資料２－２

Ａ　研究報告（詳細版）

平成２８年１月６日
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医薬品
医薬部外品　　研究報告　調査報告書
化粧品

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研究報告の概要

1．緒　　言

E 型肝炎ウイルス（HEV）は多くの国々において肝炎の原因物質であり、先進国において新興の懸念材料である。HEV はノンエンベロープ、一本鎖、＋鎖 RNA ウイルスで、ヘペウイルス科の一種である。発展途上国では、HEV（遺伝子型 1 および 2）は糞便一経口経路による伝播および飲料水の汚染に関連した、急性肝炎の主要原因である。先進国では、HEV（遺伝子型 3 および 4）は元々信じられていた以上にヒト集団において、より流行していることが知られている。HEV 遺伝子型 3 および 4 はヒトだけでなく、ブタやイノシシ、そしてシカなどの動物にも感染する。ヒトへの HEV 遺伝子型 3 および 4 の人畜共通伝播は、加熱が不十分な豚肉やイノシシ製品の消費によって、或いは感染した動物との接触によって起こり得る。遺伝子型 3 および 4 は、遺伝子型 1 および 2 よりも一般的に病原性が少ないが、重症感染症は遺伝子型 3 および 4 でも報告されている。HEV 遺伝子型 3 の慢性感染は移植レシピエントの間で新しい懸念であり、HIV および特定の血液疾患のヒトにおいても起こる可能性がある。

HEV 感染は広範囲にわたり、血液/血漿ドナーはしばしば無症候性である。従って、ウイルス血症供血のリスクがある。HEV は 2004 年以降輸血感染物質として認識されており、輸血関連症例は数か国（英国、フランス、日本、サウジアラビア、中華人民共和国）で報告されている。血液および血漿供血の最近の分析は、ヨーロッパおよび米国で HEV 感染ドナーを特定していた。結果として、HEV-RNA が医薬品の製造に使われる血漿プール中で検出されていた。

ウイルス血症供血の頻度に関する公表された報告および血漿プールに関する研究は、医薬品の製造用出発材料として使われる血漿プールが HEV で汚染され得ることを示す。その上、HEV に感染した供血が分画用血漿プールに投入されていたことを示す供血後情報の症例があった。これは血漿由来医薬品の安全性についての問題を提起する。ヒト血漿プールおよびウイルス不活性化のための処理（1646）に対する欧州薬局方モノグラフは、HEV RNA に対する検査を含めて改訂された（実施日 2015 年 1 月 1 日）。HEV RNA に対する WHO 国際標準は、核酸増幅技術（NAT）による HEV 測定法の標準化を促進して、確立された。他の血漿由来製剤の製造は、ノンエンベロープウイルスの不活化/除去のための処理工程を含む。HEV に対するそれらの有効性は、現在調査中である。HEV は細胞培養中での培養が難しく、血漿由来医薬品の製造に用いられるウイルス不活化/除去工程への HEV の感受性に関する現在の情報は限られていた。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL 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献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 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

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

E型肝炎ウイルスについての血漿由来医薬品のウイルス安全性に関するEMAワークショップが2014年10月28日～29日に開催された。ワークショップの目的は、HEVについての血漿由来医薬品の安全性に関する詳細な情報を得ることと、更なる行動が必要かどうかを決めるための基本的な情報を提供することであった。
鍵となる問題は以下の通り：
－HEV感染および輸血関連感染症の臨床経験：どの様な重篤がHEV感染症であり、その患者集団が特に危険に曝される可能性があるか。
－血液/血漿供血中のHEV検出およびHEVの疫学。
－HEVに対する抗体は有意に中和するのか。
－HEVの不活化/除去に関する研究からの最新の経験：どの工程がHEV除去/不活化に効果的であるか。どのモデルウイルスがHEV除去/不活化の評価に使用できるか。より多くのウイルスバリデーションデータが必要か。
－血漿由来医薬品のリスク評価および警告文の意義：リスク評価および/または警告文が必要か。
－NAT検査はSDプラスマに対して欧州薬局方で要求される。これは多くの他の製剤にも要求されなければならないか。

（中　略）

3．結　　論
HEV遺伝子型3は、先進国の無症候性ドナーからの血液/血漿供血において確認されている。その様な供血が血漿由来医薬品の製造工程に入ることはリスクである。遺伝子型3の感染は、しばしば無症候性か、軽度である。患者集団に関する限り、HEV遺伝子型3は免疫不全のヒト（例えば、移植レシピエント）や潜在的な肝臓障害、或いは肝臓疾患の患者に対する脅威と考えられた。HEV感染の臨床症状は多様で、まだ完全に分かっていないが、HEVは免疫不全患者において長期、或いは慢性感染につながることが知られている。
HEV遺伝子型3に罹ったヒトおよびブタの感染症は、ヨーロッパに広がっている。発生率の変動は過去に観察されており、将来の疫学を予測することは難しい。しかし、ブタの人畜共通感染ウイルスの広範な分布およびブタのHEVを低減する厳重な動物衛生対策の欠如を考慮すると、疫学的状況が近い将来大幅に改善されることは期待できない。血液/血漿ドナースクリーニングの欠如を考えると、ウイルス血症供血が血漿プールに投入する高い可能性がある。ウイルス血症量は頻繁に低、或いは中程度であったが、最高10^7IU HEV-RNAのピーク濃度が単一供血で観察されていた。
ミニプール検査（2012年、Vollmer etらを参照のこと）を用いたHEV-RNAのための血漿プールの工程内検査は、高ウイルス濃度の供血を選別排除の助けになるかもしれない。同様にHAV RNAのスクリーニングは、血漿由来医薬品の安全率に寄与する追加の安全対策として考えられた。しかし、分画用血漿プールの一般的なHEV RNAスクリーニングの勧告は、現在検討されていない。血漿由来医薬品に関する製品ガイドラインは、ノンエンベロープウイルスに対して効果的な少なくとも一つの製造工程を含んでいる。ノンエンベロープウイルスに対して効果的な工程は堅牢なHEV不活化/除去工程であるという更なる保証を得ることが現在より重要なようで、この問題に関する研究が強く奨励された。HEV-RNAスクリーニングは、HEV除去能力が非常に低いか、制限されることが予想される特定の血漿由来医薬品のために考慮されるかもしれない。これは、HEV RNA用検査を含む2015年1月からのヒト血漿プール用改訂欧州薬局方モノグラフおよびウイルス不活性化のための処理（1646）に即したものである。
製品情報の警告文に関しては、感染性病原体の可能性を完全に排除することができない一般的な警告が、血漿由来医薬品用の製品特性概要（SmPCs）および添付文書（2011年、EMA）における感染性物質の警告に関するガイドラインに含まれていた。その上、具体的な基準は血漿由来医薬品によって過去に伝播していたウイルスに対して行われ、情報は特定の製品の現状の措置がノンエンベロープのA型肝炎ウイルスおよびパルボウイルスB19に効果的であるかどうかに含まれていた。HEV感染症例は、SD-プラスマを例外として、これまでに製造した血漿由来医薬品で報告されていない。しかし、HEV感染が検出できていないことを心に留めておくべきである。それにもかかわらず、報告されたHEV感染の欠如およびHEVの不活化/除去工程の有効性に関する現在の不完全な情報を考えると、警告文におけるHEVに対する具体的な基

処理等により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、ウイルス除去膜によるろ過処理及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

準を導入することが必要、または有用であると考えられていなかった。しかし、唯一の例外はHEV用の効果的な不活化/除去工程がない、且つHEV感染が報告されていたSDプラズマかもしれない。
SDプラスマ用血漿プールのHEV RNA検査のための現行欧州薬局方要件は、HEV感染のリスクを軽減することが期待された。しかし、HEV-RNAを検査したプールからのSDプラスマの経験は、まだ限定的であった。従って、潜在的なHEV感染に関するSDプラスマの慎重な監視を継続することを勧告した。
HEVに感染した血漿供血および血漿プールの汚染の明らかな根拠を認識し、製造業者はHEVに関する利用可能な情報に基づいたそれらの血漿由来医薬品のための予備的なリスク評価を行うことを勧める。考察/検討はまた、関連製品がリスク群（例えば、移植患者、および免疫不全、或いは肝疾患の患者）に投与される可能性があるかどうかを考えるべきである。これらの予備的な工程は、製造業者が自社製品の更なる調査のための優先順位を設定することができる。
HEVの堅牢な不活性化/除去は、血漿由来医薬品のHEV-安全性に向けた重要な要因であり、製造業者は自社製造工程がHEVに対して効果的であることを保証することを勧める。これは、HEV用モデルウイルスからのウイルス除去データの推定が幾つかの症例において難しいかもしれないことが認識させた。HEVについての具体的な研究は、熱処理、沈殿、クロマトグラフィー法および30～50nm範囲のサイズを取り除くウイルスフィルタが必要と思われる。これは、HEVの感染力価測定法は技術的に困難であることが認識されており、これらのシステムはバリデーション研究のための要件を満たすためにまだ準備出来ていない。しかし、製造業者はモデルウイルスからのデータが推定することができない場合には、重要な不活化/除去工程に関するHEVの追加の研究や調査中の研究を行うことが強く奨励される。
欧州薬局方仕様に従って製造したアルブミンおよびCohnやKistler/Nitschmannが確立した分画工程による精製は優れたウイルス安全性を持っており、ウイルス特異的リスク評価はガイドライン（EMA/CHMP/BWP/706271/2010（EMA 2011））に従って期待されていない。それにもかかわらず、低温滅菌時のHEVの限定した不活性化、分画工程のHEV低減に関する限られた利用できるデータおよび個々の製造工程の特異性を考慮して、製造業者はHEV低減に関するそれらの分画工程を調査する必要がある。
十分なデータが製品毎に利用可能であるときにリスク評価を行うべきである。このリスク評価の結果は、HEVのウイルス不活化/除去方法やHEVのスクリーニングにより、不活化/除去工程の追加，削除を示す必要がある場合は考慮すべきである。
E型肝炎ウイルスに関する血漿由来医薬品のウイルス安全性は、詳細情報が利用可能になるよう検討中で、維持されるだろう。

報告企業の意見	今後の対応
E型肝炎ウイルス（Hepatitis E virus：HEV）は直径27～38nmの球状粒子で、エンベロープはなく、長さ約7,300塩基対の一本鎖RNAを内包している。万一、原料血漿にHEVが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	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。

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

25 June 2015
EMA/CHMP/BWP/723009/2014
Committee for Medicinal Products for Human Use (CHMP)

Reflection paper on viral safety of plasma-derived medicinal products with respect to hepatitis E virus

Draft

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Comments should be provided using this template. The completed comments form should be sent to Kaidi.Koiv@ema.europa.eu

Keywords	Hepatitis E virus, plasma-derived products, blood infectivity

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Reflection paper on viral safety of plasma-derived medicinal products with respect to hepatitis E virus

Table of contents

1. Introduction

Hepatitis E virus (HEV) is a causative agent of hepatitis in many countries and of emerging concern in industrialised countries. HEV is a non-enveloped, single-stranded, positive-sense RNA virus and a member of the family Hepeviridae. In developing countries, HEV (genotypes 1 and 2) is a major cause of acute hepatitis, transmitted by the faecal–oral route and associated with contamination of drinking water. In industrialised countries, HEV (genotypes 3 and 4) has been found to be more prevalent in the human population than originally believed. HEV genotypes 3 and 4 infect not only humans but also animals such as swine, wild boar and deer. Zoonotic transmission of HEV genotypes 3 and 4 to humans can occur by consumption of undercooked pork and wild boar products or by contact with infected animals. Genotypes 3 and 4 are generally less pathogenic than genotypes 1 and 2, although serious infections have been reported also with genotypes 3 and 4. Chronic infection with HEV genotype 3 is an emerging concern among transplant recipients and may also occur in persons with HIV and certain haematologic disorders.

HEV infection is widespread and blood/plasma donors are often asymptomatic. Therefore, there is a risk for viraemic blood donations. HEV has been recognised as a transfusion transmissible agent since 2004 and transfusion-related cases have been documented in several countries (United Kingdom, France, Japan, Saudi Arabia, People's Republic of China). Recent analysis of blood and plasma donations has identified HEV-infected donors in Europe and USA. Consequently, HEV-RNA has been detected in plasma pools used for production of medicinal products.

The published reports on frequency of viraemic blood donations and studies on plasma pools indicate that plasma pools used as starting material for manufacture of medicinal products can be contaminated with HEV. In addition there have been cases with post donation information, indicating that HEV-affected donations have entered plasma pools for fractionation.

This raises questions about the safety of plasma-derived medicinal products. The Ph. Eur. monograph for human plasma pooled and treated for virus inactivation (1646) was revised to include a test for HEV RNA (implementation date 1 January 2015). A WHO International Standard for HEV RNA has been established promoting the standardisation of HEV assays by nucleic acid amplification technology (NAT). Manufacture of other plasma-derived products includes process steps for inactivation/removal of non-enveloped viruses. Their effectiveness against HEV is currently under investigation. HEV is difficult to grow in cell culture and current information about susceptibility of HEV to virus inactivation/removal steps used in the manufacture of plasma-derived medicinal products is limited.

An EMA Workshop on Viral safety of plasma-derived medicinal products with respect to hepatitis E virus was held on 28-29 October 2014. The purpose of the workshop was to obtain further information on the safety of plasma-derived medicinal products with respect to HEV and to provide the basis for deciding what further action may be needed. Key questions that were addressed were:

- *Clinical experience with HEV infections and transfusion-associated infections: How serious are HEV infections and which patient populations may be particularly at risk?*
- *HEV detection and epidemiology of HEV in blood/plasma donations*
- *Do serum antibodies against HEV significantly neutralise?*
- *Latest experience from studies on inactivation/removal of HEV: Which steps are effective to remove / inactivate HEV? Which model viruses can be used to assess that? Do we need more virus validation data?*
- *Risk assessment for plasma-derived medical products and implication for warning statements: Do we need risk assessments and/or warning statements?*

- *NAT testing will be required in the Ph. Eur. for SD plasma. Should this also be required for any other products?*

2. Discussion

2.1. Transfusion-associated infections and clinical experience with HEV-infections

In industrialised countries, HEV-infection with genotypes 3 and 4 can lead to an acute self-limited hepatitis. Most infections are asymptomatic or mild. This is indicated by the large sero-prevalence of antibodies whereas only few cases of hepatitis E are notified per year in European countries where reporting is practiced. It seems therefore that only few infections with genotype 3 (0.1 to 1%) lead to acute hepatitis. Nevertheless, given the wide distribution of HEV, hepatitis E is now the most common form of acute enteric hepatitis in Western Europe and more clinical cases of endemic hepatitis E than cases of hepatitis A are diagnosed per year.

Clinical signs of hepatitis caused by genotypes 3 and 4 of HEV are indistinguishable from infections with genotypes 1 and 2. Hepatitis is commonly associated with jaundice, anorexia, lethargy, fever, abdominal pain, and vomiting. Infection may also be associated with pruritus, weight loss, and headaches. Acute hepatitis E is more frequently observed in older persons (more than 40 years old) and more than two thirds of the affected patients are men. In contrast to infections with genotypes 1 and 2, infection of immunocompetent persons with HEV genotypes 3 and 4 is mostly mild or asymptomatic and severe cases are rarely observed. Similar to hepatitis A, patients with pre-existing chronic/advanced liver disease are at risk for developing liver failure after infection with HEV genotypes 1 and 2 in developing countries. Experience with genotype 3 infection in developed countries is more limited although cases of acute hepatitis with genotype 3 have been described. HEV infection was retrospectively found in 3-13% of cases of drug-associated liver injury. It seems that HEV infection is underdiagnosed as clinicians often do not consider HEV testing of patients who have not travelled to developing countries.

Hepatitis E virus infection with genotypes 1 and 2 can lead to high mortality among pregnant women in developing countries. However, no serious infections of pregnant women with genotype 3 have been observed, so far.

The knowledge about extrahepatic clinical manifestation of HEV infections with genotypes 3 and 4 is "still emerging". Cases of arthritis, pancreatitis, bilateral brachial neuritis and encephalitis have been described and potential association with Guillain-Barré Syndrome was discussed at the 2014 EMA workshop.

Chronic hepatitis E has been defined as persistent viraemia for more than 3 months. Infection of immunosuppressed persons with HEV genotype 3 can result in chronic hepatitis. Chronic HEV infection may occur in transplant recipients, in case of haematological malignancy and in HIV-infected patients. HEV infection takes a chronic course in about 60% of solid organ transplant recipients infected with HEV. Persistent viraemia in transplant recipients can resolve spontaneously or can be treated by reduction of immunosuppression or by treatment with ribavirin. However, in some cases, infection may rapidly progress to liver fibrosis/cirrhosis in immunosuppressed patients. In one study, cirrhosis was observed in 9.4% of HEV RNA-positive solid organ recipients. In general, reduction of immunosuppression can help to clear the virus (e.g. kidney transplants). However, it may not be an option for specific transplant recipients (e.g. heart, liver) or it can be dangerous at haematopoietic stem cell transplantation because of Graft-Versus-Host-Disease (GVHD) risk.

Most HEV RNA positive blood/plasma donors do not have clinical symptoms at donation and often do not develop symptoms after donation. Therefore they are not recognised as HEV-infected at donation or thereafter. Thirty-nine transfusion-transmitted infections (TTI) with HEV have been described so far in the scientific literature. Identification of TTI may be difficult and results from antibody-testing have to be interpreted with caution. The time until seroconversion may be prolonged in immunocompromised patients. Transfusion of anti-HEV positive plasma or administration of immunoglobulins can lead to the detection of antibodies to HEV. In addition, re-activation of pre-existing chronic infections as well as re-infections of patients with an initial IgG positive status have been observed. Other possible sources of infection such as contaminated food have to be considered. Therefore detection of HEV-RNA and sequence analysis is desired in order to confirm TTI.

In France, 19 cases of post-transfusion hepatitis were declared between 2006 and October 2014. Among these cases, 14 cases were declared between 2012 and 2014 (ANSM, 2014). The clinical course of TTI with HEV ranged from mild symptoms with elevated liver enzyme values to acute cytolytic hepatitis. The assigned grades of severity were Grade 1 (non-severe) or Grade 2 (severe, not life-threatening). Most TTIs in France were observed with immunosuppressed patients. All the categories of blood components were involved in the transmission: Fresh frozen plasma (FFP) (8/19), SD-plasma (5 cases), quarantine-FFP (1 case), amotosalen-treated FFP (2 cases), red blood cell concentrates (7/19), platelet concentrates (4/19), standard platelet concentrates (2 cases), and apheresis platelet concentrates (2 cases). The HEV RNA concentrations in donations ranged from $10^{1.08}$-$10^{4.83}$ IU/ml. In one case there was evidence for transmission by SD-plasma containing 41.4 IU/ml HEV RNA. In a recent study from UK (Hewitt et al, 2014), there was a 42% transmission rate from viraemic blood components. The median viraemic concentrations of donations associated with transmission was above 10^4 IU/ml while the median concentration of donations not associated with HEV transmission was around 100 IU/ml. However, transmission cases from patients with low viraemia such as slightly above 100 IU/ml have been described. An estimate of 450 transfusion-transmitted infections per year by blood components in the UK was given.

In conclusion, HEV genotype 3 is considered mainly a threat for immunocompromised people (e.g. transplant recipients) and patients with underlying liver impairment or disease. The clinical symptoms of transfusion-transmitted infections seem similar to those from the oral route. Blood or plasma donations from patients with low viraemic HEV RNA concentrations such as 100 IU/ml may be infectious.

2.2. *HEV detection and epidemiology of HEV in blood/plasma donations*

Sero-prevalence has been studied in various populations including blood donors from several countries in Europe. Depending on the individual study and region, the IgG-prevalence in Europe ranged from 1% to 52% in South Western France. In central Italy, the overall anti-HEV IgG prevalence was 48.9% using a sensitive assay. In studies investigating sero-conversion, incidence ranged from 0.2 % infections per person year in the UK to 3.2% in South Western France. Interpretation and comparison of the various sero-epidemiological studies is difficult. A main reason is that different antibody assays show substantial variability in sensitivity and specificity. In addition, batch to batch variability of antibody assays has been reported. A WHO standard for HEV RNA is available. However, no international standard for HEV-antibodies is yet available, although an anti-HEV containing human serum has been developed as reference reagent.

Despite of the current issues with standardisation of antibody assays, it can be concluded from the many sero-epidemiological studies that the prevalence of HEV genotype 3 infection in the general

158 population and blood/plasma donors in developed countries is high. The sero-prevalence generally
159 increases with age, irrespective of gender. Prevalence of HEV genotype 3 infection in pigs (and wild
160 boar) is immense and zoonotic infection by raw or undercooked pork meat and offal or by contact to
161 pigs is considered the main transmission route responsible for the wide distribution of HEV genotype 3
162 in the human population. Sero-prevalence in vegetarians is lower than in non-vegetarians. Cases of
163 oral transmission by contaminated shellfish and fruits have been described.

