniffing implements of HCV-infected intranasal drug users recruited from a community health clinic in New York City.

Hepatitis C virus (HCV) is the most common bloodborne pathogen in the United States and is a major cause of liver-related morbidity, mortality, and liver transplantation [1]. HCV is transmitted through contact with infected blood [2] (mostly via shared needles and other drug injection paraphernalia); however, a large proportion (up to 20%) of HCV infections remain unexplained, especially among noninjection drug users [3]. One hypothesis to account for these unexplained cases involves intranasal transmission of HCV via contaminated implements, such as straws, used to snort cocaine, heroin, and other powdered drugs [4]. Implements inserted into the nasal cavity, which has been eroded by long-term drug sniffing, might come into contact with HCV-infected mucus or blood, which might then be transmited to a susceptible individual sharing the same implement [5]. Epidemiological studies of intranasal transmission of HCV have produced inconsistent findings [6, 7], in part because of the high correlation between drug sniffing and other risk factors for HCV infection. Here, we attempt to refute the intranasal transmission hypothesis by invalidating ≥1 of its virological preconditions. Specifically, we address 2 primary research questions: (1) Does HCV RNA exist in the nasal secretions of serum-positive drug sniffers? (2) If so, can HCV RNA be transferred onto the sniffing implements shared by intranasal drug users. A secondary aim was to examine clinical nasal pathologies that might facilitate intranasal HCV transmission.

Methods. Our sample included low-income, urban intranasal drug users with chronic, active HCV infection. Subjects were primarily Hispanic and African American and were recruited from a neighborhood health clinic in East Harlem, New York City, an area with a high prevalence of HCV infection (up to 29%) among noninjection drug users [3]. Eligibility criteria included (1) age, ≥18 years; (2) self-reported intranasal drug use; and (3) a positive result of a quantitative HCV PCR blood test. Overall, 38 patients enrolled in the study and provided informed consent. Study protocols were approved by 3 institutional review boards.

The following medical information was obtained from subjects: quantitative HCV RNA test result and viral load, hepatitis B antibody test results, liver enzyme levels (i.e., alanine aminotransferase level), and liver biopsy history. Subjects completed a brief survey, in either Spanish or English, that covered demographic characteristics, risk factors for HCV infection, injection and noninjection drug use, health status, and nasal pathology symptoms.

Blood samples were collected for quantitative PCR. Two nasal secretion samples (1 from each nostril) were collected with Dacron nasal swabs and placed in (1) 1 mL of TRIzol reagent (Gibco BRL) for RNA detection or (2) 1 mL of OBTI solution for blood detection. Similarly, 2 experimental sniffing implements, which consisted of new (packaged) soda straws commonly used by drug sniffers, were collected from each subject. To avoid harmful effects of sniffing powdered substances, subjects were instructed to "snort air" while mimicking their normal drug-sniffing behavior.

HCV RNA was isolated from 200 μL of serum by use of the QIAamp MinElute kit (Qiagen); HCV RNA was isolated from nasal secretions and sniffing implements using the TRIzol (Gibco BRL) on the basis of established protocols [8]. The first strand of cDNA was synthesized by ImProm-IITM Reverse Transcription System (Promega) using gene-specific downstream primers targeting the HCV p22 core region, with minor

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Reprints or correspondence: Dr. James McMahon, School of Nursing, University of Rochester Medical Center, 601 Elmwood Ave., Rochester, NY 14642 (james_mcmahon@urmc.rochester.edu).

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Table 1. Detection of hepatitis C virus (HCV) RNA and blood in biological specimens obtained from 38 patients with HCV-positive serum specimens.

Assay	No. (%) of persons (n = 38)	95% CI
Blood detection with OBTI		
Nasal secretions	28 (73.7)	57.6–85.2
Sniffing straws	3 (7.9)	2.0–21.5
HCV RNA detection with PCR		
Nasal secretions	5 (13.2)	5.3–27.8
Sniffing straws	2 (5.3)	0.5–18.2

modification of the upstream primer (410R-5′-ATGTACCCCA-TGAGGTCGGC-3′). HCV cDNA was amplified by PCR with 40 cycles of denaturation (94°C for 30 s), annealing (58°C for 30 s), and elongation (72°C for 45 s) with primers 406F-5′-TAGACCGTGCACCATGAGC-3′ and 410R. PCR products were detected by Southern blot using ^{32}P-labeled probe (5′-AGGAAGACTTCCGAGCGGTCGCAA-3′).