164 Analysis of birth cohorts by sero-epidemiological studies indicates that HEV has been present in the
165 European population for a long time. Studies from UK and the Netherlands show that HEV incidence
166 fluctuated in the past and continues to fluctuate. An increase or re-emergence of HEV infections has
167 been observed in the recent years in the Netherlands.

168 Progress has been made in developing sensitive HEV NAT assays and a WHO standard for HEV RNA is
169 available. There are now many studies on HEV-RNA in blood donations. HEV RNA was detected in 1 of
170 1595 up to 1:14250 blood/plasma donations depending on the assays and region. Plasma pools for
171 fractionation are composed of more than 1,000- 10,000 individual donations. Therefore, there is a high
172 probability that such pools contain viraemic donations. Viraemic RNA titers from serologic window
173 phase donations are usually low or moderate with not more than 10^6 IU per ml and will be diluted to
174 very low concentration in plasma pools for fractionation. However, peak concentrations exceeding
175 10^7 IU HEV RNA per ml have been observed in single donations and there is a risk that such donations
176 enter plasma pools for fractionation. HEV RNA has been detected in plasma pools from Europe as well
177 as from USA. In a study of 75 plasma pools, HEV-RNA was detected in 8 pools. HEV concentrations
178 were between 100 and 1000 genome equivalents per ml, (Baylis et al., 2012).

179 In conclusion, infections of blood donors with HEV genotype 3 are widespread in Europe and, given the
180 lack of plasma donor screening, there is a high probability of viraemic donations entering plasma pools.
181 Although the viraemic loads are frequently low or moderate, peak concentrations of up to 10^7 IU HEV-
182 RNA have been observed in single donations.

183 *2.3. Do serum antibodies against HEV significantly neutralise?*

184 HEV-antibodies can be found in plasma for about 10 years after infection. However, antibody titres
185 decline with time and IgG antibody-status may change from positive to negative. This raises questions
186 about long-term immunity. There is no licensed vaccine in Europe. A vaccine (Hecolin) produced from
187 recombinant *E. coli* has been licensed in China. With this vaccine, over 87% protection from disease
188 has been described in a 4 years observation period for healthy subjects aged 16–65 years. However,
189 protection from (sub-clinical) infection was more limited. Infection with HEV may develop after re-
190 exposure to the virus. This risk for re-infection might be higher in immunosuppressed patients. In a
191 study of solid organ transplantation patients from Toulouse region in France, 3 of 6 of HEV-infections
192 were re-infections of seropositive patients. This indicates a limited protection by serum antibodies.

193 HEV particles from blood and in vitro cell cultures have been found associated with lipids while HEV
194 particles from faeces show the typical appearance of "non-enveloped" virus particles. The particle-
195 associated lipids seem to protect the virions from antibody-neutralisation. *In vitro* neutralisation of HEV
196 derived from serum is poor and HEV can efficiently replicate in cell culture despite the presence of HEV
197 antibodies. Virions from faeces are somewhat more susceptible towards antibody-neutralisation than
198 serum-derived virus particles. Pre-treatment of virus particles with chloroform or detergent increased
199 the *in vitro* neutralization capacity of antibodies. However, the reduction capacity remained limited and
200 residual infectious virus was recovered.

Low levels of HEV-specific antibodies may be found in plasma pools. In a study from France, anti-HEV IgG was detected in nearly all minipools consisting of 96 test samples. Antibody concentrations ranged from 0.3 to 10.6 IU/ml. Five cases of HEV-transmission by SD-plasma have been reported in France Unfortunately, the antibody-concentrations from the implicated product batches or plasma pools were not reported. However, given the general anti-HEV sero-prevalence in France, it seems unlikely that none of the five implicated batches contained HEV antibody positive donations. One transmission case from Canada has been implicated by several sero-conversions in recipients of a specific SD plasma batch.

In summary, the data presented indicate that the neutralisation capacity of serum antibodies against HEV is limited. Antibodies might contribute towards reduction of HEV infectivity in product intermediates. However, it is not possible to rely on neutralizing antibodies in plasma pools or product intermediates preventing transmission of HEV by plasma-derived medicinal products.

2.4. Studies on inactivation/removal of HEV during manufacture of plasma-derived products

2.4.1 Viruses used in validation studies

HEV

HEV is a small (27-33 nm) non-enveloped RNA virus, the only representative of the hepevirus genus in the family *hepeviridae*. HEV isolates have been obtained from human plasma or human faeces, faeces from pigs or wild boar, or liver homogenates from pigs or wild boar. Unfortunately, HEV does not grow well in cell culture and establishing a suitable *in vitro* cell culture system has been difficult. Nevertheless, some cell culture systems have been developed and sufficiently high HEV titres have been achieved for investigation of virus removal/inactivation. Okamoto and co-workers adapted a genotype 3 from human faeces (JE03-1760F) to replicate to high titres in two human cell lines, A549 lung cells and PLC/PRF/5 hepatoma cells (Tanaka et al., 2007). The HEV genotype 3 strain Kernow-C1 was isolated from a chronically-infected patient and has been adapted to growth in human hepatoma cell line HepG2/C3A and a recombinant cDNA clone can be used for transfection of cells (Shukla et al., 2011, Shukla et al., 2012).

Infectivity assays are necessary for investigation of virus inactivation procedures. The propagation and detection of HEV in cell culture is hampered by the generally poor susceptibility of cultured cells to HEV, requiring relatively high virus titres for infection. This reduces the sensitivity of studies to determine the HEV reduction capacity of the manufacturing process of plasma-derived products. A classical cytopathic effect-based infectivity assay is not available for HEV and infected cells must be stained by immunological methods. Alternative read-outs for the infectivity assay such as production of HEV RNA (or antigen) can be used. However, care has to be taken that a positive read-out represents *de novo* produced virus.

Alternatively, NAT can be used for investigation of the HEV reduction capacity of manufacturing steps in cases where the mechanism for virus reduction is partitioning (virus removal), e.g. for manufacturing steps such as nanofiltration, precipitation/depth filtration, or chromatography. NAT assays are highly sensitive, thus improving the dynamic range for demonstration of logarithmic reduction factors. When using NAT for study of virus partitioning, it has to be kept in mind that NAT detects virus particle-associated RNA as well as free RNA. Therefore, care should be taken to minimize free viral nucleic in virus spike preparations. Detection of free nucleic acids can be reduced by enzymatic pre-treatment of samples.

The physical form of HEV in plasma, where the virus is present as a lipid-associated form, is different from its physical form in faeces where the virus is not lipid-associated. Similarly to plasma derived HEV, cell culture-derived HEV is lipid-associated. The difference in physical form of the different HEV spikes available should be taken into account when selecting the most appropriate virus spike for use in validation studies. For instance, the efficacy of a 35N nanofiltration step could be affected by an upstream ethanol or S/D treatment which may remove HEV associated lipids and thereby reduce the size of the virus particles. Also, partitioning during cold ethanol fractionation or adsorption to ligands may depend on whether HEV is lipid associated or not, depending on the fractionation process. Therefore, the HEV spike should be carefully selected and a pre-treatment of virus spike according to the specific manufacturing procedure should be considered.

Model viruses

Estimates of the virus reduction factors for HEV could be obtained from viral validation studies carried out with other non-enveloped viruses having similar characteristics/size. The reduction capacity of manufacturing steps for plasma derivatives has been validated using several non-enveloped model viruses such as non-enveloped RNA viruses hepatitis A Virus (HAV) or encephalomyocarditis virus (EMCV) and the small non-enveloped DNA viruses such as canine parvovirus (CPV), porcine parvovirus (PPV) or minute virus of mice (MVM). When interpreting reduction data from HAV, it should be kept in mind that, similarly to HEV, HAV particles from serum or cell culture can be associated with lipids.

In theory, there is a high probability that HEV will be removed/inactivated if effective removal/inactivation of a broad variety of non-enveloped model viruses has been demonstrated. However, given several peculiar physical properties of HEV in its different physical forms, and lack of data, accurate extrapolations from model viruses are currently not always readily possible. An important issue seems whether or not lipid association of HEV may play a role in the reduction capacity of the production step. The available data suggest that no single model virus or single virus preparation seems appropriate for all different manufacturing steps that may contribute to HEV reduction.

Feline calicivirus (FCV), murine norovirus (MNV), and cutthroat trout virus (CTV) have been suggested as specific model viruses for HEV. There are suitable cell culture systems for these viruses in order to study virus inactivation. However FCV was more susceptible than HEV toward inactivation at low pH or at high temperature and, therefore, cannot be considered as a suitable model for HEV inactivation in this respect. Experience with MNV and CTV seems yet too limited to conclude how accurate these model viruses reflect inactivation of HEV.

2.4.2 HEV reduction by specific manufacturing steps

The limited data available on this subject and the implications for further reduction studies are discussed below.

Precipitation

Reduction of non-enveloped viruses such as picornaviruses or parvoviruses has been demonstrated for several cold ethanol fractionation steps. Some well-controlled fractionation steps have been found effective for reduction of non-enveloped model viruses while others showed only moderate or non-significant virus reduction capacity. The reduction capacity depends on the specific manufacturing step and process conditions and, therefore, product-specific studies are needed.

Variable results have been reported so far when comparing reduction of model viruses with reduction of HEV. There have been cases where HEV reduction was comparable to reduction of model viruses while, in other cases, reduction differed markedly. Interpretation and comparison of data is further

complicated by the observation that different forms of HEV spike (e.g. serum derived or lipid-associated HEV particles versus HEV particles from faeces or pre-treated HEV particles) showed different partitioning. No clear partition of lipid-associated HEV particles was observed at the initial plasma fractionation steps. In summary, it seems difficult to draw general conclusions on the efficacy of specific fractionation steps for HEV reduction at this point and the relevance of data from model viruses needs to be further clarified.

In conclusion, additional research is welcomed. Product-specific investigation of selected plasma fractionation steps for HEV reduction is recommended in cases where effective reduction by other manufacturing steps has not been demonstrated. The HEV-spike should be selected according to the specific manufacturing step and pre-treatment of virus spike might be considered. As plasma-derived virus particles seem associated with lipids, non-treated virus preparations from blood or cell culture should be used for initial fractionation steps from plasma, while preparations pre-treated according to the specific manufacturing process might be considered for later steps. Virus partitioning at precipitation steps can by studied by NAT assays.

Pasteurisation

Pasteurisation is a heating procedure for 10 hours at 60°C in liquid phase. Pasteurisation has been demonstrated to inactivate effectively HAV in many cases. The actual efficacy of inactivation depends on the specific manufacturing conditions (e.g. the stabilisers present). Some heat resistant cell culture-adapted HAV-strains have been described where inactivation at pasteurisation of albumin was limited to 2-3 $\log_{10}$ while other HAV-strains show robust inactivation of more than 4 $\log_{10}$.

Inactivation of HEV by pasteurisation has been investigated in few studies, so far. Inactivation in albumin was limited to 2-3 $\log_{10}$ while effective inactivation was observed in control experiments using buffer instead of albumin. It seems therefore that albumin has a stabilizing effect on HEV. Few studies on pasteurisation of HEV in coagulation factors or other plasma proteins have been performed so far. Inactivation varied from 1.3 $\log_{10}$ in case of pasteurisation of an alpha-1 antitrypsin preparation to more than 4 $\log_{10}$ at pasteurisation of a FVIII product intermediate. These differences could be explained by the different composition (stabilisers) of the products.

The reported HEV sensitivity to pasteurisation is similar to that of the most heat-resistant HAV strains. However, it has to be considered that only few studies have been performed using such heat-resistant HAV-strains and it seems, therefore, not possible to extrapolate existing validation data from HAV towards HEV-inactivation. A heat-stable model virus such as an animal parvovirus could be selected as worst-case model for HEV. However, with this approach, there is a risk of underestimating HEV inactivation at pasteurisation.

In conclusion, more data on the effect of pasteurisation on inactivation of HEV is desired. Where further investigation of pasteurisation with respect to HEV reduction is required, a product-specific study with HEV itself should be performed. Infectivity assays are essential for such studies. The HEV spike preparation can be selected according to the specific manufacturing process.

Dry heat treatment

Dry heat treatment is the key elimination step for non-enveloped viruses in the manufacture of many complex or intermediately-purified plasma-derived medicinal products which are not processed through a parvovirus-removing nanofilter. Extrapolation of HAV inactivation data from validation of dry heat-treatment to HEV seems not possible as HAV shows significant reduction at lyophilisation. However, such an effect has not been observed with HEV. A relatively heat-stable model virus such as an animal parvovirus or murine norovirus could be considered as a worst-case scenario. However, the experience is still too limited to conclude how accurately these model viruses reflect inactivation of HEV by dry

heat treatment. Therefore, product-specific studies with HEV seem necessary. If a HEV spike is used it should be determined whether the lipid-associated form or the non-lipid associated form is most representative for the physical form of the virus at the stage of dry heat treatment. Studies should consider robust conditions, e.g. low residual moisture during dry-heat treatment.

Nanofiltration

Virus reduction by nanofiltration is based on the retention of viruses based on their particle size. Different types of filter membranes or hollow fibers are used. It is not always possible to define a unique pore size of a specific filter. Virus filters have been developed for reduction of small non-enveloped viruses such as parvoviruses. The particle size of parvoviruses is between 18 and 26nm. These filters are sometimes called small virus filters or small pore size filters. Product specific validation of these filters usually includes a parvovirus and a picornavirus such as HAV or EMCV. The particle size of non-lipid associated HEV has been specified between 27 and 33nm while the size of picornaviruses is similar or slightly smaller.

Considering the particle sizes of HEV and picornaviruses, it seems therefore reasonable to consider picornaviruses as a (worst case) model for HEV at virus filtration. No HEV-specific validation studies are required for virus filters suitable for removal of parvovirus and virus reduction data from HAV or EMCV can be considered appropriate. Effective reduction of HEV has been experimentally confirmed (Yunoki et al, 2008) and others. It seems reasonable to postulate effective reduction of HEV in cases where effective reduction of a picornavirus or a parvovirus has been demonstrated.

The so-called "medium pore size virus filters", have been designed for removal of large or medium-sized virus particles. Retention of HEV in buffer matrix was low or moderate. However, studies presented at the workshop indicated significant reduction (ranging from 3 to 4 $\log_{10}$) when virus-spiked product intermediates were applied to the filters. One of the presented studies showed increased reduction of cell-culture derived virus spike while reduction of faeces-derived or detergent-treated virus was more limited. This study implies that the size of the lipid associated particles would be greater than that of the "naked" virus particles. However it seems difficult to predict to what extent the nature of virus particles will influence the retention at a specific manufacturing process step.

Virus filters designed for reduction of medium-sized virus particles might contribute to virus safety by moderate reduction capacity for HEV. However, it is not possible to predict HEV reduction and product specific studies seem necessary. As for other process steps, where virus removal is the mechanism of virus reduction, such studies might be performed using NAT assay and consideration should be given to the appropriate spike preparation. Considering the limited reduction capacity and the potential influence of the nature of spike preparation and matrix, the use of model viruses seems not appropriate.

Low pH

HEV is stable at low pH, as can also be deduced from its route of infection. No or limited HEV inactivation was observed in IVIG after incubation at pH 4.2 and HEV was stable at pH 2.5 for 5 hours. Stability of HEV at low pH seems somewhat similar to that of animal parvoviruses such as CPV. CPV seems to be a model virus for HEV with respect to lack of sensitivity to low pH. HEV is much more stable to low pH than the calicivirus FCV which was investigated as a potential model virus for HEV.

In summary, there will be no gain in further investigation of the effect of low pH incubation on HEV inactivation as no or very limited contribution of such steps is expected during manufacture of plasma derived medicinal products.

Chromatography

In general, for chromatography steps, the achieved reduction factors can vary amongst the viruses, even within the same family. The results obtained with model viruses can therefore not be readily extrapolated to HEV. The mechanisms of partitioning and process parameters influencing virus reduction (robustness) should be understood. Product-specific studies with an appropriate HEV spike would be necessary if HEV reduction by chromatography steps is to be demonstrated. As for other partitioning steps, NAT studies could be performed.

2.5. Risk assessment for plasma-derived medical products

Risk assessments are essential for evaluating the safety of plasma-derived medicinal products. The general principles of virus risk assessments have been outlined in Chapter 9 of Guideline EMA/CHMP/706271/2010 (EMA 2011). The following considerations might be helpful for performing risk assessments with respect to HEV.

Frequency of viraemic plasma donations and virus loads.

Virus RNA concentrations from viraemic blood or plasma donations have been recently analysed in multiple studies. Viraemia is usually low or moderate with maximum titres below 10^6 IU/ml. However, some donations with more than 10^6 IU /ml have been identified and the maximum concentrations reported so far were up to 10^7 IU/ml. The frequency of viraemic donations ranged from less than 1:1000 to more than 1:14,000 depending on the individual donor population and the sensitivity of the NAT assay. However, considering that current plasma pools for fractionation can be composed of more than 10,000 donations, there is a risk that plasma pools include viraemic donations. In a worst case scenario a donation with 10^7 IU/ml would be diluted in a pool of 10^4 donations to a concentration of 1000 IU/ml.

Virus Inactivation / removal by manufacturing process:

Effective steps for inactivation/removal of HEV are considered a key factor for the virus safety of plasma-derivatives. The Guideline on plasma-derived medicinal products (EMA, 2011) requests at least one effective step with a reduction capacity in the order of 4 $\log_{10}$ or more for removal or inactivation of non-enveloped viruses. For virus filtration steps using small virus filters that have been demonstrated to remove effectively parvoviruses and/or picornaviruses (e.g. HAV, EMCV) it seems reasonable to consider similar reduction capacity for HEV. Currently, it seems more difficult to extrapolate model virus data from other manufacturing steps such as heating steps, other virus filters, precipitations or chromatographic steps and HEV-specific studies might be necessary in these cases. The specific aspects of virus inactivation/removal by individual manufacturing steps have been discussed above.

Neutralising antibodies

The *in vitro* neutralising capacity of serum antibodies against HEV is very limited. Depending on the specific product intermediate and physical state of virus particles, antibodies might moderately contribute towards reduction of infectious virus particles. However this would have to be confirmed by product-specific investigations using appropriate HEV spike preparations.

Infectious dose

Experience from transfusion-transmitted infections (TTI) was reviewed at the workshop. All kinds of blood components for transfusion (i.e. plasma, platelet concentrates, red blood cell concentrates) have

transmitted HEV. Plasma seemed the most risky component, probably because the viral load is highest in plasma. However, no information is available on the partitioning of HEV into the different components from a single blood donation. Blood components with high viraemic titres had higher probabilities for HEV transmission than low titre components. A median RNA concentration of TTIs above 10^4 IU/ml is reported. However, there is a broad variability and HEV RNA titres in blood donations or blood components from individual TTI cases ranged from more than 10^6 IU/ml down to about 100 IU/ml (Hewitt et al., 2014). Considering a volume of ca 200ml of a transfused blood product this would indicate total virus loads of at least 20,000 IU HEV RNA. The lowest TTI-associated total RNA load reported so far was of 7056 IU HEV RNA from an apheresis platelet concentrate (Huzly et al., 2013).

Although, the infectious dose represents a significant factor for risk assessment, it has to be kept in mind that it can be associated with a considerable variability depending on the individual scenario. The overall experience with transfusion-transmitted HEV infection is still limited.

Experience with transmission of HEV by plasma derived medicinal products

HEV has been in the donor population for a long time. A serologic study from Japan implied that HEV might have been transmitted in the past via coagulation factors which have not been subjected to virus inactivation/removal while there was no signal for transmission to patients receiving only virus-inactivated coagulation factors (Toyoda et al., 2007). There have been no specific case reports of HEV transmission via plasma-derived medicinal products (except S/D plasma). This lack of transmission reports is reassuring. Nevertheless, it should be kept in mind that hepatitis E can be overlooked unless specific diagnosis has been performed. Clarification of suspected transmission cases has been difficult in cases where the plasma pools tested negative for HEV RNA and where it was not possible to retest all individual donations contributing to the pool.

3. Conclusion

HEV genotype 3 has been observed in blood/plasma donations from asymptomatic donors in developed countries. There is a risk that such donations enter the manufacturing process of plasma-derived medicinal products. Infections with genotype 3 are often asymptomatic or mild. As far as patient population is concerned, HEV genotype 3 is considered a threat for immune compromised people (e.g. transplant recipients) and patients with underlying liver impairment or disease. The clinical presentation of HEV-infection can be diverse and is not yet completely known, although it is well established that HEV can lead to prolonged or chronic infection in immune deficient patients.

Infections of humans and pigs with HEV genotype 3 are widespread in Europe. Some fluctuations of incidence have been observed in the past and it is difficult to predict the future epidemiology. However, considering the widespread distribution of the zoonotic virus in pigs and the absence of stringent animal health measures to reduce HEV in pigs, it cannot be expected that the epidemiological situation will significantly improve in the near future. Given the lack of blood/plasma donor screening, there is a high probability that viraemic donations enter plasma pools. Although the viraemic loads are frequently low or moderate, peak concentrations of up to 10^7 IU HEV-RNA have been observed in single donations.

In-process testing of plasma pools for HEV-RNA using a mini-pool testing strategy (see Vollmer et al, 2012) might be helpful to screen out donations with high virus concentrations. Similarly to screening for HAV RNA, this could be considered as an additional safety measure contributing to the safety margin of plasma-derived medicinal products. However, a recommendation for a general HEV RNA

screening of plasma pools for fractionation is currently not considered. Products complying with the Guideline on plasma-derived medicinal products contain at least one manufacturing step effective against non-enveloped viruses. It seems currently more important to obtain further assurance that steps effective against non-enveloped viruses are robust HEV inactivation/removal steps, and studies on this issue are strongly encouraged. HEV-RNA screening might be considered for specific plasma-derived medicinal products where the HEV reduction capacity is expected to be very low or limited. This is in line with the revised Ph. Eur. monograph for human plasma pooled and treated for virus inactivation (1646), which from January 2015 includes a test for HEV RNA.

Concerning warning statements in product information, a general warning that the possibility of transmitting infective agents cannot be totally excluded is included in the Guideline on the warning on transmissible agents in summary of product characteristics (SmPCs) and package leaflets for plasma-derived medicinal products (EMA, 2011). In addition, specific reference is made to viruses that have been transmitted in the past by plasma-derived medicinal products and information is included on whether or not the measures in place for a specific product are effective for the non-enveloped hepatitis A and parvovirus B19 viruses. No HEV transmission cases have been reported so far with the currently produced plasma-derived medicinal products, with the exception of SD-plasma. However, it should be kept in mind that HEV transmission could go undetected. Nevertheless, given the lack of reported HEV transmissions and the currently incomplete information on effectiveness of inactivation/removal steps for HEV, it is not considered necessary or useful to introduce a specific reference to HEV in the warning statements. However, one exception might be SD plasma because there is no effective inactivation/removal step for HEV and HEV transmissions have been reported.

The current Ph. Eur. requirement for HEV RNA testing of plasma pools for SD plasma is expected to reduce the risk for HEV transmissions. However, the experience with SD plasma from HEV-RNA tested pools is as yet limited. Therefore, it is recommended to continue careful surveillance of SD plasma with respect to potential HEV transmission.

Recognising the clear evidence for contamination of plasma donations and pools with HEV, manufacturers are advised to perform preliminary risk assessments for their plasma-derived medicinal products on the basis of the available information on HEV. Consideration should also be given to whether the product concerned is likely to be administered to risk groups (e.g. transplant patients, and patients with immunodeficiency or hepatic disease). These preliminary steps will allow manufacturers to establish a priority order for further investigation of their products.

Robust inactivation/removal of HEV is the key factor towards the HEV-safety of plasma-derived medicinal products and manufacturers are advised to assure that their manufacturing processes are effective against HEV. It is recognised that extrapolation of virus reduction data from model viruses for HEV might be difficult in several cases. Specific studies with HEV seem necessary for heat-treatments, precipitations, chromatographic methods and virus filters with size exclusion in the range 30-50nm. It is recognised that infectivity assays with HEV are technically difficult and these systems are not yet ready to fulfil all formal requirements for validation studies. However, manufacturers are strongly encouraged to perform additional research or investigational studies with HEV on their key steps for inactivation/removal in the cases where data from model viruses cannot be extrapolated.

Albumin manufactured according to European Pharmacopoeia specifications and purified by established Cohn or Kistler/Nitschmann fractionation processes has an excellent virus safety record and no virus-specific risk assessments are expected according to Guideline (EMA/CHMP/BWP/706271/2010 (EMA 2011). Nevertheless, considering the limited inactivation of HEV at pasteurisation, the limited available

data on HEV-reduction during fractionation and the specificities of individual manufacturing processes, manufacturer should investigate their fractionation process with respect to HEV reduction.

A risk assessment should be performed when sufficient data is available for each product. If the outcome of this risk assessment should indicate that HEV may not be sufficiently inactivated/removed, additional measures such as improvement of virus inactivation/removal methodology or HEV testing should be considered.

The viral safety of plasma-derived medicinal products with respect to hepatitis E virus will be kept under review as further information becomes available.

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Appendix: summaries of individual presentations from the EMA Workshop on Viral safety of plasma-derived medicinal products with respect to hepatitis E virus, 28-29 October 2014

Clinical Experience with Hepatitis E Virus (Harry Dalton, University of Exeter Medical School, Cornwall, UK)

H. Dalton reviewed the clinical experience with HEV infections. Acute hepatitis E, caused by HEV genotypes 1 and 2, represents a major health issue in developing countries with high mortalities of 25% in pregnant woman and 70% in patients with chronic liver disease. In developed countries hepatitis E, caused by genotypes 3 and 4, is often asymptomatic or associated with mild symptoms. However, sporadic cases of severe hepatitis have been observed and the few studies with genotype 3 also indicate a higher risk for patients with underlying liver disease. Serious infections of pregnant woman with genotype 3 and 4 have not been observed so far. Genotype 3 may establish chronic infection in immunocompromised patients and re-infections have been observed. Hepatitis E infections have been under-diagnosed because physicians have not always been aware about HEV in developed countries. It has been found that HEV was overlooked in some cases that had been wrongly diagnosed as drug-associated liver injury. Chronic infection of immunocompromised patients, such as transplant patients and HIV infected patients, has now been repeatedly described. The knowledge about the full clinical spectrum of disease is still emerging. H. Dalton highlighted association of HEV with several extrahepatic manifestations of disease such as monoclonal gammopathy of uncertain significance, encephalitis, Bell's Palsy, ataxia/proximal myopathy, bilateral brachial neuritis, and Guillian-Barré-Syndrome.

HEV Experience from the Netherlands (Hans L Zaaijer, Sanquin and Academic Medical Centre – Clinical Virology, Amsterdam NL)

H. Zaaijer presented experience from diagnostic HEV-testing in the Netherlands (NL) from 2009-2014. Most HEV-infections were autochthonous and an average diagnostic laboratory in the Netherlands detects now more clinical cases of endemic hepatitis E than hepatitis A. In a first study on 5239 donors, 27% were found IgG positive. HEV RNA was detected in 1:3000 donations. However, recent experience from monthly analysis of screening of donations for SD-plasma showed even higher frequency of HEV RNA positive donations (up to 1:611). This and the different distribution of RNA or antibody positive donations among age-groups of donors indicated fluctuations of HEV incidence in NL with a recent increase. Pigs are still considered the predominant source for human infections. So far, there are no governmental actions against HEV-positive blood donations. H. Zaaijer pointed out that the main source of endemic HEV infection for patients probably is contaminated food or water, and it would be more important to uncover and eliminate this source of HEV. There seems a negligible significance of HEV genotype 3 infection for immune competent babies, children, pregnant women and adults <30 years, while HEV genotype 3 is considered a threat for immune compromised children and adults.

Transfusion Transmission: Hepatitis E Virus (Richard Tedder, Hepatitis E Study Group, Transfusion Microbiology Service, NHBSTT and Blood Borne Virus Unit, PHE, Colindale, UK)

R. Tedder presented the recent studies from UK investigating HEV in blood donations and transmission cases. In a study on 9382 minipools (consisting of 24 donations) from blood donations collected in 2013, 0.03% of donations were HEV RNA positive and 79 donors could be identified. The median viral RNA load of viraemic donations was 3.9 x 10^3 IU/ml (ranging from 50 to 2.37 x 10^6 IU/ml). A look back could be completed on 43 of 60 recipients and, in 18 cases (42%), transmission could be confirmed by identity of the virus sequences from donor and recipient. HEV genotype 3 (mainly clade2) was found in all cases. All kinds of blood components (plasma, red blood cells, platelets) were involved. The median viraemic concentrations of donations associated with transmission was above 10^4 IU/ml while the median concentration of donations not associated with HEV transmission was around 100IU/ml. However, transmission cases from patients with low viraemia (between 100IU/ml and 1000IU/ml) were observed. There was only one clinical case of mild post transfusion hepatitis. Ten recipients developed prolonged or persistent infection. An estimate of 450 transfusion-transmitted infections per year by blood components in UK was given. However, zoonotic HEV transmission via food was estimated to result in 100,000 infections per year in England.

HEV Infections Associated with Transfusion/Blood-derived Products/Organ Transplants- Situation and Cases in France (Wahiba Oualikene-Gonin, ANSM, France).

An overview about the HEV cases from the French hemovigilance database was presented. Nineteen cases of post-transfusion hepatitis E have been registered between 2006 and October 2014. Among these cases, 14 cases were declared between 2012 and 2014. All the categories of blood products were involved in the transmission: (FFP (8/19) = FFP-SD (N = 5), FFP-Quar (N = 1), FFP-IA (N = 2); RBC (7/19); Platelets (4/19) = MPC (2), PCA (2)). Viral RNA load of donor ranged from $10^{1.08}$-$10^{4.83}$ IU /ml. The clinical course of transfusion transmitted HEV-infections ranged from mild symptoms with elevated liver enzyme values to acute cytolytic hepatitis. The assigned grades of severity were Grade 1 (non-severe) or Grade 2 (severe, not life-threatening). Most transfusion transmitted infections (TTIs) in France were observed in immunosuppressed patients. One recipient, a solid organ transplant patient, developed a chronic HEV-infection. In addition, two cases of suspected transmission via plasma-derivatives were presented. However, it was not possible to confirm these cases by sequence analysis, as the affected plasma pools tested negative for HEV-RNA and it was not possible to test all individual plasma donations. One transmission case in 2013 by a kidney graft could be confirmed by sequence analysis.

Hepatitis E in recipients of allogeneic hematopoietic stem cell transplantation (HSCT) and organ transplantation (Annemiek van der Eijk, Department of Viroscience, Erasmus MC, Rotterdam, NL)

A cross-sectional study was performed of all living adult solid organ transplant (SOT) recipients (n=1188) for whom serum or EDTA-plasma samples were available in the Erasmus Medical Center biobank. In 12 (1%) patients, hepatitis E virus infection was identified; in 10 patients, chronic infection developed. In a retrospective study from Erasmus Medical Centre, 8 infections were found in 328 recipients (2006-2011) of allogenic hematopoietic stem cell transplantation and 5 recipients developed chronic infection. All infections were by genotype 3. Five of 8 patients were misdiagnosed with graft versus host disease (GVHD), and 3 with drug induced liver disease. Three patients had positive HEV

IgG status before transplantation, which did not protect them. Four patients died with HEV viraemia and signs of ongoing hepatitis and there have been cases with rapid development of cirrhosis. The 4 surviving patients cleared HEV after a median period of 6.3 months. One patient was diagnosed with HEV reactivation after a preceding infection prior to allogenic HSCT. This patient was treated with ribavirin and cleared HEV infection up until this moment. Histopathology showed fibrosis in the periportal area of the liver, inflammation, and necrotic hepatocytes (councilman bodies). In conclusion HEV should be always included in the differential diagnosis of transplant patients presenting with liver enzyme abnormalities. Although the symptoms of graft rejection or graft versus host disease (GVHD) and HEV-infections are similar, the effects of modulating immunosuppression are contrary.

Hepatitis E Virus in solid organ transplant patients (Nassim Kamar, Toulouse University Hospital France)

Whereas chronic HEV infection has been defined in the literature by detection of HEV in serum or stool 6 months after diagnosis, N Kamar proposed to define chronic HEV infection by persisting HEV replication beyond 3 months after infection. A multicentre study involving 85 recipients of solid organ grafts was presented. 29 patients cleared the virus within the 6 months after diagnosis and 56 developed chronic hepatitis. The main clinical symptom was fatigue. In 8 cases, there was a progression towards cirrhosis. In contrast to chronic HCV infections, more rapid progression towards cirrhosis in 2-3 years has been observed. Chronic infection is usually observed in highly immunosuppressed patients. The use of tacrolimus versus cyclosporin A and low platelet counts could be associated with a risk of developing chronic infection. Some immunosuppressive drugs (cyclosporin, tacrolimus) promote virus replication *in vitro* while other (mycophenolic acid) inhibit virus replication. Patients who developed a chronic infection had lower serum concentrations of IL-1 receptor antagonist and IL-2 receptor compared to those with resolving hepatitis. A considerable quasispecies heterogenicity of viral RNA sequences has been observed in chronically infected patients. Chronic infections of solid organ recipients can be managed by reduction of immunosuppression or by treatment with ribavirin. Treatment with pegylated interferon is another option but should not be done after heart, kidney or lung transplantation because of the risk for graft rejection. Two cases have been observed where ribavirin treatment failed to clear the virus. Three cases of re-infections of patients with a former positive antibody status have been identified. One of these re-infections resulted in chronic infection.

HEV in Europe and Latin America (José M. Echevarria, National Centre of Microbiology, Spain)

In Europe, the anti HEV seroprevalence ranged from 1.1 to 14% depending on the geographic region and the individual study. The different performance of available anti HEV assays seems to be, at least in part, responsible for the various outcomes. The prevalence of HEV in pigs is immense and zoonotic transmission by pig meat is considered the main source of infection. Epidemiology of hepatitis E virus infection in Latin America is more complex. In Chile, Argentina, Brazil, and Bolivia, infections were by genotype 3 while in Caribbean regions infections were due to genotype 1. Seroprevalence ranged from 5 to 20% but was found up to 30-70% in some studies. Isolated Amazonian population showed a distinct pattern of seropositivity with 30% in the age group of 21 to 30 years.

Detection of HEV infections and epidemiology in Italy (Anna Rita Ciccaglione, Istituto Superiore di Sanità, Italy)

The Italian national surveillance system for acute viral hepatitis (SEIEVA) collects data from Local Health Units covering 72.6% of the Italian population. Between 2007 and 2011, 49.4% of 6761 notified acute viral hepatitis cases were attributed to HAV and only 1.2% to HEV. Travel to endemic countries (India, Bangladesh) and consumption of seafood were the most prominent risk factors. However, HEV seems to be under-diagnosed or under-reported. Serum samples from 84 patients with non-A to non-C acute hepatitis were tested for HEV infection and 38 of them (33.3%) could be attributed to HEV. Genotypes 1 and 3 were identified in positive serum samples. In 2014, a study on 313 blood donors from Abruzzo (a rural region of central Italy located in the Apennines mountains) found two HEV genotype 3-RNA positive donors and 153 (48.9%) anti-HEV IgG-positive donors. In this study, the only risk factor independently-associated with anti-HEV IgG positivity was the consumption of raw dried pork-liver sausage. In another study conducted in 2013 on blood donors from Lazio, central Italy, anti-HEV IgG prevalence was found to be much lower (9%).

Hepatitis E Virus Assay Standardization (Sally A. Baylis, Paul-Ehrlich-Institut, Langen, Germany)

In 2009, the Paul-Ehrlich-Institut started to develop an HEV RNA standard on behalf of the WHO. The 1st WHO International Standard (IS) for HEV RNA (code number (6329/10) was established in October 2011 and was assigned a unitage of 250,000 international units (IU)/ml based on the collaborative study data. The standard has been derived from a HEV genotype 3a RNA-positive plasma donation from Japan. The PEI is currently developing a WHO international reference panel representing all four HEV genotypes. A secondary standard for HEV RNA has been prepared for the Biologicals Standardization Programme (BSP127) of the European Directorate for the Quality of Medicines and HealthCare to support the implementation of HEV NAT testing for S/D-treated plasma; there are now at least 10 commercially-available NAT assays and several of them have a CE mark according to Directive 98/79/EC on in vitro diagnostic medical devices.

Performance of IgG tests is very variable and also batch to batch variability has been observed. The consequences are a lack of comparability of results from different assays, significant discrepancies in performance and poor concordance between assay results. The serological WHO international reference reagent (95/584) was prepared at NIBSC and established by the ECBS in 1997. It is a lyophilized preparation of pooled sera from a patient in the US who developed acute hepatitis following travel to India. It was not established as an IS because the number of laboratories able to participate in the collaborative study was limited (n=7). There is a need for a validation of all existing serology tests and the poor performance of existing assays has led to underestimation of the seroprevalence of HEV, a matter which was highlighted at the consensus workshop on HEV at NIH in 2012 as well as by the WHO SAGE working group on the HEV vaccine in 2014.

Investigation about potential HEV-transmission through SD-plasma and HEV epidemiology in Canadian blood donors (Anton Andonov, Public Health Agency of Canada, Canada)

Anton Andonov presented a study indicating HEV transmission via SD Plasma. A serological follow up of 17 patients treated with 40 litres of SD-plasma for thrombotic thrombocytopenic purpura showed anti-HEV IgG/IgM in two cases who also became viraemic one month post exposure while no markers of HEV infection were observed in patients treated with cryo-poor plasma. None of the patients demonstrated any clinical signs of viral hepatitis during the 6-month period of observation. HEV

seroprevalence in Canadian swine herds is high ranging from 38% to 88%. The number of laboratory confirmed human HEV cases reported from 2006 to 2013 in Canada fluctuated between 10 and 41 per year. The majority of these were travel related. During the same period of observation only a dozen of autochthonous HEV cases belonging to genotype 3a have been confirmed both by serology and PCR. A recent study on 14,000 blood donors found anti- HEV IgG in 5.9% which is lower than the seroprevalence seen in other surveys of blood donors in North America. Seroprevalence was significantly higher in older age groups and males. None of 14,000 blood donors were viraemic for HEV (threshold of detection 250 IU/ml).

SD plasma and neutralization of HEV antibodies (Jaques Izopet, University of Toulouse, France)

In France, 558 testing pools (96 donations) for SD plasma corresponding to 53,234 plasma donations from Nov 2012 to Dec 2013 were tested for HEV-RNA (sensitivity 23 IU/ml) and antibodies. Twenty-two pools were HEV RNA positive indicating that HEV RNA was detected in 1 of 2200 donations. The median viral RNA titre of positive donations was $10^{4.4}$ IU/ml with individual values ranging from 468 IU/ml to 5 x 10^6 IU/ml. Frequency of positive donations was higher in South France than in North France and higher in males than in females. Nearly all 96-pools were positive for anti HEV with concentrations ranging from 0.3 to 10.6 U/ml. The proportion of subgenotypes characterized in France corresponded to that observed in pig populations. Preclinical trials with candidate vaccine in rhesus macaques indicated that a level of more than 100 IU/ml anti-HEV correlated with 50% reduction in infection. Experience with HEV vaccine from China indicates that protection against HEV infection by immunity is not absolute in immunocompetent individuals. With this vaccine, over 87% protection from disease has been demonstrated for healthy subjects aged 16–65 years in a 4 years observation period. However, protection from (sub-clinical) infection was more limited. In a prospective study of 263 solid-organ recipients at Toulouse University Hospital, six HEV infections were found in a 1 year follow up. Three of them were re-infections as patients had a positive IgG status at the beginning. Re-infection resolved spontaneously in two cases while one case showed chronic infection. The experience with solid organ transplant recipients shows that serum antibodies do not protect immunocompromised patients. An *in vitro* antibody neutralisation assay has been developed. The neutralisation capacity of antibody positive plasma depended on the virus spike preparation. A limited neutralisation capacity (1-2.2 $\log_{10}$) could be measured using plasma with 50 IU/ml anti-HEV and HEV-spike without lipid-associated virus particles while virus reduction was always below 1 $\log_{10}$ using lipid-associated virus spike.

HEV reduction in Virus Inactivation/Virus Elimination steps of plasma products manufacturing processes (Benoît Flan, LFB, France)

An infectivity assay has been developed at the Laboratoire de Virologie, Toulouse (J. Izopet) using HepG2/C3A cells and an adapted HEV genotype 3f isolate. The read out is *de novo* production of viral RNA. With this system, a virus stocks with 10^8 – 10^{10} HEV RNA copies/ml – corresponding to 5 $\log_{10}$ $TCID_{50}$ / ml could be obtained. A study on pasteurisation (at 58 ± 1°C) of a 20% albumin using cell-culture derived virus spike showed 2$\log_{10}$ inactivation after 10 hours. Inactivation kinetics was similar to delayed inactivation of some heat-resistant HAV strains reported in the literature (Farcet et al. Transfusion 2012 52:181-7). Pasteurisation of HEV in an intermediate from alpha-antitrypsin production showed only 1.3 $\log_{10}$ reduction indicating that the specific matrix or composition of stabilisers can influence HEV inactivation. Cell culture derived virus spike in PBS was significantly removed (≥4.55 log10 reduction of infectivity) by Pall DV50 filters while reduction was lower (3 $\log_{10}$)

when a faeces-derived virus spike or an NP40 treated virus spike was used. This indicated that the lipid-association of virus particles can influence particles size and virus retention; in these latter conditions HEV reduction was higher than HAV (CHCl3 treated) reduction (1.4 $\log_{10}$). Product-specific investigation of HEV-reduction at Planova 35N filtration of von Willebrand factor using a detergent-treated spike showed more than 2.5 $\log_{10}$ reduction of HEV infectivity and Planova 35N filtration of an immunoglobulin intermediate using ethanol treated virus spike showed 3 $\log_{10}$ reduction of HEV RNA. In vitro neutralisation experiments were performed using intravenous immunoglobulin (IVIG) preparations. There was no inactivation of cell culture-supernatant derived virus while faeces derived virus was moderately neutralised (1.8$\log_{10}$ and 2$\log_{10}$ reduction of infectivity). Virus stocks were also pre-treated with ethanol in order to simulate potential HEV-contaminants from IVIG production. There was a combined effect of ethanol-treatment and neutralisation leading to 2.8$\log_{10}$ overall reduction of infectivity. In addition to virus reduction data, a review of viraemic titres from blood/plasma donation was presented and TTI were reviewed in order to define an infectious dose for HEV (correspondence between HEV RNA and infectivity) for the HEV risk assessment of plasma-derived medicinal products.