HCV cDNA was amplified from randomly selected HCV-positive blood samples with use of high-fidelity Pfu polymerase (Perkin Elmer) using 410R and 406F primers and cloned into a TA cloning vector (Invitrogen). The pTA_HCV was used to prepare standard curves ranging from 1×10^4 to 10 copies of HCV mRNA, which were run in parallel to each set of samples. The intensity of DNA bands was evaluated by densitometry using the Kodak Image Analysis System; the HCV load for the test sample was calculated on the basis of the numeric value derived from the HCV titration curve. HCV load was calculated as the number of copies per milliliter for blood specimens and as the number of copies per sample for nasal secretions and implements.

Traces of blood in nasal secretions and sniffing implements were detected by Hexagon OBTI Kit (BLUESTAR Forensic). Titration curves were prepared using human hemoglobin (Sigma) in 2-fold dilutions ranging from 10 to 0.1 μg/mL. The concentration of blood in each sample was established by comparing the OBTI intensity between the sample and the hemoglobin titration curve.

Nasal cavity pathology was assessed for each patient by anterior nasal examination, rendering diagnoses on 8 nasal pathologies. Rhinitis was diagnosed on the basis of the classic symptoms of mucosal and nasal secretion appearance [9]. Rhinosinusitis was defined by symptomatic inflammation of the paranasal sinuses and nasal cavity [10].

Sample prevalences of HCV RNA and occult blood in nasal secretions and on sniffing implements were estimated. Ninety-five percent CIs were calculated around point estimates using the adjusted Wald method. Descriptive statistics were calculated for sample descriptors and measures of nasal pathology. Our limited sample size precluded statistical tests of significance (e.g., associations between virological and clinical variables).

Results. All 38 patients had chronic, active hepatitis C. The serum HCV load ranged from 250 to 5,000,000 copies/mL (median, 5000 copies/mL). Recent liver biopsies had been performed for 6 patients; all indicated chronic liver disease, with stages ranging from 1 to 4. Recent alanine aminotransferase levels were available for 17 patients; the mean level (± SD) was 46.7 ± 26.7 U/L (range, 16–118 U/L). Antibody screening revealed that 34% of subjects were positive for antibodies to HIV, and 45% were positive for antibodies to hepatitis B virus.

Trace amounts of blood were detected in 28 (74%) of 38 nasal secretion samples (range, 0.1–10 μg/mL) and on 3 (8%) of the 38 sniffing implements (range, 0.1–2 μg/mL). HCV RNA was detected in 5 nasal secretion samples (13%; HCV RNA level range, 10–100 copies/sample) and on 2 sniffing implements (5%; HCV RNA level, 50 and 100,000 copies/sample). Prevalence estimates suggest a wide discrepancy between the presence of blood (74%) and the presence of HCV RNA (13%) in the nasal secretion samples (table 1). Of the 5 HCV RNA–positive nasal secretion samples, only 3 had traces of occult blood; of the 28 samples containing occult blood, 25 were negative for HCV RNA (figure 1).

The prevalence of rhinitis in this cohort was high (71%) (table 2). In contrast, the prevalence of rhinosinusitis (11%) is consistent with that of the general population. More than 40% of subjects experienced rhinorrhea or nasal congestion at least once per week, 8% reported nose bleeds at least once per week, and 8% and 16% reported mucosal lesions and crusting, respectively. Approximately one-half of the subjects attributed these symptoms to intranasal drug use. Four persons (11%) were observed to have nasal septal perforations; 1 (3%) had a nasopalatal perforation; and 6 (16%) displayed symptoms of saddlenose deformation. These pathologies have been associated with advanced nasal cavity deterioration associated with chronic intranasal drug use [11].