Hepatitis E Virus: Baxter inactivation / removal data. Thomas R. Kreil, Baxter BioScience, Austria

RNA transcripts from the recombinant Kernow-C1 clone were used to transfect HepG2/C3A cells and supernatants from transfected cells could be used to infect fresh HepG2 cells. Read out for infectivity assay was by immunofluorescence analysis. When investigating the suitability of HEV RNA as read out, there was a virtual increase in HEV-concentration from inoculated CHO cells which are not permissive for HEV replication. This increase probably represented desorption of virus particles from inoculated cells. Therefore, NAT read-outs should be interpreted with care. An alternative virus spike was HEV from an infected pig liver homogenate. Partitioning steps were investigated by NAT using both virus spikes. 3.6 and 4.1 $\log_{10}$ reduction could be demonstrated for FVIII immuno-affinity chromatography and HEV was removed to below the limit of detection (up to >4.2 $\log_{10}$) for a Cohn II+III extraction step from IgG-purification. Reduction at the fractionation step was comparable to that of HAV and FCV. At pasteurisation of albumin, HEV inactivation was at least 3 $\log_{10}$. Treatment of virus stocks with solvent/detergent (SD) and C18 column chromatography to remove SD reagents resulted in reduction of infectivity by less than 1 $\log_{10}$. As expected, HEV was removed to below the detection limit by Planova 20N filtration.

HEV Reduction by Selected Manufacturing Steps of CSL Behring's Plasma-derived Products (Albrecht Gröner, CSL Behring, Germany)

An *in vivo* assay has been developed at the Friedrich-Loeffler-Institut – Federal Research Institute for Animal Health, Germany. In an inactivation study for VWF/FVIII intermediate (pasteurisation of stabilised aqueous solution of VWF/FVIII for 10 h at 60°C), samples of the intermediate spiked with a filtered liver homogenate from a wild boar with HEV genotype 3 prior to pasteurisation were pasteurised and inoculated into piglets. Read outs for infection of piglets were the time course of HEV RNA in faeces and detection of HEV RNA in bile after termination of study. This study showed inactivation of HEV in the order of at least 4 $\log_{10}$. Combined precipitation and adsorption steps from the VWF/FVIII manufacturing process were studied using NAT demonstrating an overall removal capacity of 3 $\log_{10}$. In summary, the overall reduction capacity was found comparable to that indicated by studies with HAV or B19V/CPV. Furthermore, cell culture derived cutthroat trout virus (CTV) was spiked into Ig-Matrix and infectivity could be removed below the detection limit by Pall DV20 filtration.

Plasma Products HEV Program Update (Rodrigo Gajardo, Grifols, Spain)

A double/complementary approach to study HEV removal/inactivation in different plasma derivatives production processes steps was presented i.e. investigation of new virus models for HEV and development and application of an HEV infectivity assay. The new HEV infectivity assay was described. The calicivirus murine norovirus (MNV) was investigated as a model for HEV inactivation by dry-heat treatment. Inactivation kinetics were slower than that of HAV, however, MNV was inactivated (around 4-5 $\log_{10}$) after treating of FVIII/vWF at 48-72h at 80°C. In addition inactivation of HEV was studied by infectivity assay. A mean reduction factor of 3.7 $\log_{10}$ (2 runs, residual infectivity detected) could be achieved using a detergent pretreated virus spike, with similar inactivation kinetics. HEV removal at partitioning steps (precipitation/depth filtration) from IVIG production were also studied using HEV infectivity assay and an overall reduction capacity of 6 $\log_{10}$ could be observed. Nanofiltration of another IVIG product using filters designed for parvovirus removal were found effective for removal of HEV by infectivity (≥ 5.4 $\log_{10}$). Finally a comparison of HEV removal/inactivation results with other non-enveloped viruses was made showing similar results.

Experiences of HEV elimination during the manufacturing process steps and the suitable model viruses (Mikihiro Yunoki, Japan Blood Products Organization, Japan)

Evidence indicating that anti-HEV IgG / IgM may have no or only weak neutralising activity against HEV infection was reviewed. Adsorption experiments of HEV to protein G indicated that lipids may be attached to viral particles and inhibit (interfere with) IgG binding. Maternal antibodies failed to protect against mother to piglets infection and transfusion transmitted infection (TTI) with anti HEV containing donation has been observed in Japan.

Hepatitis E virus isolates in albumin solutions were inactivated slowly at 60°C for 10 h and the $\log_{10}$ reduction factor (LRF) varied from 1·0 to >3·0. Heat stability of HEV depended on the concentration of albumin. Non-detergent treated HEV spike from serum was found to be more resistant. The virus was slowly inactivated in a freeze dried fibrinogen containing stabilisers and the LRFs were 2·0 and 3·0, respectively, after 72 h at 60° C, but inactivated to below the detection limit within 24 h at 80 °C with an LRF of more than 4·0. Studies on partitioning at ethanol fractionation steps showed different behaviour of virus spikes according to their origin (serum, faeces) or pretreatment. It was found difficult to predict HEV-reduction from data with EMCV or CPV and reduction of HEV was more limited than that of model viruses. HEV was stable at 5hours incubation at pH 3.0 or pH2.5. Filtration experiments showed effective removal at Planova 20N filtration while reduction at Planova 35N filtration was limited to about 3 $\log_{10}$.

PPTA perspective on risk assessment for plasma-derived medicinal products and implications for warning statements (Ilka von Hoegen, Plasma Protein Therapeutics Association)

I. von Hoegen summarised PPTA's point of view. PPTA member companies have demonstrated HEV reduction capacity of manufacturing process steps such as virus filtration/nanofiltration, heat (pasteurisation, dry-heat) treatment, and partitioning steps. No HEV transmission by plasma-derived medicinal products (PDMPs) has been reported with virus-inactivated products and a recent study (Modrow et al. Vox Sang 100:351-8, 2011) failed to detect HEV RNA in different coagulation factor concentrates. Toyoda and collaborators have reported a suspected HEV transmission by non-virus inactivated coagulation factor concentrate in Japan. The warning statements in the Guideline on the

warning on transmissible agents in summary of product characteristics (SmPCs) and package leaflets for PDMPs (EMA/CHMP/BWP/360642/2010 rev. 1) make specific reference to viruses that have been transmitted in the past by PDMPs but do not, for instance, make a specific reference to vCJD. PPTA does not consider the addition of a warning statement on HEV as justified as no HEV transmission has been reported for "state-of-the-art" PDMPs. The warning statement should indicate the remaining potential risk of transmitting infective agents by PDMPs, i.e. the general statement in the SmPC and package leaflet, "the possibility of transmitting infective agents cannot be totally excluded. This also applies to unknown or emerging viruses and other pathogens," is considered appropriate and sufficient.

Risk assessment for plasma-derived medical products and implication for warning statements, IPFA Perspective (Françoise Rossi, International Plasma Fractionation association).

F. Rossi pointed out that HEV is not an emerging virus and the virus has been in the donor population for a long time. Infections are most of the time inapparent and there has been no report of transmission associated with the use of plasma-derivatives. An overall risk analysis shows that blood/plasma donations can contain HEV RNA. However viraemia is usually low or moderate and due to exposure to HEV in a significant part of the donor population, plasma pools also contain HEV antibodies which may contribute to the safety of plasma products through neutralisation. There is some indication that neutralization can contribute to HEV reduction in the context of IgG. However, virus particles associated with lipids are non neutralisable. Experimental data, reported so far, indicate significant removal/inactivation of HEV during manufacture of plasma-derived medicinal products and product-specific evaluation was not generally recommended. Only for the few products with lower safety margin, a scientific evaluation could/would be beneficial. When performing theoretical risk assessments, care should be taken not to overestimate the risk and data are available which indicate a minimum infectious dose in the order of 10,000 IU HEV RNA. Specific warning statements for HEV were not recommended for the SmPC as the objective is to inform on "established/proven risk only".

In conclusion, available information and risk assessment for plasma-derived medicinal products according to the current state of knowledge support the safety regarding the HEV transmission risk Implementation of additional regulatory measures (such as pool NAT testing or product-specific validation studies) will not contribute to improving safety for patients.

医薬品 研究報告 調査報告書

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研究報告の概要

○感染症最新情報 パルボウイルスB19

ドイツの供血者と受血者を対象として実施された研究では、パルボウイルスB19(B19V) DNA陽性の血液成分を輸血された424名の受血者において、B19V輸血伝播(TT-B19V)の証拠は得られなかった。ヒトにおけるB19V感染は経上気道感染が一般的であるが、輸血による伝播が実証されている。B19Vの自然感染は一般的に良性であるが、赤血球産生が亢進している患者やHIV感染患者が感染したとき、及び胎児が子宮内感染したときは深刻な状態となりうる。血液製剤によってもたらされるB19Vの脅威を評価するために、Institute of Transfusion Medicine(ドイツ、リューベック)のDavid Julは、以前に得たB19V感染供血者に関するデータに基づく遡及調査を行い、TT-B19Vが起きていた可能性について調査した。424名の受血者のうち、132名については輸血前のB19V IgG抗体は陰性であった。これら132名の受血者のうち、67名についてはフォローアップ検体が入手可能であった。このうち65名は、B19V DNA濃度が10^4 IU/ mL plasma未満の供血者から採血した血液成分を輸血されていたが、TT-B19Vの兆候は認められなかった。供血者と受血者におけるB19Vの遺伝子配列の一致により、受血者2名のTT-B19Vが示された。これらの受血者はそれぞれ、B19V DNA濃度が3.4×10^6 IU/ mL plasmaまたは1.8×10^4 IU/ mL plasmaの赤血球製剤の輸血を受けていた。両名に明らかな感染兆候または症状は認められなかった。著者らは、「本調査では、血漿中のB19V DNA濃度が低い($<10^4$ IU/ mL)血液成分を介するTT-B19V感染の発生は確認されなかった。TT-B19V例のうち1例については、供血者が中和抗体を保有していたにもかかわらず、B19V DNA濃度が中等度の赤血球を介して感染が発生していた。しかしながらその臨床的意義は低かった」と結論付けた。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

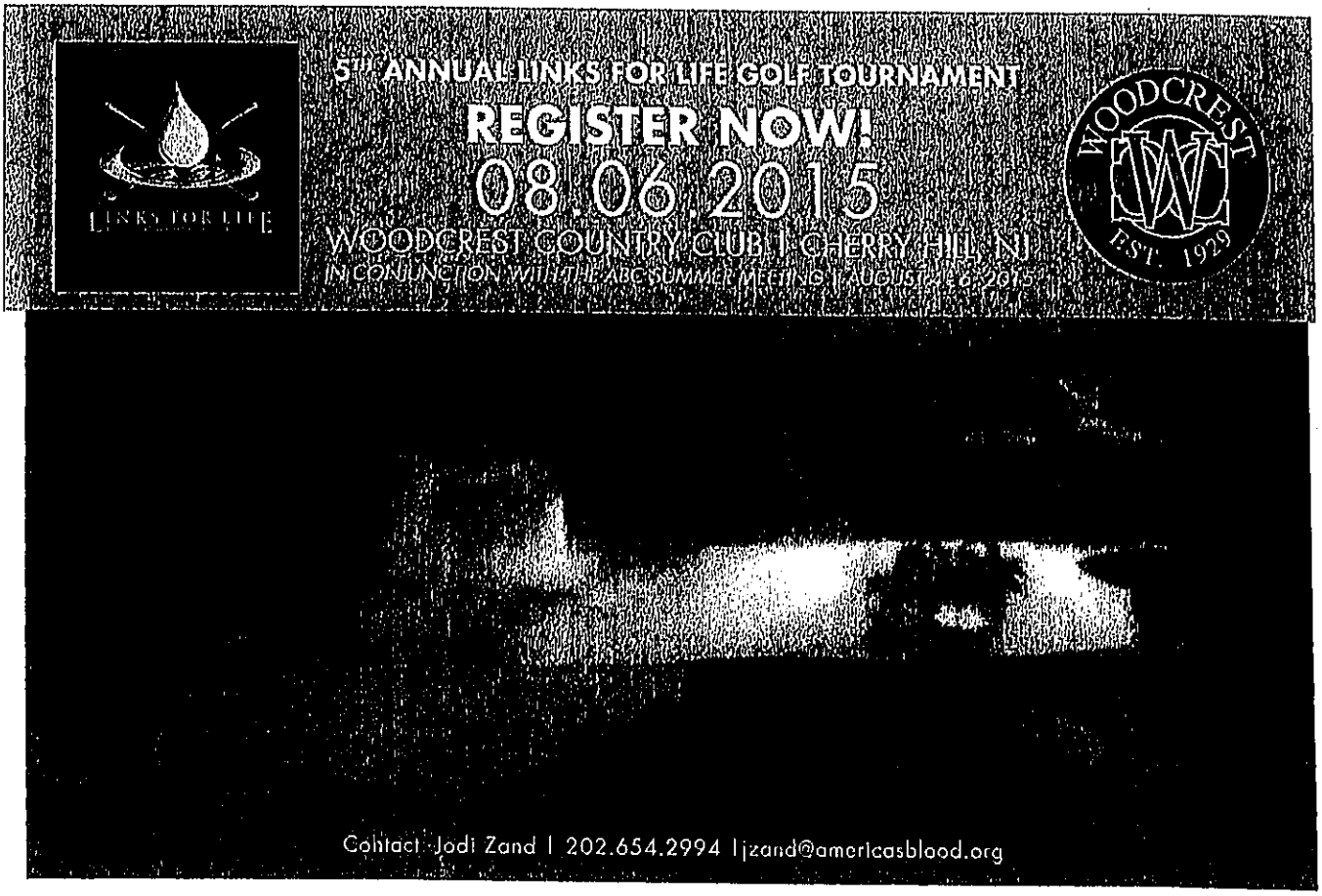
血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

血漿中のパルボウイルスB19(B19V) DNA濃度が低い($<10^4$ IU/ mL)供血者から得た血液製剤による輸血ではB19V感染は確認されなかったという報告である。

今後の対応

日本赤十字社では、以前よりRHA法によるパルボウイルスB19(B19V)抗原検査(検出感度:10^{10}～10^{11}IU/mLレベル)を導入、ウイルス量の多い血液を排除してきた。また、2008年には検査法をより感度の高いCLEIA法(検出感度:10^6～10^7IU/mLレベル)に変更した。今後も引き続き、B19Vに関する新たな知見及び情報の収集に努める。



INFECTIOUS DISEASE UPDATES

PARVOVIRUS B19

A study conducted in German blood donors and recipients did not find evidence of transfusion-transmitted parvovirus B19 (TT-B19V) infection among 424 recipients of B19V DNA-positive blood components. B19V infection in humans is typically acquired via the upper respiratory tract, but transmission through transfusion has been demonstrated. While natural B19 infections are generally benign, severe morbidity can be associated with infection in patients with accelerated erythropoiesis, of HIV-infected patients, and when the developing fetus is infected *in utero*. To assess the threat posed by blood products by B19V, David Jul, of the Institute of Transfusion Medicine, in Lubeck Germany, performed a look-back study based on previously acquired data on B19 infected donors and investigated the extent to which potential TT-B19V infections might have occurred. In 132 out of 424 recipients, the researchers could detect no anti-B19V IgG before transfusion. In 67 out of these 132 susceptible recipients, a follow-up sample was available. Sixty-five of these received blood components from donors with $<10^4$ IU B19V DNA/ml plasma and had no evidence of TT-B19V infection. Homology in genome sequences in donors and recipient provided evidence for TT-B19V infection in two recipients; the patients received RBC

(continued on page 11)

INFECTIOUS DISEASE UPDATES (continued from page 10)

containing 3.4 x 10^6 and 1.8 x 10^4 IU B19V DNA/ml plasma, respectively. Neither had signs or symptoms clearly attributable to the transmissions. "TT-B19V infections through blood components with low (<10^4 IU/ml plasma) B19V DNA concentrations did not occur in our study. One of the TT-B19V infections occurred from RBC with intermediate B19V DNA concentration despite the presence of potential neutralizing antibodies in the donor, but its clinical significance was low," concluded the authors.

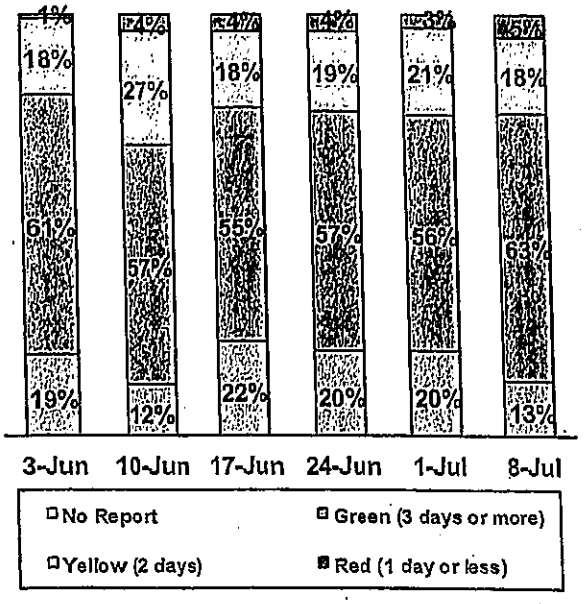
Citation: Jul D, *et al.* Look-back study on recipients of Parvovirus B19 (B19V) DNA-positive blood components. Vox Sang. 2015 June 5. [Epub ahead of print]

MALARIA

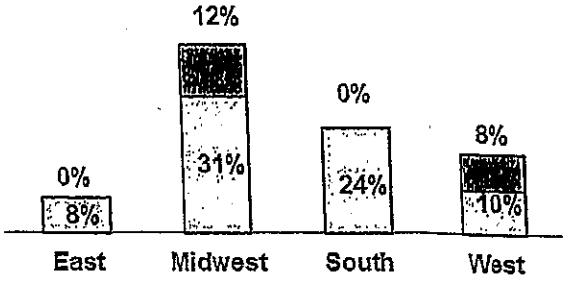
The Centers for Disease Control and Prevention recently posted an updated malaria information and prophylaxis table, available online. This table is now updated with the most current information from the Health Information for International Travel 2014 (CDC's Yellow Book). Those interested may sign up for CDC e-mail updates here to receive e-mails regarding the most current malaria prophylaxis information. (Source: CDC e-mail updates, 7/6/15)

STOPLIGHT®: Status of America's Blood Centers' Blood Supply

Total ABC Red Cell Inventory



Percent of Regional Inventory at 2 Days Supply or Less, July 8 2015



Percent of Total ABC Blood Supply Contributed by Each Region
East: 20%; Midwest: 25%; South: 24%; West: 31%

Daily updates are available at:
www.AmericasBlood.org

別紙様式第2-1

No. 13

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
				2015. 5. 7	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	Sobata R, Matsumoto C, Uchida S, Suzuki Y, Satake M, Tadokoro K. Vox Sang. 2015 Apr 30. doi: 10.1111/vox.12263. [Epub ahead of print]	公表国 日本
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			

研究報告の概要

○ヒトT細胞白血病ウイルス1型(HTLV-1)の輸血伝播(TT-HTLV-1)が起こるウイルス量、および白血球除去によるTT-HTLV-1の予防効果の推測

背景と目的: 保存前白血球除去(LR)を行った血液製剤によるヒトT細胞白血病ウイルス1型(HTLV-1)の輸血伝播(TT-HTLV-1)リスクは、TT-HTLV-1を起こす血液製剤のプロウイルス量が判明していないため、不明である。我々はHTLV-1陽性献血者におけるHTLV-1プロウイルス量の分布に基づき、輸血による感染が起こるプロウイルス量を推測した。また、TT-HTLV-1を予防する目的のLRの効果についても考察する。

材料と方法: 2008年から2011年に東京近郊で献血され、化学発光酵素免疫測定法(CLEIA)法と間接蛍光抗体(IF)法でHTLV-1抗体が確認された300名の献血検体について、LR前に採取した血液からゲノムDNAを抽出し、HTLV-1 pX領域とヒトCD81遺伝子を対象としたリアルタイムPCRによりプロウイルス量を測定した。輸血による感染が起こるプロウイルス量は、HTLV-1抗体陽性の献血者から輸血を受けた患者の遡及調査から得たTT-HTLV-1の過去の頻度データ、および献血者におけるHTLV-1プロウイルス量の分布パターンを用いて推測した。

結果: HTLV-1プロウイルス量は、白血球100細胞当たり0.01未満～25.0コピーと幅があった。過去データからTT-HTLV-1の頻度は80%であることが示された。抗体陽性検体300例の80%が感染性を有すると仮定すると、TT-HTLV-1が成立するには9 x 10^4コピー以上のHTLV-1プロウイルスを含む細胞が必要と推測される。

結論: LR後の残存HTLV-1感染細胞数は、TT-HTLV-1成立に必要なウイルス量より大幅に少ない。したがって、LRはTT-HTLV-1の発生率を最小限に抑えることに有効と考えられる。

報告企業の意見	今後の対応
保存前白血球除去製剤中のヒトT細胞白血病ウイルス1型(HTLV-1)のプロウイルス量を測定して、保存前白血球除去がHTLV-1の輸血伝播リスクの発生率を低減するとの報告である。	日本赤十字社では、既に全製剤に保存前白血球除去を適応しており、HTLV-1の抗体スクリーニング検査を行っている。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

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VoxSanguinis JRC2015T-015 ISBT International Society of Blood Transfusion

Vox Sanguinis (2015)

ORIGINAL PAPER

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Estimation of the infectious viral load required for transfusion-transmitted human T-lymphotropic virus type 1 infection (TT-HTLV-1) and of the effectiveness of leukocyte reduction in preventing TT-HTLV-1

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Vox Sanguinis

Background and Objectives The risk of transfusion-transmitted human T-lymphotropic virus type 1 infection (TT-HTLV-1) after prestorage leucocyte reduction (LR) remains unknown, as the proviral load in the blood component that would cause TT-HTLV-1 is undetermined. On the basis of the distribution of HTLV-1 proviral load among HTLV-1-sero-positive blood donors, we attempted to estimate the proviral load for transfusion-related infectivity. We also discuss the effectiveness of LR in preventing TT-HTLV-1.