Discussion. Our findings revealed a high prevalence of blood (74%) in the nasal secretions of HCV-positive long-term drug sniffers. We also confirmed that HCV RNA was present in the nasal secretions of a substantial proportion (13%) of this cohort. Most significantly, this study demonstrated that both blood and HCV particles can be transferred onto sniffing implements (i.e., straws) during simulated intranasal drug use. Studies have shown that HCV can remain viable on environmental surfaces for up to 16 h, but little is know about the quantity of virus required for transmission [12]. The prevalences of HCV in the nasal secretions and on sniffing straws are likely conservative estimates. It is reasonable to assume that HCV will be present in the nasal secretions with greater frequency and quantity during episodes of active drug sniffing, which may exacerbate discharge of nasal fluids and blood.

		Occult Blood in Nasal Secretions		
		Pos.	Neg.	
HCV RNA in Nasal Secretions	Pos.	3	2	5
	Neg.	25	8	33
		28	10	38

Figure 1. Hepatitis C virus (HCV) RNA and occult blood in nasal secretions.

Table 2. Frequency of nasal pathology symptoms among intranasal drug users.

Symptom	No. (%) of subjects (n = 38)
Findings of an anterior nasal clinical examination	
Loss of nasal hairs	4 (10.5)
Rhinitis	27 (71.1)
Rhinosinusitis	4 (10.5)
Presence of nasal crusting and/or scabbing	6 (15.8)
Sores or erosion of nasal mucosa	3 (7.9)
Saddlenose deformation	6 (15.8)
Nasopalatal perforation	1 (2.6)
Nasal septum perforation	4 (10.5)
Self-reported nasal pathology	
Frequency of nosebleeds in the past year	
Never, or rarely	26 (68.4)
Once or a few times per month	9 (23.7)
Once or a few times per week	2 (5.3)
Once or more per day	1 (2.6)
Experienced a runny or stuffy nose in the past year	
Never or rarely	16 (42.1)
Once or a few times per month	6 (15.8)
Once or a few times per week	13 (34.2)
Once or more per day	3 (7.9)
Reason for nasal symptoms	
Allergies	19 (60.0)
Cold or influenza	10 (26.3)
Drug sniffing	21 (55.3)
"Have you ever noticed any of the following problems with your nose due to drug sniffing?"	
Scabs in the nose	14 (36.8)
Sores in the nose	8 (21.1)
Poor sense of smell	13 (34.2)
Sinus pain	13 (34.2)
Headaches located in the forehead	16 (42.1)
Double vision	5 (13.2)
"Has a doctor or other health care professional ever told you that the inside of your nose is damaged in any way from sniffing drugs?"	7 (18.4)

Data in table 1 contradict the assumption that, in persons with HCV-positive serum specimens, detection of blood implies the presence of HCV. This discrepancy may be explained by 2 factors. First, the 2 assays (PCR and OBTI) were not performed on the same samples. Second, the OBTI assay for blood detects immune complexes between human hemoglobin (hHb) and monoclonal anti-hHb antibodies, which can occur even in the absence of viable cells. In contrast, PCR can only detect HCV RNA from intact particles. Therefore, the discrepancy between the high prevalence of occult blood and relatively low detection of HCV RNA in nasal secretions may be associated with the rapid deterioration of viral RNA in the nasal environment or the destruction of viral particles by mucosal immunity. If the viability of HCV particles in nasal secretions is moderated by nasal pathology or immunity, this might help explain conflicting epidemiological findings in which these moderating factors are not considered.

This study establishes the validity of 2 primary virological preconditions necessary for intranasal HCV transmission: (1)

the presence of blood and HCV in the nasal secretions of intranasal drug users, and (2) the transference of blood and HCV from the nasal cavity onto sniffing implements, which are often shared by intranasal drug users. Moreover, the frequency and severity of nasal pathologies observed in this cohort might aggravate conditions that facilitate intranasal HCV transmission. Consequently, these findings lend important virological and clinical support to the intranasal HCV transmission hypothesis. In addition, detection of HCV in nasal secretions advances the debate regarding potential iatrogenic and nosocomial transmission of HCV in the context of ear, nose, and throat and related clinical practices. More research is needed to confirm intranasal transmission as a mode of viral infection and to determine its impact on the wider epidemic of HCV infection.

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Potential conflicts of interest. All authors: no conflicts.

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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	－		研究報告の公表状況	Vox Sanguinis (2008) 95, 94-100	公表国		使用上の注意記載状況・その他参考事項等
販売名（企業名）	－				英国		
研究報告の概要	E型肝炎ウイルス（HEV）はヘペウイルス属に分類され、エンベロープがなく、直径約27～34nmのウイルスで、糞便から経口、食物媒介および血液媒介経路で伝播され、ヒトの肝炎の原因となる。 今までの報告では、56℃30分間の加熱で4つのHEV株が不活化され、別の報告では糞便由来の3つのHEV分離株は、56℃または60℃60分の加熱で不活化されたが、熱抵抗性の特性は株間でわずかに異なったとされている。 本研究では、日本で発見された遺伝子型3と4の4つのHEV分離株を用いて、アルブミン及びフィブリノゲンにおける液状加熱、乾燥加熱およびウイルス除去膜ろ過によるHEV不活化/除去能を検討した。 その結果、25％アルブミン（液状加熱段階直前に採取）では、60℃で5時間の加熱を行ったが、いずれのHEV分離株も熱抵抗性を示し、5時間加熱後も感染力が検出され、LRF（log reduction factor）はそれぞれ2.0、2.0、1.0および2.2以上であった。 フィブリノゲン（2.0 w/v％塩酸L-アルギニン含有、乾燥加熱段階直前に採取）では、60℃で72時間処理したところ、感染力が検出された。 また、ウイルス除去膜では、いずれのHEV分離株も、孔径19nmおよび15nmでは検出限界以下まで除去されたが、35nmでは大量のHEVが検出され、HEVの粒子サイズは既に電子顕微鏡分析で報告されているように約35nmであることが示唆された。					
	報告企業の意見		今後の対応				
	HEVの不活化、除去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HEVに関する情報に留意し、関連情報の収集に留意していく。				

(5)

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ORIGINAL PAPER

Extent of hepatitis E virus elimination is affected by stabilizers present in plasma products and pore size of nanofilters

M. Yunoki,[1,2] S. Yamamoto,[1] H. Tanaka,[1] H. Nishigaki,[1] Y. Tanaka,[1] A. Nishida,[1] J. Adan-Kubo,[1,2] M. Tsujikawa,[1] S. Hattori,[3] T. Urayama,[1,2] M. Yoshikawa,[1] I. Yamamoto,[1] K. Hagiwara[3] & K. Ikuta[2]

[1]*Infectious Pathogen Research Group, Hirakata Research Laboratory, Research & Development Division, Benesis Corporation, Hirakata, Osaka, Japan*
[2]*Department of Virology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan*
[3]*Department of Veterinary Microbiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan*

Vox Sanguinis

Background and Objective To investigate the physico-chemical properties of hepatitis E virus (HEV) with regard to inactivation/removal, we have studied four isolates with respect to sensitivity to heat during liquid/dry-heating as well as removal by nanofiltration.

Materials and Methods Hepatitis E virus in an albumin solution or phosphate-buffered saline (PBS) was liquid-heated at 60°C for a preset time. HEV in a freeze-dried fibrinogen containing stabilizers was also dry-heated at 60 or 80°C for a preset time. In addition, to clarify the removal of HEV, the purified virus in PBS was filtered using several types of virus-removal filter (nanofilters) that have different pore sizes. HEV infectivity or genome equivalents before and after the treatments were assayed by a semiquantitative cell-based infectivity assay or quantitative polymerase chain reaction assay, respectively.

Results Hepatitis E virus isolates in albumin solutions were inactivated slowly at 60°C for 5 h and the resultant log reduction factor (LRF) was from 1·0 to ≥ 2·2, whereas the virus in PBS was inactivated quickly to below the detection limit and the LRF was ≥ 2·4 to ≥ 3·7. The virus in a freeze dried fibrinogen containing trisodium citrate dihydrate and L-arginine hydrochloride as stabilizers was inactivated slowly and the LRF was 2·0 and 3·0, respectively, of the 72 h at 60°C, but inactivated to below the detection limit within 24 h at 80°C with an LRF of ≥ 4·0. The virus in PBS was also confirmed as to be approximately 35 nm in diameter by nanofiltration. These results are useful for evaluating viral safety against HEV contamination in blood products.

Conclusion The sensitivity of HEV to heat was shown to vary greatly depending on the heating conditions. On the other hand, the HEV particles were completely removed using 20-nm nanofilters. However, each inactivation/removal step should be carefully evaluated with respect to the HEV inactivation/removal capacity, which may be influenced by processing conditions such as the stabilizers used for blood products.