Materials and Methods The HTLV-1 proviral load in 300 HTLV-1-sero-positive blood donors was determined by real-time polymerase chain reaction analysis. The proviral load required for transfusion-related infectivity was estimated using historical TT-HTLV-1 frequency data from a retrospective study on patients who had received blood from HTLV-1-sero-positive blood donors and the distribution pattern of HTLV-1 proviral load among blood donors.

Results HTLV-1 proviral loads ranged between <0·01 and 25·0 copies per 100 leucocytes. Historical data showed TT-HTLV-1 frequency to be 80%. Assuming that 80% of the 300 sero-positive samples are infectious, it is estimated that the transfer of $\geq 9 \times 10^4$ cells containing the HTLV-1 provirus is required to establish TT-HTLV-1.

Conclusion The residual number of HTLV-1-infected cells after LR is substantially lower than the viral load necessary for TT-HTLV-1. LR therefore appears to be effective in minimizing the incidence of TT-HTLV-1.

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Key words: HTLV-1, leucocyte reduction, proviral load, transfusion, transfusion-transmitted infection.

Introduction

Human T-cell lymphotropic virus type 1 (HTLV-1) was the first human retrovirus to be identified; it causes adult T-cell leukaemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [1, 2]. Approximately 20 million people worldwide are currently infected with HTLV-1, and the infection is endemic to south-western Japan, the Caribbean basin, South America and parts of Africa [3, 4]. In Japan, the number of carriers is estimated to be at least 1·08 million [5]. The lifetime incidence of ATL among HTLV-1 carriers after a long latent period is estimated to be 2·5–5% [6, 7]. Almost 1000 new cases of ATL are diagnosed each year in Japan [8, 9]. However, effective therapy for ATL is limited, and the median survival of patients with ATL is 13 months [10].

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HTLV-1 infects lymphocytes when viral DNA integrates into the host genome via reverse transcription of viral RNA. HTLV-1-infected lymphocytes produce very few cell-free infectious virions, and efficient transmission of HTLV-1 requires direct cell-to-cell contact. Therefore, HTLV-1 transmission between individuals occurs through the transfer of HTLV-1-infected lymphocytes [11, 12]. The major routes of HTLV-1 infection are mother-to-child transmission, mainly via breast milk [13–15], and sexual transmission, which occurs predominantly from male to female [16, 17]. Blood transfusion with HTLV-1-positive cellular components has also been identified as a prevalent route of infection [18, 19]. From 1986 to 2007, all Japanese Red Cross (JRC) blood centres used particle agglutination assays (PA) to screen for anti-HTLV-1 antibodies (anti-HTLV-1) in donated blood samples to prevent transfusion-transmitted HTLV-1 infection (TT-HTLV-1). In 2008, PA was replaced with a highly sensitive chemiluminescence enzyme immunoassay (CLEIA).

In Japan, no established cases of TT-HTLV-1 have been reported since the introduction of these screening tests. However, PCR-positive but HTLV-1-sero-negative carriers reportedly exist [20]. Additionally, the window period of HTLV-1 infectivity is reportedly relatively long (51 days) [19]. Therefore, the possibility of TT-HTLV-1 resulting from screened blood should be considered in HTLV-1-endemic areas.

In 2007, universal prestorage leucocyte reduction (LR), which removes >99·9% of all leucocytes in blood components by filtration, was introduced for all blood components in Japan to reduce the incidence of adverse transfusion reactions resulting from leucocytes. The number of residual leucocytes after LR should be $<1 \times 10^6$ per unit for >95% of units currently issued from JRC blood centres. The benefits of LR in reducing the risk of non-haemolytic febrile transfusion reactions [21], alloimmunization to leucocyte antigens [22] and transmission of cytomegalovirus [23] have been reported. In addition to these benefits, LR of blood components is expected to reduce the risk of TT-HTLV-1. In support of this conjecture, post-LR whole blood from asymptomatic carriers reportedly shows a marked (3–4 $\log_{10}$) reduction in HTLV-1 provirus [24]. Although the number of subjects was small, a lookback study in England showed that TT-HTLV-1 frequency in recipients was significantly lower for LR components than for non-LR components [25]. However, the effectiveness of LR in preventing TT-HTLV-1 remains unknown, in part, because the proviral load in blood components that would cause TT-HTLV-1 has yet to be quantified.

In this study, we determined the amount of HTLV-1 provirus among HTLV-1-sero-positive blood donors. We then used these data and historical data of TT-HTLV-1 frequency in a retrospective study of patients who received blood from HTLV-1-sero-positive blood donors [26] to estimate the proviral load necessary for infectivity. We also discuss the effectiveness of LR in preventing TT-HTLV-1.

Materials and methods

HTLV-1-sero-positive blood samples

We collected 300 blood samples that had each tested positive for anti-HTLV-1 based on CLEIA and been confirmed by indirect immunofluorescence assay (IFA). These samples were donated sometime from July to October 2008 or April 2010 to March 2011 in the Greater Tokyo area, a non-HTLV-1-endemic area in Japan. Of these samples, 169 were from males; 131 were from females; and the mean donor age was 45·6 years (range, 16–65 years).

CLEIAs (CL4800 Testing System; Fujirebio, Tokyo, Japan) were performed in accordance with the instructions provided by the manufacturer. Samples with an anti-HTLV-1 CLEIA cut-off index ≥1·0 were defined as reactive. IFA was conducted following the method of Hinuma *et al.* [27] with some modifications and with mixed targets of HTLV-1-infected and non-HTLV-1-infected cells [28].

Each blood sample was obtained before the associated donation was subject to the LR filtering process. Informed consent to undergo tests for HTLV-1 infection had been obtained from each blood donor at the respective blood collection site.

Extraction of genomic DNA from blood clots

Because anti-coagulated whole-blood samples were not available, we used the clotted blood samples that remained in the serum-separation tubes that had been used for serological screening tests for major transfusion-transmissible pathogens in donated blood. After the removal of the serum, blood clots were homogenized using a BioMasher (Nippi, Tokyo, Japan); homogenates were then suspended in an equal volume of phosphate-buffered saline. QIAsymphony DNA Midi kits (Qiagen, Gaithersburg, MD, USA) were then used to extract genomic DNA. Spectrophotometry was used to assess DNA quantity and quality. Genomic DNA extracted from blood clots was very pure (average value of 260/280 nm absorbance ratios; 1·80).

Quantification of HTLV-1 proviral load

To detect and quantify HTLV-1 proviral DNA, we performed real-time PCR analysis using a TaqMan probe

(TaqMan PCR) designed for the HTLV-1 pX region. The primers and probe were as follows: sense primer, 5′-TG GACAGAGTCTTCTTTTCGGATA-3′ (nt 7341–7364 [29]); antisense primer, 5′-CACCAGTCGCCTTGTACACAGT-3′ (nt 7406–7385); and TaqMan MGB probe, 5′-FAM-CCAGTCT ACGTGTTTGG-MGB-3′ (nt 7366–7382).

In addition to TaqMan PCR for HTLV-1, TaqMan PCR for human CD81 DNA was performed simultaneously in the same reaction tube to monitor PCR inhibition and estimate the amount of cellular DNA in the reaction. The number of cells involved in each reaction was calculated on the basis of the finding that a haploid human DNA contains one copy of the CD81 gene [30, 31]. The primers and probe used to detect exon 5 of the CD81 gene were as follows: sense primer, 5′-CCAGCACACTGACTGCTT TGA-3′; antisense primer, 5′-GCCCGAGGGACACAAAT TG-3′; and TaqMan MGB probe, 5′-VIC-CACCTCAGTGCT CAAG-MGB-3′.

The PCR products of the HTLV-1 pX region isolated from HUT102 cells, an HTLV-1-infected T-cell line, and the coding sequence of CD81 isolated from leucocytes of a healthy donor, who had given informed consent, were cloned into a plasmid vector using a TOPO TA Cloning kit (Invitrogen, Carlsbad, CA, USA). After purification, plasmid DNA concentration was determined, and a dilution series was used to construct a standard curve.

Each TaqMan PCR was performed in a 50 μl reaction mixture comprising approximately 1 μg of DNA sample, 25 μl of QuantiTect Probe PCR Master Mix (Qiagen), 0·4 μM of each primer and 0·2 μM of TaqMan MGB probe. TaqMan PCR conditions were a 10-min initial PCR activation step at 95°C and 45 amplification cycles, each of 95°C for 15 seconds and at 60°C for 45 seconds. Each sample was analysed in duplicate. The Applied Biosystems StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was used for TaqMan PCR and all data analysis. HTLV-1 proviral load was calculated from the number of copies per 100 leucocytes via the following formula: HTLV-1 proviral load = [(number of copies of HTLV-1 pX region DNA)/(number of copies of DNA coding CD81/2)] × 100.

To determine the detection sensitivity of TaqMan PCR, we spiked cloned HTLV-1 pX region DNA into 1 μg of DNA solution extracted from blood clots of non-HTLV-1-infected individuals and performed TaqMan PCR for the HTLV-1 pX gene. The 95% detection limit of TaqMan PCR was estimated to be approximately 20 copies per 1 μg of blood clot DNA sample. TaqMan PCR was performed retrospectively with archived samples.

Estimation of proviral load for infectivity

Okochi *et al.* reported that seroconversion occurred in approximately 80% of recipients transfused with one unit of non-LR red cell concentrate (RCC) within 5 days of collection from blood donors subsequently identified as HTLV-1-positive [26]; these blood donors were confirmed as HTLV-1-sero-positive by IFA, the same method used in the current study. However, information on proviral load of these blood donors was not available. To estimate the risk of TT-HTLV-1, we supposed that the efficacy of transmission is dependent on the number of HTLV-1-infected cells transfused. That is, we supposed that 80% of fresh non-LR RCCs from IFA-positive blood donors in Japan each contained a sufficient amount of provirus to cause TT-HTLV-1 and that other 20% did not. Furthermore, we assumed the distribution pattern of HTLV-1 proviral load among HTLV-1-sero-positive blood donors in our study to be the same as that reported by Okochi *et al.* [26]. Therefore, 80% of our blood samples from HTLV-1-sero-positive blood donors are expected to be in the same category as units bearing an infection risk.

Results

Determination of HTLV-1 proviral load among HTLV-1-sero-positive blood donors

The HTLV-1 pX gene was detected in all of the 300 samples enrolled in this study, and HTLV-1 proviral loads ranged from <0·01 to 25·0 copies per 100 leucocytes, with a mean value of 2·0 (median 0·79; interquartile range 0·16–2·86) copies per 100 leucocytes (Fig. 1). Although most of the samples in this study had a high anti-HTLV-1 titre based on CLEIA results, the proviral

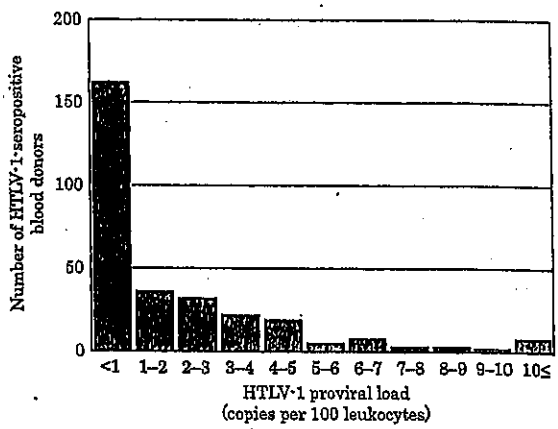

Fig. 1 Distribution of HTLV-1 proviral load among 300 HTLV-1-sero-positive blood donors. The HTLV-1 proviral loads in 300 HTLV-1-sero-positive blood donors ranged from <0·01 to 25·0 (mean 2·0, median 0·79; interquartile range 0·16–2·86) copies per 100 leucocytes. In 54·0% of the HTLV-1-sero-positive blood donors used in this study, the HTLV-1 proviral load was estimated to be <1·0 copy per 100 leucocytes.

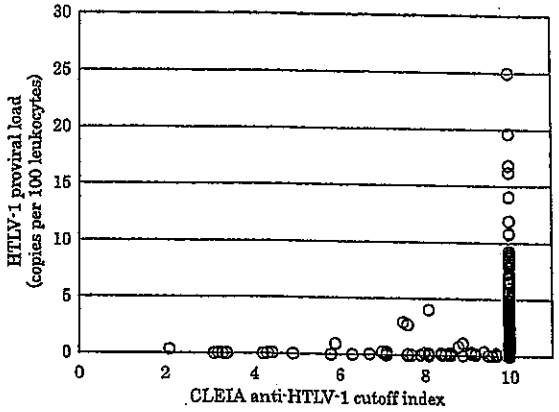

Fig. 2 HTLV-1 proviral load and anti-HTLV-1 level. The samples with high proviral load (≥5·0 copies per 100 leucocytes) showed high anti-HTLV-1 levels measured by chemiluminescence enzyme immunoassay. The upper limit of the cut-off index for detecting the anti-HTLV-1 is 10·0.

load was highly variable. Notably, each sample with a high proviral load (≥5·0 copies per 100 leucocytes) was at the upper limit of the CLEIA cut-off index (Fig. 2).

Estimation of proviral load needed for transfusion-related infectivity

We assumed that 80% of blood samples enrolled in the current study were infectious and they would each contain ≥0·09 copies of the HTLV-1 provirus per 100 leucocytes (Fig. 3). The number of leucocytes per unit of RCC is reportedly between 1×10^8 and 1×10^9 before LR [32, 33]. We estimated that when the number of leucocytes per unit of RCC is 1×10^8 before LR, non-LR-RCC units that cause TT-HTLV-1 contain $\geq 9 \times 10^4$ (0·09% of 1×10^8) cells with integrated HTLV-1 provirus (Fig. 3). Therefore, we estimated that the minimum infectious proviral load for TT-HTLV-1 is approximately 9×10^4 copies.

Discussion

We used TaqMan PCR analysis that has high sensitivity over a wide quantification range, to measure the HTLV-1 proviral load in peripheral blood from 300 HTLV-1-sero-positive blood donors. Using our method, we showed that the proviral loads in HTLV-1-sero-positive blood donors ranged from <0·01 to 25·0 (mean 2·0, median 0·79; interquartile range 0·16–2·86) copies per 100 leucocytes. For 54·0% of the blood samples analysed, the HTLV-1-infected leucocytes were estimated to constitute <1·0% of the total leucocyte population. Each sample with a high proviral load showed correspondingly high anti-HTLV-1 levels.

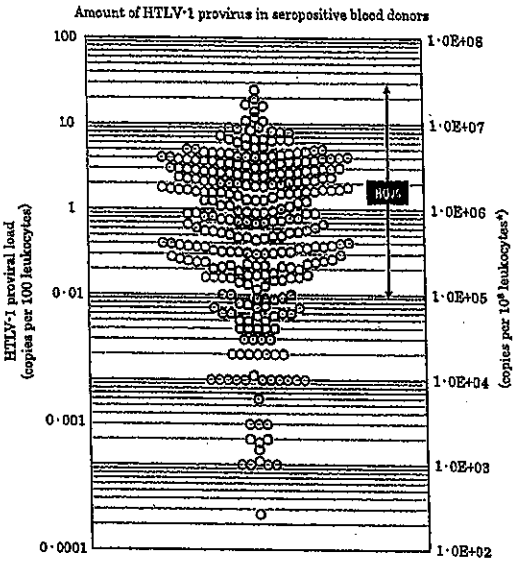

Fig. 3 Estimation of HTLV-1 proviral load required for transfusion-related infectivity. According to the report by Okochi *et al.* [26], it is estimated that 80% of our blood samples would have infectious potential. The minimum infectious virus load based on Okochi *et al.*'s report is indicated by the dotted line. It was estimated that a minimum of 9×10^4 HTLV-1-infected cells were present in units that caused TT-HTLV-1. *Number of leucocytes per unit of non-leucocyte reduction-red cell concentrate.

In a study conducted before 1986, which is when the anti-HTLV-1 screening of donated blood started in Japan, seroconversion was reported in 79·2% of 48 recipients of blood transfusions who had received one unit of fresh non-LR-RCC from HTLV-1-sero-positive blood donors within 5 days of donation [26]. In a prospective study in Jamaica, a 44% seroconversion rate was reported in recipients receiving non-LR, HTLV-1-positive cellular components. For recipients who received non-LR, HTLV-1-positive cellular components with a storage time of <6 days, seroconversion was seen in 80% of the cases [19]. A retrospective study in the US showed that non-LR, RCC used within 5 days of donation from HTLV-1/HTLV-2-sero-positive blood donors had a transmission efficiency of 80% [34]. Together, these studies showed that the efficacy of transmission is high within 6 days of donation and decreases as storage time of components increases.

This finding is consistent with *in vitro* lymphocyte culture data that demonstrate that the infectivity of preserved blood samples is reduced compared with that of fresh blood samples [35]. On the other hand, seroconversion was not observed in any recipients of non-LR, fresh-frozen plasma (FFP) from HTLV-1-sero-positive blood donors [18, 19, 26, 34, 36]. It is predicted that FFP, which contains an average of $1 \times 10^{6-7}$ leucocytes, does not

contain enough viable lymphocytes to cause TT-HTLV-1. Moreover, the infectivity of HTLV-1 in FFP is expected to be lost during the freeze–thaw process. Similarly, studies of mother-to-child transmission via breast milk have shown that the freeze–thaw process could eliminate the infectivity of HTLV-1 from the milk of mothers carrying the virus [37, 38]. These observations indicate that a large number of viable HTLV-1-infected lymphocytes are necessary to establish HTLV-1 infection via transfusion. Okochi *et al.* [26] conclude that the rate of seroconversion is associated with the number of lymphocytes transfused and that 1×10^8 lymphocytes per unit are necessary for infection via transfusion; however, the exact infectious dose remains unknown.

We expected that 80% of fresh non-LR RCC units from anti-HTLV-1-positive donations would be infectious on the basis of data described in the literature presented above. When the cut-off point was set at 80% of the maximum proviral load among those samples, infectious samples were estimated to contain ≥0·09 copies of HTLV-1 provirus per 100 leucocytes. Based on fact that the number of leucocytes per unit of non-LR RCC is $\geq 1 \times 10^8$, we estimated that a minimum of 9×10^4 HTLV-1-infected leucocytes were present in the cellular components that caused TT-HTLV-1.

In 2007, LR was introduced for all blood components in Japan. In >99% of the blood components supplied from JRC blood centres, residual leucocyte numbers are within acceptable limits (1×10^6 per unit) [39]. Furthermore, in >95% of blood component units, the actual number of residual leucocytes is $<1 \times 10^5$, even in whole blood which contains a larger number of leucocytes than individual components [32, 39, 40].

The HTLV-1 proviral loads in our blood samples, which came from HTLV-1 carriers, ranged from <0·01 to 25·0 copies per 100 leucocytes. We subsequently calculated that for every 1×10^5 leucocytes from HTLV-1-sero-positive blood donors, <1·0 to $2{\cdot}5 \times 10^4$ of the cells are infected with HTLV-1. Even the upper limit of this range is substantially lower than the proviral load that we estimated was needed for transfusion-related infectivity. Thus, LR appears to be effective in minimizing the incidence of TT-HTLV-1. In case where the HTLV-1 proviral load in donated blood is extremely high, LR may not suffice to eliminate the risk of TT-HTLV-1. However, on the basis of the finding in the current study that blood samples with high proviral loads also had high anti-HTLV-1 levels, it is thought that donated blood with a high HTLV-1 proviral load is undoubtedly eliminated by CLEIA screening.