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Correspondence: Mikihiro Yunoki, PhD, Infectious Pathogen Research Group, Hirakata Research Laboratory, Research & Development Division, Benesis Corporation, 2-25-1 Shodai-Ohtani, Hirakata, Osaka 573-1153, Japan
E-mail: yunoki.mikihiro@mk.mt-pharma.co.jp

Introduction

Hepatitis E virus (HEV), classified in the genus *Hepevirus*, is a causative agent of human hepatitis. The virus capsid is non-enveloped and the nucleocapsid containing positive-sense single-stranded RNA has a diameter of 27–34 nm [1]. HEV is also endemic in humans, swine and several wild animals such as deers and boars, suggesting that hepatitis E is a zoonosis [2,3].

The virus has been shown to be transmitted by faecal-oral, food-borne and blood-borne routes [1,4–7]. Four genotypes of HEV that infect humans have been identified, three of which, genotypes 1, 3 and 4, have also been isolated from swine and commercial swine liver [1,8,9]. Zoonotic food-borne transmission of HEV was shown to be one reason for the occurrence of a severe form of hepatitis E in Hokkaido, Japan, and HEV genotype and the presence of an underlying disease influenced the severity of the hepatitis E infection [10]. In addition, the prevalence of HEV RNA or anti HEV immunoglobulin G (IgG)-positive blood donors in Hokkaido was 0·01% (56/432,167) and 3·9%, respectively [11]. These reports also suggested that a small but significant proportion of blood donors in Japan with or without elevated alanine aminotransferase (ALT) levels are viremic and are potentially able to cause transfusion-associated hepatitis E. Note that anti-HEV IgG and HEV levels in pooled plasma have not been reported yet. Thus, these data may indicate the need for precautions against the potential risk of transfusion-transmitted HEV infection, as previously discussed [12]. In addition to foods, the safety of plasma-derived products with respect to HEV may be an important issue and each product should be evaluated for safety against HEV contamination.

Huang *et al.* reported that four HEV strains in culture media containing 2% calf serum were inactivated, and that residual infectivity was not detected after heating at 56°C for 30 min [13]. Emerson *et al.* reported that three HEV isolates derived from faeces including genotypes 1 and 2 were inactivated after 60 min at 56 or 60°C, but the heat-resistance properties differed slightly between the strains used. A strain that was slightly more resistant to heating showed some residual infectivity (< 1%) after 1 h at 56°C [14]. Tanaka *et al.* also reported that an HEV isolate in a faecal suspension in Tris-HCl buffer was inactivated and that residual infectivity was not detected after heating at 70°C for 10 min, whereas residual infectivity was detected after 30 min at 56°C [15]. Unfortunately, these studies did not evaluate the log reduction of infectivity and kinetic pattern of inactivation.

There have been no reports of HEV transmission via plasma-derived products that contain various kinds of proteins at high concentrations and also various types of stabilizers. However, investigative methods with log reduction and/or general information on HEV regarding the contamination of blood products have been required. In this study, we investigated the impact on the ability to inactivate HEV during liquid/dry-heating and viral particle removal by nanofiltration in plasma protein preparations using four HEV isolates found in Japan and belonging to genotypes 3 and 4.

Materials and methods

Viral isolates

Isolates from four different HEV clusters were used, that is, genotype 3_{SP} [swJB-E, cluster SP (3e), GENBANK (in preparation by Yamate *et al.*)], genotype 3_{US} [swJB-M, cluster US (3a), GENBANK (in preparation by Yamate *et al.*)], genotype 3_{JP-a} [swJB-N, unclassified cluster, GENBANK (in preparation by Tsunemistu *et al.*)], and genotype 4_{JP} [swJB-H, cluster JP (4c), GENBANK (in preparation by Yamate *et al.*)] (Table 1). These viruses were derived from faeces of infected swine in Japan. The origins of swJB-H, swJB-E and swJB-M were naturally infected swine faeces, while swJB-N was from faeces of experimentally infected swine (Highland strain, kindly provided by Dr Hiroshi Tsunemitsu, National Institute of Animal Health, Japan).