In the current study, we accurately measured HTLV-1 proviral load in samples from sero-positive blood donors and estimated the minimum infectious proviral load required for TT-HTLV-1 based on the assumption that the risk of TT-HTLV-1 depends on the viral load in the donated blood component. However, an overlap in the viral load range between infectious and non-infectious components has often been observed in transfusion-related infections caused by other viruses [41, 42]. Moreover, transfusion-related infectivity will vary depending not only on the blood component factors, but also on the clinical state of the recipient. TT-HTLV-1 risk might be amplified in immunocompromised recipients and neonates. Furthermore, proviral load during the serological window period may be higher than that in a sero-positive HTLV-1 carrier, and transfusion-related HTLV-1 infectivity might be higher with units from the window period, which is the period when anti-HTLV-1 is absent as has been observed in the early stage of HIV-1 infection [43]. Thus, we cannot rule out the possibility that proviral loads lower than the minimum infectious dose estimated here may cause TT-HTLV-1.

Although there have been no documented cases of HTLV-1 transmission occurring as a result of a needle-stick injury, it is well known that the related virus, HTLV-2, is endemic among intravenous drug users [44]. Proviral loads in the peripheral blood from HTLV-1-infected individuals have been reported to be higher than those in HTLV-2-infected individuals [45, 46]. In a rabbit model, the infection and replication abilities of HTLV-1 were reportedly higher than those of HTLV-2 [47], and 0·01 ml of HTLV-1-infected blood containing $1{\cdot}7 \times 10^4$ lymphocytes was capable of transmitting HTLV-1 [48]. Thus, the possibility remains that small amounts of contaminated blood are able to transmit HTLV-1 in the same manner as HTLV-2. However, the infectivity of HTLV-1-positive blood components that have undergone production processes and storage is predicted to be far less than that of HTLV-1-positive fresh peripheral blood, as seen for transfusion-related HBV infection [41].

Screening for anti-HTLV-1 in blood donors is an effective strategy for preventing TT-HTLV-1 in Japan. It seems that even if an HTLV-1-infected blood donation slipped through the screening system of JRC blood centres, LR would eliminate the risk of TT-HTLV-1 in almost every such case. Importantly, the combination of serological screening and LR appears to have virtually eliminated the risk of TT-HTLV-1 in Japan. In areas where LR is a standard practice and HTLV-1 prevalence among blood donors is and has been extremely low over a long period, discontinuing anti-HTLV-1 screening of blood donations on the basis of strict quality control of LR process could be considered from a cost-benefit point of view. Alternatively, selective screening of blood donation based on

donor's ethnicity could be an option under the standard practice of universal LR.

In conclusion, we estimated that the minimum infectious load of HTLV-1 provirus for TT-HTLV-1 is $\geq 9 \times 10^4$ copies. LR decreases the number of HTLV-1-infected leucocytes below this level in most blood components contaminated with HTLV-1. LR in addition to serological screening of donated blood minimizes the risk of TT-HTLV-1.

Conflict of interest

The authors declare no conflict of interests.

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No. 17

医薬品 研究報告 調査報告書

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				2015. 5. 7	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	Dodd RY, Foster GA, Stramer SL. Transfus Med Rev. 2015 Jul;29(3):153-161. doi: 10.1016/j.tmrv.2015.03.001. Epub 2015 Mar 22.	公表国
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研究報告の概要

○ウエストナイルウイルスから輸血の安全性を守る: 2003～2012年の米国赤十字社の経験

ウエストナイルウイルス(WNV)は、米国では1999年に初めて出現し、その後数年間で西半球全体に急速に広がり、多数のヒト感染と深刻な疾患を引き起こした。2002年には、輸血によって伝播することが明らかになり、その後1年以内に米国の全供血に対して、WNV RNAの核酸増幅検査を実施することになった。米国赤十字社(ARC)は、米国の供血の約40%を担当し、前述のような検査の結果を詳しく監視し、反応を示した供血者を調べ、その結果を供血者管理の方針の開発と変更に反映させている。本稿は、2003～2012年の10年間のARCの検査プログラムの結果について記述する。

全体では、流行期に2,700万以上の供血の検査を行い、1,576件がWNV RNA陽性であった。感染供血者の時間的および地理的分布について述べる。安全性を最大限に高めるため、個別の供血検査の期間を開始・終了する方法を開発しバリデートした。供血者におけるWNV感染の特性を探り、ウイルス力価の分布を調べた結果、720,000コピー/mL以下であることが判明した。また、抗体出現の時系列に沿って力価の分布を調べた。感染の初期と確認された供血者について症状の出現を調べ、26%が少なくとも3つの特徴的な症状を呈していた。検査プログラムは輸血によるWNV伝播の予防に有効であり、検査の開始以降に報告された全13症例のうち1例のみがARCによるもので、検査結果が出る前に輸血された顆粒球製剤が原因であった。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
2003～2012年に米国赤十字社(ARC)が実施したウエストナイルウイルス検査プログラムの結果では、2,700万以上の供血の検査を行い、1,576件がウエストナイルウイルスRNA陽性であり、実施された検査プログラムは輸血によるWNV伝播の予防に有効であったとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としているほか、ウエストナイル熱の既往がある場合、治癒後6カ月は献血不適としている。また、ウエストナイルウイルス感染の国内発生に備え、平成17年10月25日付血液対策課発事務連絡に基づく緊急対応(献血制限、NAT検査)のほか、厚生労働科学研究「血液製剤及び献血血の安全性確保と安定供給の維持のための新興・再興感染症に関する総合的研究」班と共同して対応について検討している。今後も引き続き情報の収集に努める。

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Original Articles

Keeping Blood Transfusion Safe From West Nile Virus: American Red Cross Experience, 2003 to 2012

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ABSTRACT

West Nile virus (WNV) appeared for the first time in the United States in 1999 and rapidly spread across the Western hemisphere within a few years causing hundreds of thousands of human infections and significant disease. In 2002, it was found to be transmissible by blood transfusion, and within less than a year, nucleic acid testing for WNV RNA was in place for all US donations. The American Red Cross (ARC) collects approximately 40% of blood donations in the United States and closely monitors the results of such testing and evaluates donors found to be reactive. This review describes the 10-year results of the ARC testing program during the period 2003 to 2012. Overall, more than 27 million donations were tested during the transmission periods with 1576 RNA-positive donations identified. The temporal and geographic distributions of the infected donors are described. Methods to initiate and discontinue periods of individual donation testing were developed and validated to maximize safety. The nature of WNV infection among donors was investigated, and the distribution of viral titers was defined and was found to be no greater than 720 000 RNA copies per milliliter. The distribution of titers by time sequence of appearance of antibodies was determined. Donors who were identified as being in the earliest stages of infection were evaluated for the appearance of symptoms, and 26% developed at least 3 characteristic symptoms. The testing program has been successful in preventing transmission of WNV by transfusion, and only 1 of the 13 reported cases since the initiation of testing was attributable to the Red Cross; it was from a granulocyte product transfused before availability of the test result.

Contents

Until 1999, the West Nile virus (WNV), a mosquito-borne *Flavivirus*, was endemic to many parts of Africa, Southern Europe, the Middle East, Southwest Asia, India, and Australia (Kunjin strain). However, in that year, an unexpected outbreak occurred in Queens, New York, marking the first autochthonous cases in the Americas. A total of 17 confirmed and 20 probable human cases, with 4 deaths, had been reported by September 28, 1999 [1]. West Nile virus spread rapidly throughout the east coast and as far west as the Rocky Mountains [2], with a total

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of 4305 clinical human cases reported by the end of 2002. Subsequently, WNV spread throughout the Western hemisphere. It is generally recognized that the US outbreak is unique and that, in other areas of endemicity, outbreaks are usually geographically and temporally restricted and do not bear comparison to the US situation. The bases for the explosive and sustained nature of the US epidemic remain unclear; likely multiple factors are at play including climate; migratory bird patterns; and hybrid (human and bird) feeding patterns of the primary mosquito vector, *Culex pipiens*, in the United States [3]. Although it was recognized that WNV infection was almost always acute, the size and rapid expansion of the epidemic in the United States led to concerns that transfusion transmission was possible. In 2002, Biggerstaff and Petersen [4] estimated that the mean potential risk of transfusion transmission in Queens, NY, at that time was 0.18 to 0.27 per 1000 component units. The model was applied again during the 2002 outbreak with 6 high-incidence metropolitan areas estimated with a risk of 0.15 to 1.23 infections per 1000 units [5]. The initial publication was immediately followed by a report of the first 23 cases of transfusion-transmitted WNV infection and disease [6]. To date, 2002 was the year with the highest overall number of human WNV infections nationally, closely followed by 2003 and subsequently 2012 (as estimated by WNV neuroinvasive disease [WNND] cases reported to the Centers for Disease Control and Prevention) (Table 1).

As a result of these findings, blood organizations, the diagnostics industry, regulators, and public health agencies worked together to develop and implement nucleic acid tests (NATs) for WNV-RNA in donated blood. Methods became available and were broadly implemented under Investigational New Drug protocols by the summer of 2003. The testing program, relying on a combination of testing minipools (MP-NAT) and individual donations (ID-NAT), has been remarkably successful and has been credited with the identification and interdiction of 4355 WNV-positive donations through the end of 2014, many of which are considered to be infectious (Table 1).

Early experience revealed that there continued to be a small number of breakthrough WNV infections among blood recipients and that they were attributable to low-level donor viremia that escaped detection by MP-NAT (involving 6 or 16 samples) during outbreak periods. Accordingly, criteria were developed to convert from MP-NAT to periods of ID-NAT referred to as triggering; resumption of MP-NAT occurs at the conclusion of outbreak activity, as determined by a variety of measures discussed in a recent AABB Association Bulletin [7]. These approaches were validated and modified as needed; their proper use has been shown to have essentially eliminated transfusion-transmitted WNV risk nationally.

The emergence of WNV has offered an object lesson in the management of a major emerging infection event in the United States. Analysis of data from blood donor testing has provided information about the distribution of infections, the significance and occurrence of asymptomatic infection, and the limits of infectivity by the intravenous route. It has also supported public health investigations and has demonstrated the value of having available platforms for high-throughput NAT. This review uses the ARC system as an example of success through validation and ongoing hemovigilance.

Emergence of WNV in the United States and development of a national donor testing program

Initially, procedures were established to defer donors and/or recall their donations in the event that they reported symptoms suggestive of WNV. (It was subsequently shown that such a policy had little to no value [8].)

Two manufacturers developed WNV-RNA screening tests, designed to run on existing automated NAT platforms. Gen-Probe (now Hologic) adapted their Procleix transcription-mediated amplification (TMA) method to detect WNV-RNA, Procleix WNV Assay, using the automated TIGRIS platform, marketed by Novartis (now Grifols), whereas Roche developed the cobas Taq-Screen real-time RT-PCR, running on the cobas s201 system. One of these candidate tests was used in a study of plasma samples from donations implicated in the 23 transfusion-transmission cases noted above [9]. In addition, routine surplus donation samples were collected from 6 ARC blood centers during the period September 3 to 28, 2002 [10]. A total of 48 620 samples were selected for evaluation using ID-TMA. Overall, 46 RNA-positive samples were identified, for a frequency of 0.95 per 1000 (similar to the estimated rate described by Biggerstaff and Petersen) [5]. These early data demonstrated that only a minority of RNA-positive samples could be detected by MP-NAT (16/46, 35%; Table 2) with the remainder requiring ID-NAT for detection. A caveat was that all 30 of the ID-NAT-only detectable donations were antibody positive (immunoglobulin M [IgM] and/or immunoglobulin G [IgG]). At that time however, routine programs had already been initiated based on MP-NAT [11].

Routine testing was initiated nearly nationwide in June to July 2003 before the start of the WNV season. Table 1 shows the number of WNV-RNA–positive donations reported each year, 2003 to 2014, along with the number of reported WNND cases; it should be noted that there is a close relationship between these 2 numbers each year. Also shown are 13 identified "breakthrough" infections attributed to transmission by blood transfusion after the implementation of WNV-NAT screening [12-19]. Only 1 of these 13 cases (in 2010) was caused by a component (granulocyte concentrate) from the ARC, which was transfused before the test result was available [18]. Of note, of the 36 total transfusion-transmitted WNV cases, only 2 were WNV IgM positive [9,19].

Materials, Methods, and Results

The Early ARC Testing Program and Its Contributions

The ARC collects approximately 40% of all blood for transfusion in the United States in a coordinated, centrally managed system that includes 35 blood regions in 44 States, Puerto Rico, and Washington, DC. Figure 1A shows the overall pattern of detection of 1576 WNV-RNA confirmed-positive donations that occurred mainly in the June-October period. As noted above, 2003 and 2012 had the greatest number of detected positives, paralleling the number of nationally reported clinical cases. Figure 1B shows the geographic distribution of donors of the 1576 RNA-positive donations by residential zip

Table 1
Yearly statistics for WNV in the US: example of a rapidly emerging agent and a successful intervention, 2002–2014

Year (No.) Reported	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
WNND (18725)	2946	2866	1148	1309	1495	1227	689	386	629	486	2873	1267	1262[+]
WNV-RNA confirmed-positive donations (4355)[*]	N/A	714	224	417	437	481	218	161	182	139	752	307	303
Transfusion cases[^] (36)	23	6	1	0	2	0	2	0	1[***]	0	1	0	0

WNND, West Nile virus neuroinvasive disease.
N/A, not available; prospective testing not introduced until 2003.
[*] 2003–2005 reported from CDC ArboNet [12]; 2006–2014 reported from the AABB WNV site http://www.aabb.org/research/hemovigilance/Pages/wnv.aspx) [13].
[^] All transfusion-transmission cases were identified from May to October [14–19].
[***] 1 WNV NAT-untested granulocyte [18].
[+] Data available through Dec 16 2014; www.cdc.gov/westnile/StatsMaps/.

Table 2
Number of WNV-NAT confirmed-positive blood donations by year and category, ARC, 2002–2012

ID-NAT*	MP-NAT*	IgM and/or IgG	2002**	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total***
+	−	−	0	10	4	8	13	9	5	2	9	7	26	93
+	+	−	16	283	62	90	98	62	45	23	15	27	112	817
+	+	+	0	36	9	19	34	19	4	6	7	3	17	154
+	−	+	30	107	24	33	60	38	31	17	34	26	119	489
Time period from first to last positive			Sep 3–Sep 28	Jun 29–Dec 1	Jun 16–Oct 16	Jun 30–Oct 25	May 23–Oct 23	Jun 6–Nov 17	Jun 26–Nov 24	Jul 15–Oct 21	Apr 14^–Nov 2	Jul 7–Oct 11	Jun 5–Nov 8	May 23–Dec 1
Total tested			48620	2935249	2386630	2345449	2817815	3196527	3000498	1843114	3674626	1749696	2844199	26793803
Total confirmed positive			46	436	102	150	209	129	90	49	67	65	279	1576+
Rate (per 10000)			9.46	1.49	0.43	0.64	0.74	0.40	0.30	0.27	0.18	0.37	0.98	0.59

* ID-NAT, individual-donation testing required for detection; MP-NAT, detectable by mini-pool NAT.
** Retrospective testing on frozen samples from six high incidence regions [11].
*** Totals exclude data from 2002.
^ April 14 case was a laboratory-related exposure.
+ A total of 23 of the 1576 confirmed-positive donors were not antibody tested due to lack of index sample.

code (note that the ARC does not collect blood in New Mexico, Colorado, Wyoming, or the Dakotas): the distribution clearly parallels national disease reporting.

The overall performance characteristics of the ARC testing program are summarized in Table 3. Nucleic acid test–reactive minipools (MPs) are resolved by ID-NAT of each pooled donation sample. If no individual sample is reactive, the initial pool result is considered false positive. West Nile virus–reactive individual samples are further tested. A WNV-confirmed-positive donor is defined as having repeat NAT reactivity using the same or alternate NAT assay or reactivity in an IgM antibody assay; in both cases, reactivity must occur using an independent sample from the index donation or from a follow-up sample [11,20].

Together, the use of repeat NAT and IgM at index was shown to confirm 99% of all WNV-confirmed-positive donors. According to data generated from 2003 to 2005 in a multicenter US blood donor study, 1559 WNV-reactive donors were followed, of which 1019 confirmed positive [21]. Of these, 1009 (99%) confirmed by index sample results; only 10 (1%) of the 1019 required follow-up sampling for confirmation. Figure 2 shows the number of WNV-reactive donations that confirmed and those that did not in the ARC, by year, 2002–2012.

Table 3 includes information about presumed viremic donations (PVDs); their relevance is described later. The ARC defines a PVD as an initially reactive donation that repeats as reactive on the original sample from the donation or one that has a signal-to-cutoff ratio greater than or equal to 17 (the latter is applied to the Procleix WNV Assay; samples having an signal-to-cutoff ratio <17 must be repeated to determine if they are a PVD). Note that the specificity and thus the positive predictive value (PPV) of differing test methods and interpretations vary, with ID-NAT being less specific than MP-NAT. A PVD as defined has a PPV of 95% with nearly 100% specificity (99.9997%).

During the first year of routine testing by the ARC (2003), 436 RNA-confirmed-positive donations were identified among 2.94 million donations tested during the WNV season or 1.49 per 10000 of which 117 (27%) required ID-NAT for detection (Table 2). Even in 2003, it became apparent that such low-titer, RNA-positive donations were capable of transmitting WNV infection by transfusion. A total of 6 cases were confirmed for 2003, none attributable the ARC, which elected to initiate ID-NAT in any blood region after the identification of 4 RNA-positive donations and a detection frequency of 1 or more positive donations per 1000, the first use of a WNV trigger [11]. Individual donation NAT continued until there was a period of 7 consecutive days without a positive in that blood region. In an effort to validate this approach, donations (30501) collected within the states with the highest incidence of NAT-positive donations, Nebraska and Kansas during parts of August, September, and October, were subjected to ID-NAT; 181 confirmed positive. Of these, 96 (53%) were nonreactive at a 1:16 dilution when the corresponding MP was tested (ie, MP-NAT), 92% of which were IgM positive and potentially offering a reduced risk of transmission. In that same study, retrospective ID-NAT was performed on frozen samples from 18037 donations collected in Nebraska in July and August. During that time, 80 donations were RNA positive by MP-NAT; ID-NAT, using existing ID-NAT triggering criteria, identified 21 additional RNA-positive donations (20%) [22]. During 2004, 102 RNA-positive donations or 0.43 per 10000 were identified during a shorter WNV season (June–October); however again, approximately 30% of the positive donations identified prospectively required ID-NAT for detection [11]. A single breakthrough transfusion-transmission case was reported in 2004, again from outside the ARC. The 2003-2004 ARC prospective testing experience indicated that existing triggering criteria lacked adequate sensitivity; retrospective data had shown that yield could be increased by greater than 50% by ID-NAT. Table 2 and Figure 3 show the increasing proportion of RNA-positive donations identified by ID-NAT as a result of the increasing sensitivity of triggering criteria.

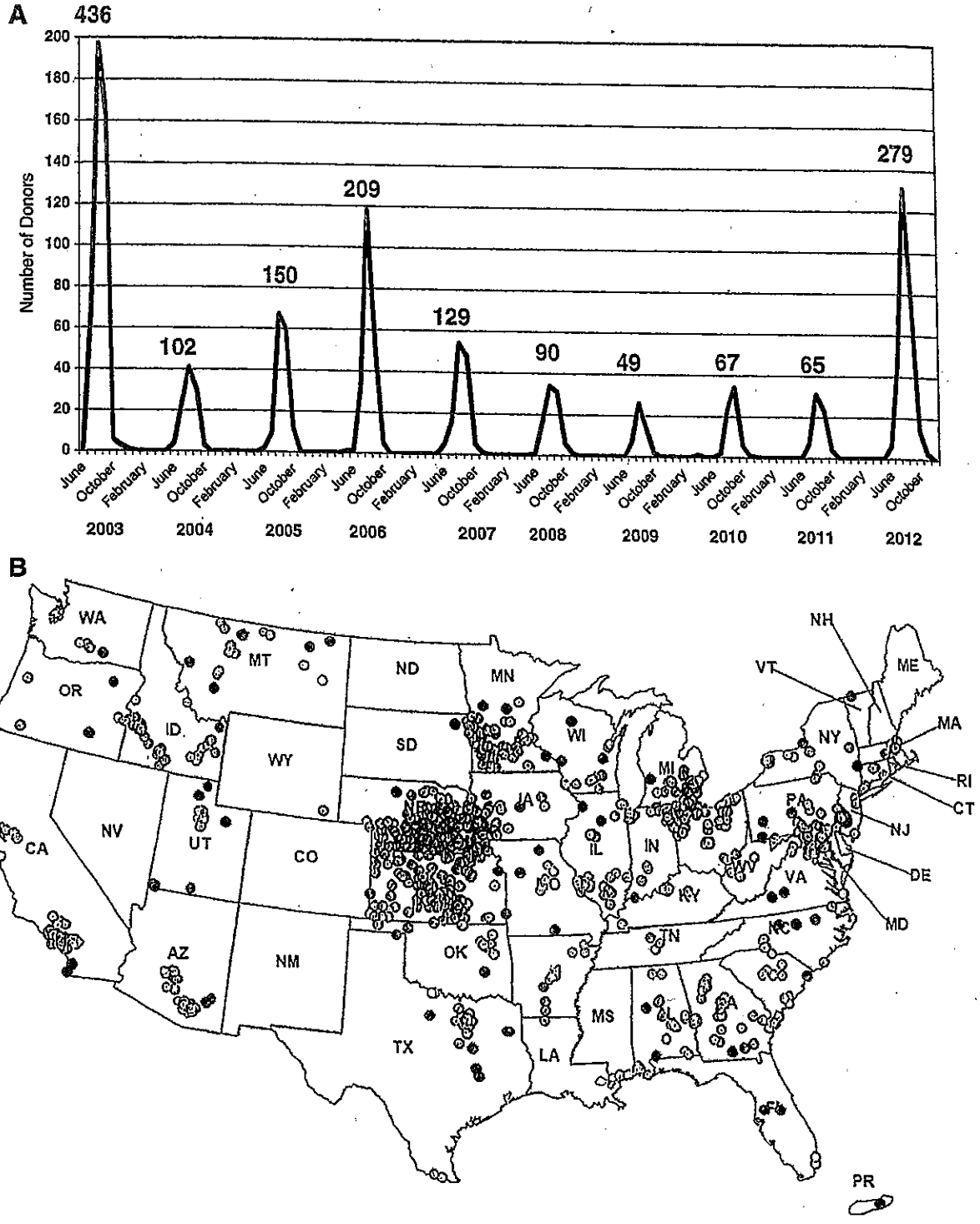


Fig 1. 1576 donors with WNV-RNA confirmed-positive donations by year (A) and residential zip code (B), ARC, 2003 to 2012.