Table 1 Details of viral isolates used

Genotype[a]	Isolation ID	Viral titre: HEV genome[b]	Viral titre: HEV infectivity[c]	Used for
3_{JP-a}	swJB-N2	6·3	3·8	Liquid-heating, nanofiltration
3_{US}	swJB-M5	7·2	4·8	Nanofiltration, dry-heating
	swJB-M8	8·4	5·3[d]	Liquid-heating
3_{SP}	swJB-E8	7·5	4·8	Dry-heating
	swJB-E10	7·7	5·8[e]	Liquid-heating, nano-filtration
4_{JP}	swJB-H1	7·0	–	Nanofiltration
	swJB-H1/H7	7·0/7·4	4·8[f]	Liquid-heating
	swJB-H7	7·4	3·2[d]	Liquid-heating
	swJB-H8	6·8	3·8	Liquid-heating
	swJB-H21[g]	7·2	3·8	Liquid-heating

[a]The genotypes and clusters of isolates were grouped as described by Takahashi *et al.* and Lu *et al.* [24,25].
[b]Genome amount is indicated by log copies per mL. For swJB-M, specific primer sets and probes '(sense primer F2: 5′-TCGTGTACAAACCGAGATTC-3′, anti-sense primer R2: 5′-GCCCGGCAATATTGTTCTA-3′, Probe Flu2: 5′-GATGCAACCCCGGCAGTTGGTTTTC-FITC-3′ and Probe LC2: 5′-LCRed640-GCCCTGAGGTACTCTGGAATCATCCTATCC-3′)' were designed and used. For the other isolates, the primer set and probe (HEB6, HE87 and FAMlabeled probe FHEB8) designed by Jothikumar *et al.* [26] were used.
[c]Infectivity titre is given as log dilution non-detectable end-point per mL.
[d]Mean titre of two ('three) independent experiments.
[e]Mixture of H1 and H7 used.
[f]This isolate is derived from faeces of an experimentally infected piglet.

Isolation and purification of virus

Faecal samples (10 g) were resuspended with 100 ml of phosphate-buffered saline (PBS) and centrifuged at 1600 *g* for 10 min and the supernatant retained. Pellets were resuspended in 50 ml of PBS and the suspension was centrifuged again under the same conditions. Resultant pellets were resuspended with 25 ml of PBS and the suspension was centrifuged again. All these three supernatants were pooled and were filtered using an AP filter (AP2504700, Millipore, Billerica, MA, USA). After centrifugation at 10 000 *g* for 30 min, the supernatant was filtered through four sequential filters (5·0 μm; SMWP04700, 1·2 μm; RAWP04700, 0·8 μm; AAWP04700, and finally 0·45 μm; HAWP04700, Millipore). Then polyethylene glycol (PEG) 6000 (Wako Pure Chemical Industries, Osaka, Japan) and sodium chloride up to final concentrations of 8% (w/v) and 2·4% (w/v), respectively, were added to the final filtrate. The solution was stirred for 10 min and incubated overnight at 4°C. The solution was centrifuged at 10 000 *g* for 30 min and the precipitate was resuspended with one-tenth the volume of the original solution of PBS prior to the addition of PEG. The solution was sonicated and centrifuged at 4000 *g* for 15 min at 4°C. The resultant supernatant was filtered in two steps (0·45 μm; SLHV033RS and 0·22 μm; SLGV033RS, Millipore), and the filtrate was aliquoted and stored at −80°C as HEV stock. Isolated HEV samples were allocated an isolation ID and preparation lot number.

Hepatitis E virus stocks were further purified for filtration experiments. The viral stocks in PBS were treated with 1% (v/v) Tween-80 (Wako Pure Chemical Industries) and 0·3% (v/v) Tri-n-butyl Phosphate (TNBP, Sigma, St. Louis, MO, USA) for 1 h at 30°C and then the solutions were ultracentrifuged at 150 000 *g* for 3 h at 4°C. The precipitates were resuspended in PBS and subsequently sonicated and centrifuged at 4000 *g* for 15 min at 4°C. The supernatants were filtered by sequential 0·22 and 0·1 μm filtration [SLGV033RS (0·22 μm) and SLVV033RS (0·1 μm); Millipore] and the filtrate was aliquoted and stored at −80°C as purified HEV stock. In addition, HEV Genotype 3_{Sp} derived from the culture media of infected A549 cells was treated with detergent alone, as described above, and subsequently used for filtration experiments.