Continuing Efforts to Enhance the Sensitivity of WNV Testing

The continued (albeit rare) occurrence of WNV-breakthrough transfusion-transmitted infections emphasized the need to establish effective ID-NAT implementation criteria (triggers) and then when to revert back to MP-NAT. However, the need for sensitivity must be balanced against available resources. Key parameters for the development and use of such criteria include the geographic areas where WNV is being transmitted, the rate of transmission, and the absolute yield of ID-NAT. During the earlier years of the US outbreak, broad surveillance was undertaken, and WNV activity in mosquitoes, birds, animals, sentinel animals, and humans was collected and reported (eg, http://diseasemaps.usgs.gov/wnv_us_human.html). Ultimately, the most useful and consistent WNV surveillance measures have been based upon WNV-RNA detection among donors. Policies were developed around the finding of PVDs. Most blood centers collect 1000 to 4000 units per week from a restricted area, and detection of PVDs may be infrequent. Thus, information about WNV activity in surrounding and

Table 3
Performance characteristics of WNV-NAT based on the number of reactive donations, ARC, 2003–2012

	# Tested	# TMA-reactive	# Confirmed positive	# False positive	% Specificity	% PPV
MP-NAT	23 910 576	1256	992	264	99.999	78.98
ID-NAT	2 883 227	1362	584	778	99.973	42.88
All NAT	26 793 803	2618	1576	1042	99.996	60.20
PVDs	26 793 803	1542	*1469	73	100.000**	95.27

* There were 107 confirmed-positive donations that were not classified as PVDs, of those, 92(86%) were ID-NAT-only reactive; thus, the overall sensitivity of the PVD designation is 93.2%. PVDs had the highest positive predictive value (PPV).
** Actual value 99.9997%.

overlapping collection areas was shown to be necessary to guide decisions. To this end, AABB established a real-time geographic reporting tool for PVDs (Table 1; http://www.aabb.org/research/hemovigilance/Pages/wnv.aspx). In addition, an email network was established to assure functional contact among blood centers, so that ID-NAT could be triggered based on activity in overlapping and neighboring areas.

Validation Studies for Triggering

Triggering policies, including multiple-site activity based upon the AABB program continued, although after 2008, most establishments moved towards ID-NAT after detecting one or two PVDs within a week, continuing until one or two weeks had elapsed without any further PVDs [23]. In 2007, the ARC performed a validation study to assess different approaches to triggering in order to select the most effective.

During the 2007 WNV season, the sensitivity of the then recommended trigger criteria (2 PVDs and 1:1000 rate) was assessed by the ARC against a trigger of one PVD in endemic locations (ie, regions known to have recurring WNV outbreaks or in areas where outbreaks would be predicted for 2007). The yield could then be evaluated against the 2007 recommended trigger to determine if ID-NAT-reactive donors would have been detected by the recommended trigger. Six blood collection regions were selected for prospective study. Entire collection regions were used to define the geographic area; these ranged from several hundred to 1000 or more donations per day. Each of the six regions converted to ID-NAT in response to a trigger of one PVD; a total of 136388 donations were tested of which 73 were confirmed positive; 42 (58%) required ID-NAT for detection, and of those, 30 were fully characterized by antibody testing including five that were antibody-negative (both IgM and IgG), 13 IgM-positive/IgG-negative and 2 IgM-negative/IgG-positive (AABB Association Bulletin 08–03) [23].

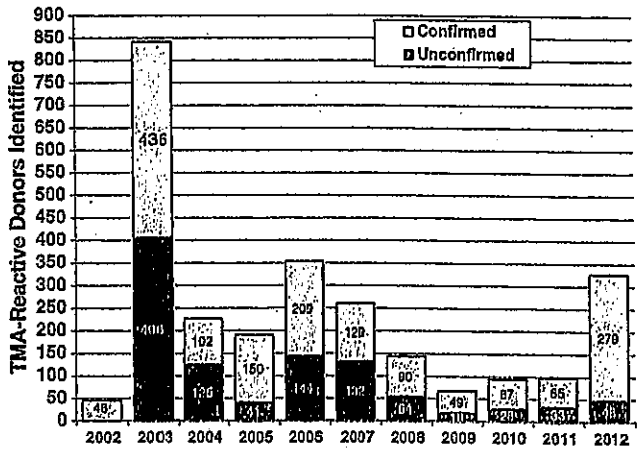


Fig 2. Number of donors whose donations tested WNV TMA-reactive by confirmation status, ARC, 2002 to 2012 (includes retrospective testing for CY 2002).

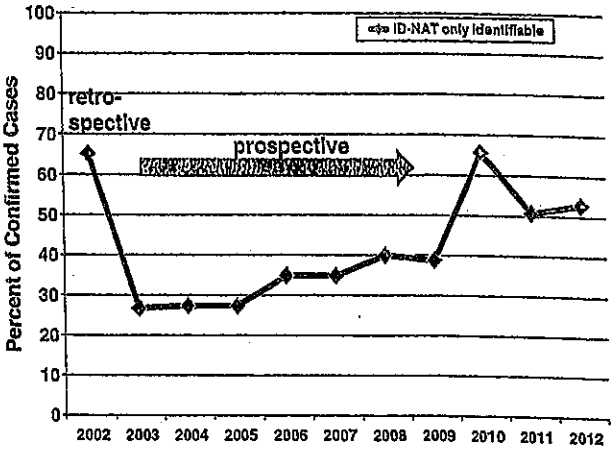


Fig 3. Percent WNV confirmed-positive donations requiring ID-NAT for detection by year, ARC, 2002 to 2012 (includes retrospective testing for CY 2002).

A criterion including the use of a 1:1000 frequency coupled with the detection of two PVDs missed 25 of 30 ID-NAT identified donations including 3 of the 5 antibody-negative donations. Using two PVDs without a rate requirement detected 20 of the 30 WNV-RNA-positive donations including all antibody-negative donations. The data demonstrated that the previously developed trigger missed RNA-positive donations and that a trigger based only on detection of PVDs without a rate function was demonstrably more sensitive. This particular study did not explore the optimal conditions for returning to MP-NAT, but recommended continuation until at least seven days without detection of a PVD and for a longer period if WNV activity (eg, as assessed by clinical cases, mosquito or bird activity) continued in the blood region.

In 2008, a second evaluation was performed, primarily to define the sensitivity of criteria not only for triggering, but also for "detriggering", or returning to the use of MP-NAT [24]; six ARC blood collection regions with a high incidence of WNV were evaluated. Overall, 209353 donations were tested. At each site, ID-NAT was triggered when a single PVD was detected and continued until no WNV activity among humans, animals or mosquitoes within the region was reported. Additionally, nine regions with historically low or absent levels of WNV activity triggered after two PVDs in a week. Among the regions that triggered on one PVD, 68 RNA-confirmed-positive donations were found, of which 36 (53%) were detectable only by ID-NAT (similar to the 2002 retrospective experience and 2007 validation results; Table 2, Fig 3). Of the 36, 26 (72%) would have been missed by a 7-day detrigger and 33 (92%) would have been missed by a two-PVD trigger and 7-day detrigger. Interestingly, a study based upon mathematical simulation came to similar conclusions, which were that rate-based triggering was inconsistent, that triggering on the basis of a single PVD was more sensitive and that prolonging the period of ID-NAT improved sensitivity [25].

Table 1 demonstrates that the implementation of triggering criteria and communication strategies appear to have been quite successful, although there have been two breakthrough cases since 2010. However, one represented the necessary transfusion of a granulocyte component prior to the availability of the (positive) test result in a region that had triggered WNV ID-NAT [18]. The use of validated triggering/detriggering procedures has been recommended by the FDA, although with no specific approach (http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM189464.pdf). Consequently, the AABB has actively provided information and data-based recommendations on this topic, most recently in 2013 [7]. Again, emphasis was placed upon effective communication mechanisms between blood centers to assure awareness of WNV activity in overlapping/neighboring regions. Figure 3 illustrates the outcome of actions related to ARC studies, showing the increasing proportion of

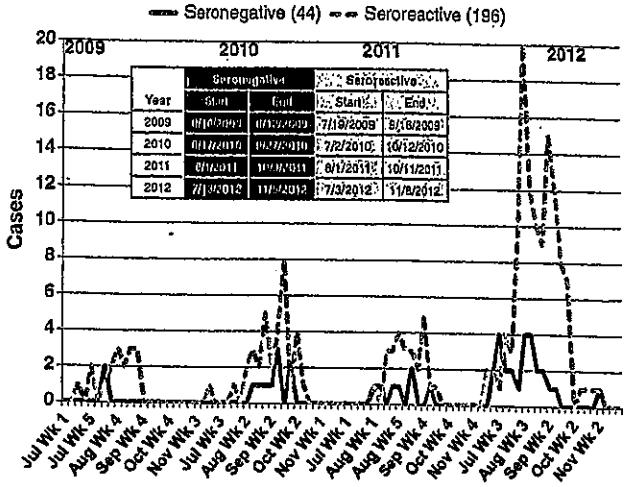


Fig 4. Start and end dates of identification of WNV-RNA confirmed-positive donations that required ID-NAT for detection (MP-NAT negative).

samples that were detected by ID-NAT as triggering and detriggering algorithms were strengthened. Figure 4 provides detail for the 2009–2012 WNV seasons including characterization of the 240 RNA-positives detectable by only ID-NAT following criteria changes (analogous to AABB Association Bulletin 13–02) [7]. Specifically for the 4-year period, 18% of the 240 yield donations were seronegative and hence likely infectious with ID-NAT required for detection of the 240 RNA-positives from July 3 to November 8. Note the longer time period required for detection of all WNV-RNA confirmed positives for the 10-year period, 2003 to 2012, was May 23 to December 1 (Table 2). The periods required for ID-NAT are likely even longer than we have documented.

Distribution of WNV markers among donors, American Red Cross, 2003 to 2012

Table 2 provides a breakdown of all test results by year, showing the reactivity by ID- or MP-NAT and the presence or absence of WNV IgM and/or IgG antibodies in the index samples. During the period 2003 to 2012, almost 27 million donations were tested during the WNV seasons: 1576 donations were RNA-confirmed positive, a rate of 0.59 per 10 000 screened donations (range, 0.18-1.49). The overall performance characteristics of the testing systems for the entire period of routine testing are summarized above in Table 3. As previously stated, the specificity and PPV of ID-NAT are poorer than those for MP-NAT, but are nevertheless comparable to other routine NAT procedures and markedly superior to those for serologic tests.

Investigation of donors with reactive test results

The ARC has routinely recalled blood donors with WNV NAT-reactive results to obtain samples for additional testing (including WNV confirmatory status, if this could not be determined from the index donation). In addition, donors complete questionnaires about risk factors and symptoms. These studies have permitted careful characterization of the early stages of WNV infection as identified by routine NAT and have contributed significantly to the development and modification of donor management policies [8,20,26].

In 2002, when it became apparent that WNV could be transmitted via blood transfusion, mitigation strategies were implemented. FDA Guidance recommended that attempts be made to avoid transfusion of frozen blood products collected in areas of high incidence and that prospective donors be deferred if they had experienced fever and headache in the seven days preceding their presentation for donation. This policy was based upon the assumption that fever and headache were associated with the early stages of WNV infection and would indicate an increased risk for viremia. However, Orton et al [8] investigated the frequency of symptoms among 389 WNV-RNA-positive and 387 RNA false-positive donors in 2003 and 2004. Overall, symptoms were reported by 61% of the RNA-positive donors and by 20% of the false-positives, who served as controls, suggesting a net frequency of 41%. Most importantly, though, only 9% of RNA-positive donors and 5% of controls reported headache and fever in the seven days prior to donation; the difference was not statistically significant. As a result, this deferral requirement was eventually eliminated.

A more extensive study was performed later by Zou et al [20] characterizing the development of symptoms among a subset of 576 WNV-RNA-positive donors who were nonreactive for IgM antibodies, thus being in the earliest stages of infection. They were compared with 418 control donors who had false-positive reactivity for WNV-RNA. Subjects were considered to be symptomatic for WNV on the basis of the occurrence of at least three of eight "indicator" symptoms; 29% of the subjects and 3% of controls met this definition for a net frequency of 26%. The net frequencies for the most common single symptoms were new rash, 26%; headache, 24%; and generalized weakness, 24%. Fever was reported with a net frequency of 15%, as were severe muscle pain and joint pain. A similar study by Custer et al [27] found a net frequency of only 13% of donors with three or more symptoms, perhaps due to differences between confirmatory definitions in the two studies. In particular, the Custer study did not specifically study IgM-negative donors, and reported on symptoms occurring over a longer time period, including the time prior to donation. Nevertheless, the distribution of symptoms was very similar in the two studies.

Dynamics and immunology of WNV infection among donors

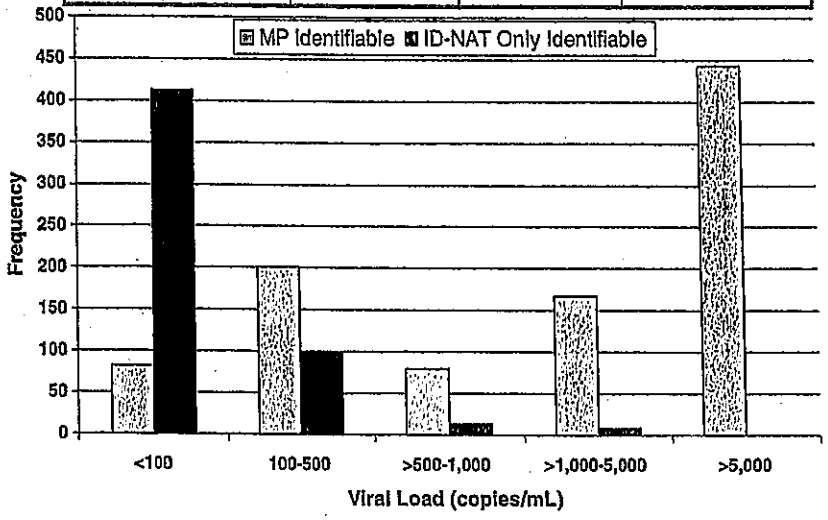
WNV loads were determined for each RNA-positive donation from 2003 to 2012 (National Genetics Institute, Los Angeles, CA). Overall, among the 1576 confirmed-positive donors, 1508 had samples for which quantitative RNA results were available; viral loads ranged from 5 to a maximum of 720 000 copies per milliliter. Among these samples, 973 could be detected by MP-NAT, with mean and median titers of 24 810 and 3500 copies per milliliter, whereas 535 were identifiable only by ID-NAT with mean and median levels of 88 and 5 copies per milliliter (Fig 5A). Figure 5B provides the viral load distributions for 1477 of the 1576 confirmed-positive donations that had a quantitative load reported (ie, ≥100 copies per milliliter), again indicating the higher viral load observed for those donations that are MP-NAT-detectable (and antibody-negative).

Routine ARC testing during the first two WNV seasons including approximately 5.3 million donations with 538 donations found to be positive for WNV-RNA. Of these, 359 (67%) were non-reactive for IgM antibody. Of the 436 RNA-positive donors identified in 2003, 350 participated in follow-up studies and 335 (96%) were IgM-positive at index or seroconverted during follow-up. Follow-up of 186 donors indicated that 169 (89%) retained IgM reactivity for 100 days or longer. In the cohort representing collections from 2002, 59% had IgM reactivity for more than 398 days [11]. The specificity of the commercial IgG antibody testing (Focus Diagnostics, Cypress CA) was uncertain, but there were no confirmed RNA-positive samples that were IgG positive in the absence of IgM (Abbott Laboratories, Abbott Park IL).

A subset of 186 WNV RNA-positive donors identified in 2003 were further characterized [26]. In 76 of the 186 cases, RNA was detected at follow-up between two and 39 days post-donation. On the basis of an estimated doubling time of 15.8 hours from three closely followed donors early in infection (see below), the dynamics of infection were estimated from a "time zero" when the RNA load was estimated at 1 copy per milliliter. Mean times from this point to the first detection of RNA by ID- and MP-NAT were estimated at 2.2 and 4.8 days, the mean time of RNA detectability by MP-NAT to index donation was back-calculated at 7.9 days and the onset of IgM and IgG was 15.7 and

A

	Donors with PCR results at index	Mean viral load (copies/mL)	Median viral load (copies/mL)
MP-NAT Identifiable	973	24,810	3500
ID-NAT Only Identifiable	535	88	5



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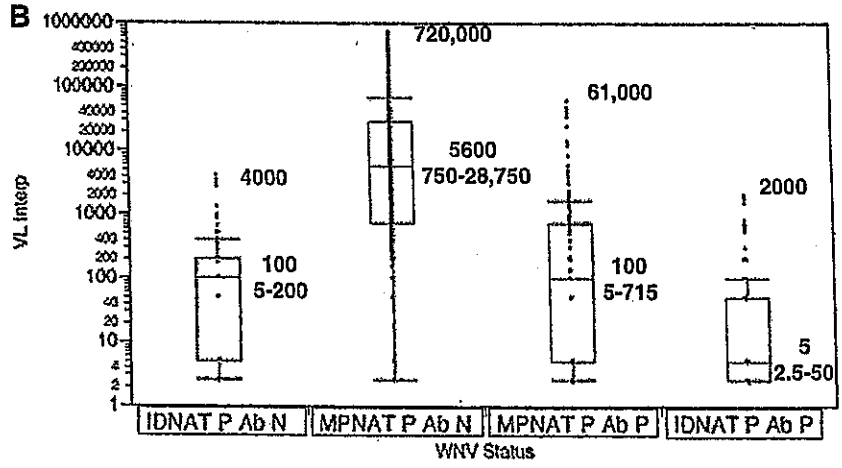

Fig 5. Index viral load distributions, ARC, 2003 to 2012; 1576 WNV confirmed-positives. WNV loads by category (ID vs MP-NAT): 1508 with available samples (A), and WNV loads by category (ID vs MP-NAT): 1477 of 1508 with viral loads greater than 100 copies per milliliter (B).

15.0 days, respectively with minimum values of 6.5 and 10.5 days. The mean duration of WNV-RNA was estimated at 20.5 days (maximum of 56.4 days).

Another study by Busch and colleagues determined the incidence of WNV from the peak prevalence of IgM antibodies among blood donors in North Dakota. With this information and the frequency of WNV-RNA-positive donations, they estimated a mean 6.9-day period during which RNA was detectable by MP-NAT and prior to the development of antibody [28]. More recently, we have examined accumulated data from 635 WNV RNA-positive donors identified during 2006 to 2012 using ID-NAT for primary testing. Antibody results were available from all to better define viral and antibody dynamics. As a part of this study, we also examined which of the 635 donations would also have been detected at a 1:16 dilution (MP-NAT by using the existing MP tubes). In this analysis, we assumed that the proportion of samples with any given set of results was directly proportional to the period during which that pattern was present. This is a simple extension of the window-period – risk concept.