Quantitative HEV RNA assay for each isolate

The total HEV RNA in each sample was extracted using the RNeasy Mini Kit (cat. 74104; Qiagen GmbH, Hilden, Germany) and then quantified by polymerase chain reaction (PCR) using specific primers. The copy number of swJB-M was quantified using specified primers and probes set from the light cycler (LC) RNA Amplification Kit Hybridization Probes (Roche Diagnostics, Basel, Switzerland) and LC quick system 350S (Roche Diagnostic). The assay conditions were as follows: reagents; 4·0 μl of 5× LC reverse transcription (RT)-PCR Mix HybProbe (Roche Diagnostic), 3·2 μl of 25 mM $MgCl_2$, 2·0 μl of 5 pmol/μl primer F+R, 2·0 μl of 2 pmol/μl probe Flu+LC, 3·4 μl of water, 0·4 μl of LC RT-PCR enzyme mix and 5·0 μl of template (total 20 μl), and reaction; 55°C 10 min, 95°C 30 second, 45 cycles of 95°C 5 second, 60°C 15 second, 72°C 13 second and subsequently 40°C 30 second. The copy number of ORF3 for swJB-N, swJB-E and swJB-H (genotypes 3_{JPn}, 3_{Sp} and 4_{JP}) was also quantified using a QuantiTect Probe RT-PCR Kit (Qiagen) and Applied Biosystems 7500 (Applied Biosystems, Foster City, CA, USA). The assay conditions were as follows: reagents; 25 μl of 2× QuantiTect Probe RT-PCR Master Mix (Qiagen GmbH), 1·0 μl of 20 μM primer Mix, 0·5 μl of 10 μM Probe, 0·5 μl of QuantiTect RT Mix, 13·0 μl of water and 10 μl of template (total 50 μl), and reaction; 50°C 30 min, 95°C 15 min, 45 cycles of 95°C 15 second and 60°C 35 second.

Infectivity assay for HEV

Infectivity of HEV was assayed according to Huang *et al.* [13] with minor modifications. A549 cells (kindly provided by Dr Takaaki Nakaya, Research Institute for Microbial Diseases, Osaka University) were cultured in DMEM (cat. 11995-065, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (cat. SH30071·03; Hyclone, Logan, UT, USA), 100 U/ml penicillin, 100 μg/ml streptomycin (cat. 15140-122, Invitrogen) and Insulin-Transferrin-Selenium-X (ITS-X) supplement (cat. 51500-056, Invitrogen) at 37°C in 5% CO_2 in air. The composition of the medium used for the viral assay was Dulbecco's modified Eagle's medium (DMEM) containing 2% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, ITS-X supplement and 30 mM $MgCl_2$ (cat. 135-00165, Wako Pure Chemical Industries) at 37°C in 5% CO_2 in air. For the infectivity assay, A549 cells were seeded in a 12-well microplate ($3·6 \times 10^5$ cells/ml, 2 ml/well). After an overnight culture, the cells were inoculated with serial 10-fold dilutions of the virus stock solution (0·3 ml/well). On day 7 of culture, HEV RNA in cultured cells was assayed using the HEV RNA assay method described above. The infectivity of each stock of isolate used was determined from the dilution end-point where no RNA was detected.

Heat sensitivity of HEV during liquid- and dry-heating

Hepatitis E virus isolates were ultracentrifuged at 150 000 *g* for 3 h at 4°C. The resultant pellets were resuspended with PBS or a 25% albumin solution that was collected just before the heating step in the manufacture of Kenketsu Albumin-Wf (Benesis, Osaka, Japan) as a stabilizer. These samples were aliquoted at 0·5 ml per tube and incubated in a water bath at 60°C for preset times (0, 0·5, 1, 2 and 5 h). After quickly cooling, the residual infectivity of the sample was determined as described above.

The HEV precipitates described above were also resuspended with a Fibrinogen solution containing 1·3% (w/v) trisodium citrate dihydrate and 2·0% (w/v) L-arginine hydrochloride as a stabilizer that was collected just before the dry-heating step in the manufacture of Fibrinogen HT-Wf (Benesis). The HEV solutions were aliquoted at 2·0 ml/vial and freeze-dried using an optimized freeze drying cycle (programme) for this product (freeze dry systems cat. 7948020 and 7934024, Labconco, Kansas City, MO, USA). The freeze-dried samples in the vials were closed under vacuum. The vials were then heated at 60 or 80°C in a drying oven (cat. DK43; Yamato Scientific, Tokyo, Japan) for 72 h. The heated samples were cooled quickly and stored at 4°C until the assaying. Residual infectivity was assayed as described above. In addition, the residual water content of mock-infected samples prepared using the same freeze drier programme and conditions without spiking with HEV were assayed using the loss on drying test method described previously [16].

Removal of HEV by nanofiltration

Hepatitis E virus stocks that were detergent-treated, as described above, were thawed, concentrated, if required, sonicated and filtered using 0·22 μm (0·22 μm; SLGV033RS, Millipore) and Bemberg Microporous Membrane (BMM) filter (Planova®-75N (72 ± 4 nm, 0·001 m^2); Asahi Kasei Medical, Tokyo, Japan) immediately prior to nanofiltration. The viral samples were subjected to nanofiltration using BMM-35N (35 ± 2 nm), -20N (19 ± 2 nm) and -15N (15 ± 2 nm; Asahi Kasei Medical) under conditions where 2-ml samples were applied to 10^{-5} m^2 filters with 50 kPa and dead end filtration. The quantities of HEV RNA before and after filtration were measured using the quantitative HEV RNA assay described above.

Results

Viral preparations

Isolates from four different clusters including two genotypes were prepared and each isolate was evaluated regarding genome and infectious titre in the stocks.

We evaluated the appropriateness of the method to determine the HEV infectious titre by semiquantitative PCR (data not shown). The levels of HEV RNA in the infected cells were higher at 3 and 7 days post-infection (dpi) than at 0 dpi. The titres obtained were not consistent on 3 dpi whereas the results were consistent on 7 dpi. Therefore, we decided that the titre of HEV should be determined on 7 dpi. According to our data, about 1000 copies of the genome per infectious unit were observed in our system. The infectious titres in the HEV stocks of the viruses are summarized in Table 1.

Heat sensitivity of HEV

The heat-inactivation kinetics of HEV isolates from four clusters including two genotypes during liquid-heating using 25% albumin and PBS at 60°C for 5 h was evaluated. All isolates in PBS were inactivated below the detectable infectivity limit within 30 min at 60°C and showed a rapid inactivation. The log reduction factor (LRF) of genotype 3_{JPn}, 3_{Sp}, 3_{US} and 4_{JP} was ≥ 2·7, ≥ 3·7, ≥ 3·7 and ≥ 2·4, respectively. In contrast, all HEV isolates in the 25% albumin solution showed heat resistance, and residual infectivity was detected even in the samples heated for 5 h and the LRF was 2·0, 2·0, 1·0 and ≥ 2·2, respectively (Fig. 1).

The heat-inactivation kinetics of Genotype 3_{US} and 3_{Sp} in fibrinogen during dry-heating was also evaluated. The water content of freeze-dried samples containing the two HEVs was < 0·3%. Residual infectivity was not detected with the LRF

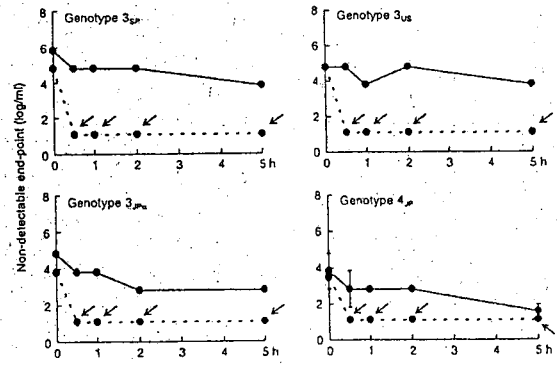

Fig. 1 Inactivation kinetics of the four HEV isolates during liquid-heating. Solid lines: HEV in 25% albumin. Broken lines: HEV in PBS. Arrow: infectious virus was not detected. Genotype 4_{JP}: $n = 3$.