Table 4 presents the number of donations in each category by sequence of RNA and antibody appearance. Of the 635 RNA-positives, 396 or 62% were detectable only by ID-NAT. Of these, 71, or 11% of the total were window-period donations and 86, or 13% had IgM antibodies only and thus might have been infectious. Using the published 6.9-day estimate of the duration of MP-NAT detection (MPs of 16), other time-

Table 4
Marker patterns for 635 WNV-RNA confirmed-positive donations detected during periods of ID-NAT, ARC, 2006 to 2012*

Marker patterns at index	No. of samples	Estimated duration (days)
ID-NAT: RNA only	71	2.3
MP-NAT: RNA only	213	**6.9
MP-NAT: RNA + IgM	12	0.4
MP-NAT: RNA + IgM + IgG	5	0.2
MP-NAT: RNA + IgG	9	0.3
ID-NAT: RNA + IgM	86	3.1
ID-NAT: RNA + IgM + IgG	226	7.3
ID-NAT: RNA + IgG	13	0.4

* 888 total WNV confirmed-positive donors were identified of which 868 had samples available for further index donation testing; 635 (73%) of the 868 were identified during periods of ID-NAT. Of the 868 total, 71 (8.2%) were ID-NAT–positive/antibody-negative, 382 (44%) were MP-NAT–positive/antibody-negative, 90 (10.4%) were MP-NAT–positive/antibody-positive, and 325 (37.4%) were ID-NAT–positive/antibody-positive. Those indicated as ID-NAT–positive, were negative when tested by MP-NAT.

** Busch et al [28].

period estimates were calculated by direct proportion, based upon the number of observations. The appearance of antibodies at 9.2 days after the first detection of RNA was essentially coincident with the end of the period of detection by MP-NAT, at 10.1 days. The early window-period, which could only be detected by ID-NAT, was estimated at 2.3 days (nearly identical to our earlier observation of 2.2 days) [26]. Assuming that this period represents a 16-fold mean increase in RNA titer, suggests that the doubling period for WNV is 13.8 hours comparable to our earlier unpublished doubling time of 15.8 hours from three closely followed donors. This approach does not account for donors who did not donate because they developed symptoms but it does give an overview of the dynamics of asymptomatic infections. The data suggest that RNA is potentially detectable by ID-NAT for a total of about 21 days (nearly identical to the earlier 20.5-day estimate), and within this period for about 8 days by MP-NAT. Again, these figures are in agreement with the earlier ARC follow-up study results and those published by Busch et al, based upon repeated follow-up of RNA-positive donors [26,28].

It might be anticipated that these data, along with the recognition that to date only two of 36 WNV transfusion transmissions were associated with an RNA-positive donation that was also IgM antibody-positive [9,19], could be used to support a relatively short deferral period followed by the potential for donor reentry. However, Busch et al found that four of 75 donors had very low-level RNA, detectable only by multiple replicate testing, one of whom was reactive at 104 days; all were IgG-positive [29]. On this basis, US regulators required a minimum deferral period of 120 days for RNA-positive donors (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm074111.htm). Nevertheless, it is clear that such a long period of RNA detection is not the norm among healthy donors, as demonstrated in the other studies discussed above. More recently, however, Lanteri et al have suggested that the total period during which RNA is detectable is greater if the test is performed on whole blood, rather than plasma [30]. Among 54 followed donors, 42% had RNA persisting in whole blood for two months and some up to three months, whereas 100% of the 54 donors had cleared WNV RNA from their plasma within the first three weeks post-index. The phenomenon of WNV-red cell association had been reported previously [31].

Epidemiologic significance of WNV NAT

Relatively early in the WNV epidemic, it was recognized that WNV-NAT screening results had epidemiologic value [28,32]. Of particular importance is the fact that donor testing provides rapid and standardized information about recent infection, along with information about the area of residence of the infected donors both on a large scale and locally. The former is clearly illustrated by comparing maps of reported WNND with those reported for donors using PVDs (Table 1). It has also become clear that the frequency of WNV-RNA, confirmed-positive donations also directly correlates with the frequency of clinically reported disease. This was first shown by Busch and colleagues in a review of testing data from 2003. For each State, the number of WNV infections was imputed from the proportion of infected blood donors multiplied by the State's population. These estimates were shown to be broadly correlated with the frequency of WNND in each State [28]. More recently, we have shown a similar correlation between the number of reported WNND cases and confirmed-positive donations reported to the AABB Biovigilance program from 2006 through 2012 (Fig 6). A number of clear outliers have been investigated; it seems likely that these are due to variation in implementation of triggering policies for ID-NAT. More specifically, in Nebraska, the majority of blood was collected by the ARC, which employed a stringent and conservative triggering policy in that area, identifying 138 of the 155 confirmed positives in that State. In contrast, in Texas, the ARC identified only 17 of the 297 confirmed-positive donors.

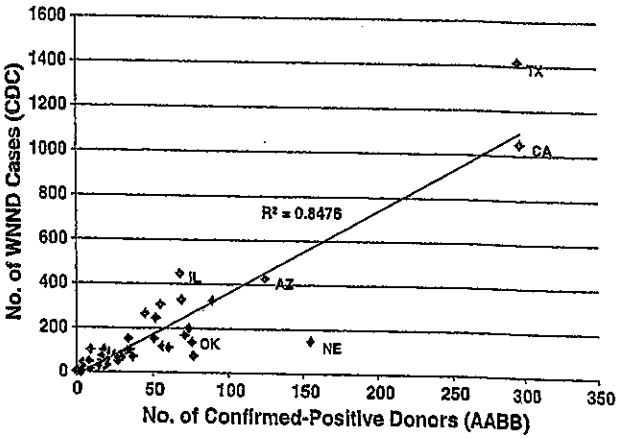

Fig 6. WNV confirmed-positive donors reported to AABB vs. neuroinvasive disease cases reported to CDC ArboNet by US State/Territory.

Discussion and Commentary

Although the potential threat of emerging infections to blood safety has been recognized at least since 1985, the WNV outbreak in the US had a number of unexpected features. As an acute, mosquito-borne infection, it contrasted with the general expectation in the last century that any major new threat to blood safety would be a chronic, parenterally/sexually transmitted agent. Also, the size and rapid geographic expansion of the epidemic were completely unexpected. These factors drove a rapid response, once transfusion-transmitted risk was recognized. WNV-RNA NAT was an appropriate intervention, as platforms were already in use and because reagents could be designed and validated rapidly, such tests were available within nine months [33]. The judgment that the then-traditional approach of testing donors for antibodies was demonstrated as not appropriate and validated by subsequent experience, as described here.

Not only was the outbreak unexpected and unpredictable but it is clear that the dynamics of the epidemic are variable in space and time. There do not seem to be simple explanations for this, other than that there are complex relationships between mosquitoes, birds, humans and environmental conditions [3]. A consequence has been a need for flexibility in the management of testing policies. A particularly important issue is the fact that MP-NAT is not sensitive enough to identify an acceptable proportion of WNV-infectious donations, as demonstrated by breakthrough infections. On the other hand, resource and logistic constraints do not permit full-time ID-NAT. Consequently, mechanisms had to be developed to determine when and where to implement ID-NAT and when to revert to routine MP-NAT. As a result, a number of studies, which are described above, were performed in order to validate effective approaches. These procedures appear to have prevented any breakthrough infections from tested blood in the ARC system.

Do these observations offer any information for the future? It might have been hoped that, after 15 years, there might be some signals about WNV in North America and indeed, in 2011, it might have been reasonable to suppose that the infection had equilibrated and that we could anticipate a few hundred clinically apparent cases each year, but 2012 dispelled that illusion, with a 5-fold increase in cases and an unexpectedly high incidence in Texas [34]. Clearly, we will continue to have to manage an unstable and unpredictable situation, at least in the foreseeable future. Could we be impacted by similar outbreaks of other infections? Certainly, the explosive outbreaks of chikungunya virus in the Indian Ocean and more recently in the Caribbean suggest caution [35], but currently this particular virus is carried only by *Aedes* spp. mosquitoes, which have very different feeding patterns from the culicine mosquitoes that carry

WNV [3,36]. Autochthonous outbreaks of dengue virus have occurred on the US mainland, most recently in Florida, but the outbreaks have been small and apparently self-limited [37]. It is generally accepted that the human-mosquito-human transmission route is relatively ineffective in the US as a result of a predominantly indoor lifestyle. But we really do not know what impact other arboviruses might have if introduced and there are suitable mosquitoes and amplifying hosts. Management of blood safety in such an outbreak should however be successful, if recognized and acted upon promptly, as was our experience with WNV.

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別紙様式第2-1

No. 8

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
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一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		ABC NewsLetter, June 12, 2015 (#22)	ブラジル

研究報告の概要

○デングウイルス輸血伝播に関する最大規模の研究の報告(REDS-III study)

輸血により伝播した(TT)デングウイルス(DENV)の調査を目的とする大規模研究により、流行地域においては多数の無症候性DENV感染者が供血を行っており、頻繁にDENV RNA陽性血液が輸血されていることが確認されている。しかしながら、輸血による感染と重篤化は稀である。これらの結果は、DENV RNAに対しての供血者のスクリーニングが輸血安全性の有意な上昇につながる可能性は低いことを示唆している。

DENV RNA陽性血液の輸血による臨床的転帰を明らかにするために、著者らはDENV RNA陽性血液の輸血を受けた受血者(症例群)のカルテとRNA陰性血液のみを輸血された受血者(対照群)のカルテを比較した。

39,134名の供血者のうち、リオデジャネイロの供血者の0.51%、レシフェの供血者の0.80%においてDENVによるウイルス血症が確認された。RNA陽性血液42製剤が35名の受血者に輸血され、このうち16製剤はDENV感染の既往がないと考えられる16名に輸血された。著者らは、これらの16名からTT-DENV感染者を6名特定した。この結果、輸血によるDENVの感染率は、対照群受血者におけるウイルス血症陽性率0.93%を大幅に上回る37.5%となった。

著者らは、「我々の研究結果は、季節的流行期間中は相当数の無症候感染者が供血を行っており、受血者にRNA陽性血液の成分が輸血されていることを確認するものである」と報告した。

データにより、輸血伝播と、輸血されたRNA陽性血液製剤におけるウイルス量、受血者の人口統計学的背景、血液成分の種類、または輸血までの保存期間との間には関連性がないことが示された。輸血によりDENVに感染した患者の臨床症状と、対照群の臨床症状との間に差異は認められず、重篤なデング熱を発症した患者はいなかった。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
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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

デングウイルス(DENV)流行期において供血者の0.5%以上がDENV RNA陽性となる状況においては、DENV RNAスクリーニングが輸血の安全性を高めることにつながる可能性は低いこと、DENV感染既往が無い患者にDENV RNA陽性血液を輸血した場合、かなりの頻度でウイルス血症が起こるものの重篤な症状は発症せずDENV RNA陰性血液を輸血された患者と臨床的に有意な違いは認められないという報告である。

今後の対応

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。問診でデング熱の既往があった場合には、治癒後1ヶ月間献血不可としている。今後も引き続き情報の収集に努める。

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2015 #22 June 12, 2015

INSIDE:

REDS-III Investigators Report Results of Largest Transfusion-Transmitted Dengue Study

The largest study to-date investigating transfusion-transmitted (TT) dengue virus (DENV) confirms that a large number of asymptomatic, dengue-infected blood donors give blood in an epidemic area and that recipients frequently receive dengue RNA-positive blood; however, infection and significant illness are unusual. They suggest screening donors for dengue RNA in these settings would not likely lead to an important boost in transfusion safety.

As many as 400 million people are infected with the mosquito-borne DENV annually, and it is a leading cause of illness and death in the tropics and subtropics. In recent years, concern over the TT-DENV has increased, but few cases have been reported and the true burden and clinical consequences of TT-DENV are controversial. Brazilian and US researchers led by Ester C. Sabino, MD, of the University of São Paulo, Brazil, conducted a linked donor-recipient study in Rio de Janeiro and Recife during explosive dengue epidemics in 2012 to characterize rates of TT-DENV from dengue RNA-positive blood donations and clinical signs and symptoms of TT-DENV.

The researchers collected samples from Brazilian blood donors and recipients from February to June 2012 during dengue outbreaks and retrospectively tested for dengue RNA by transcription-mediated amplification. The donations from participating donors were linked to recipients at participating hospitals. To define clinical outcomes from transfusion of RNA positive blood, the authors compared the charts of transfusion recipients who received DENV RNA-positive blood (case group) with recipients receiving only RNA-negative units (control group).

In 39,134 blood donors, DENV-4 viremia was confirmed in 0.51 percent of Rio de Janeiro and 0.80 percent of Recife donations. Forty-two RNA-positive units were transfused to 35 recipients. Sixteen of these RNA-positive units were transfused to 16 patients considered susceptible to dengue. The authors identified six cases of TT-DENV among these 16 patients, leading to a TT-dengue rate of 37.5 percent, much higher than the 0.93 percent rate of viremia in non-exposed recipients.

"Our findings confirm that during seasonal epidemics, substantial proportions of asymptomatic donors with infection are donating blood and recipients are receiving RNA-positive components," wrote the authors.

(continued on page 3)

REDS-III TT-DENV Study (continued from page 1)

The data showed no association between transmission and viral load in the transfused RNA-positive units, recipient demographics, component type, or duration of storage prior to transfusion. There was no difference in clinical symptoms between patients with TT-DENV and control recipients, and none developed severe DENV.

"This very large and complicated study, funded by the National Heart, Lung, and Blood Institute under the REDS-III international program and incredibly well executed by Dr. Sabino and our Brazilian team, illustrates how challenging it is to establish the rate of transfusion transmission and disease consequences to infected recipients for a virus like dengue, which is vector borne, such that very high background rates of infection are occurring in hyper-endemic regions like Brazil," said Michael Busch, MD, PhD, director of Blood Systems Research Institute, a study co-author.

He added that the "results indicate that screening donors for dengue RNA in these settings is probably not warranted." Further, he suggested that studies like this one be conducted to address the transfusion transmission risk and disease consequences of similar viruses like chikungunya and Zika viruses. These viruses are spreading widely in the Americas but no TT cases have been reported. He recommended that such studies must enroll transfused patients and test linked donor samples in settings with epidemics, such as Central and South America.

"We believe that such studies are critical to guide policies on donor screening and pathogen reduction, rather than implementing expensive interventions with minimal or no evidence regarding disease consequences for recipients," said Dr. Busch. "This is especially important in resource-limited settings with very large community outbreaks of these diseases, which need to be addressed by broader public health interventions."

The authors note that their study has limitations, including the small number of DENV RNA-positive donations transfused to recipients deemed "susceptible." Additionally, the study was conducted in a hyper-endemic setting with high rates of past exposure, meaning that the results should not be generalized to populations in non- or low-endemic areas.

Citation: Sabino EC, *et al.* Transfusion-transmission of dengue virus and associated clinical symptomatology during the 2012 epidemic in Brazil. J Infect Dis 2015 June 8. [Epub ahead of print]

We Welcome Your Articles

We at the *ABC Newsletter* welcome freelance articles on any subject relevant to the blood banking community. Writers are encouraged to submit short proposals or unsolicited manuscripts of no more than 1,100 words. While ABC cannot pay for freelance pieces, the writer's name and title will be included at the end of the story, brief news item, or commentary. If proposing a story, please write a few paragraphs describing the idea and sources of information you will use, your present job and background, and your qualifications for writing on the topic. ABC staff cannot guarantee all stories will be published, and all outside writing will be subject to editing for style, clarity, brevity, and good taste. Please submit ideas and manuscripts to ABC Publications Editor Betty Klinck at newsletter@americasblood.org. You will be sent a writer's guide that provides information on style conventions, story structure, deadlines, etc.

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No. 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	新医薬品等の区分	総合機構処理欄
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一般的名称	新鮮凍結人血漿	研究報告の公表状況	Lin HH, Lee SS, Yu ML, Chang TT, Su CW, Hu BS, Chen YS, Huang CK, Lai CH, Lin JN, Wu JC. Hepatology. 2015 Jun;61(6):1870-9. doi: 10.1002/hep.27742. Epub 2015 Apr 20.	公表国 台湾
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			

研究報告の概要

○B型肝炎ウイルスの流行地における国家予防接種プログラムに伴うD型肝炎ウイルスの疫学的変化

台湾のB型肝炎ウイルス(HBV)ワクチン接種世代の注射薬物使用者においてヒト免疫不全ウイルス(HIV)感染が発生した後の、高リスク群および低リスク群におけるD型肝炎ウイルス(HDV)感染の疫学的変化を調査することを目的とした。2001年から2012年までの期間において、HDV感染の有病率、遺伝子型およびリスク因子を決めるために、HBs抗原が陽性となった2,562例を対象に前向き多施設コホート研究を行った。

HDV感染の有病率は、HIVに感染している注射薬物使用者では74.9%、HIVに感染していない注射薬物使用者では43.9%、同性との性交渉を行っている男性HIV感染者では11.4%、HIVに感染している異性愛者では11.1%、HBs抗原陽性者全体では4.4%であった。HDV有病率については、HIVに感染している注射薬物使用者において38.5%(2001-2004)から89.8%(2009-2012)への有意な増加傾向が認められた(P=0.0002)。多変量解析では、注射薬物使用、HCV感染、HIV感染、250 IU/mL以上の血清HBs抗原値、薬物使用期間および加齢がHDV感染に関連する重要因子であった。注射薬物使用者に多く認められたHDVの遺伝子型は4型であった(72.2%)。これに対し、非注射薬物使用者では遺伝子型2型が最も多く認められた(73.3%)。1987年以降に出生し、HBs抗原が陰性となったHIV感染集団では、過半数(52.9%)が同抗原に対する抗体を有しており、抗体価は10 mIU/mL未満であった。対照群との比較において、HIV感染集団のHBs抗原の血清陽性率は有意に高かった(8.1% vs. 0.0%; P=0.02)。

結論:HBVワクチン接種世代において、注射薬物使用者およびHIV感染者がHDV感染の高リスク群およびリザーバーとして浮かび上がった。これらの高リスク群におけるHDV感染の流行再燃を防ぐための有効な対策が必要とされている。

使用上の注意記載状況・その他参考事項等

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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
HBVワクチン接種世代の注射薬物使用HIV感染者は、HDV感染の高リスク群でありリザーバーであったという報告である。	今後も引き続き、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。

HEPATOLOGY

JRC2015T-021

Changing Hepatitis D Virus Epidemiology in a Hepatitis B Virus Endemic Area With a National Vaccination Program

Hsi-Hsun Lin,[1,2] Susan Shin-Jung Lee,[3,4] Ming-Lung Yu,[5*] Ting-Tsung Chang,[6,7*] Chien-Wei Su,[1,3,8] Bor-Shen Hu,[9] Yaw-Sen Chen,[10] Chun-Kai Huang,[2] Chung-Hsu Lai,[2] Jiun-Nong Lin,[2] and Jaw-Ching Wu[1,11]

The emergence of hepatitis D virus (HDV) infection in the era of widespread HBV vaccination has not been described before. We aimed to investigate the changing epidemiology of HDV infection among high- and low-risk populations after an outbreak of human immunodeficiency virus (HIV) infection among injection drug users (IDUs) in Taiwan. A prospective, multicenter, cohort study of 2,562 hepatitis B surface antigen (HBsAg)-positive individuals was conducted to determine the prevalence, genotype, and risk factors of HDV infection from 2001 through 2012. The prevalence rates of HDV infection were 74.9%, 43.9%, 11.4%, 11.1%, and 4.4% among HIV-infected IDUs, HIV-uninfected IDUs, HIV-infected men who have sex with men, HIV-infected heterosexuals, and the general population of HBsAg-positive subjects, respectively. A significant increase in the trend of HDV prevalence from 38.5% to 89.8% was observed in HIV-infected IDUs (odds ratio = 3.06; 95% confidence interval: 1.68-5.56; $P = 0.0002$). In multivariate analysis, injection drug use, hepatitis C virus infection, HIV infection, serum HBsAg level ≧250 IU/mL, duration of drug use, and older age were significant factors associated with HDV infection. HDV genotype IV (72.2%) was the prevalent genotype circulating among IDUs, whereas genotype II was predominant in the non-IDU populations (73.3%). In the HIV cohort born after 1987 who were HBsAg negative, over half (52.9%) had antibody to hepatitis B surface antigen antibody levels of <10 mIU/mL and there was a significantly higher HBsAg seroprevalence in the HIV cohort, compared to the control group (8.1% vs. 0.0%; $P = 0.02$). *Conclusion:* In the era of HBV vaccination, IDUs and HIV-infected individuals have emerged as high-risk groups and a reservoir for HDV infection. Effective strategies are needed to curb the reemerging epidemic of HDV infection in these high-risk groups. (HEPATOLOGY 2015;61:1870-1879)

Hepatitis D virus (HDV) is a defective, single-stranded RNA virus that requires hepatitis B surface antigen (HBsAg) envelope for assembly and transmission.[1,2] Studies show that most patients with hepatitis B virus (HBV) and HDV dual infections have more severe liver disease, more rapid progression to cirrhosis, and increased frequency of hepatic decompensation and hepatocellular carcinoma

Abbreviations: Abs, antibodies; ALT, alanine transaminase; anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to hepatitis B surface antigen; AST, aspartate transaminase; CAH, chronic active hepatitis; CHB, chronic hepatitis B; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; IDUs, injection drug users; LC, liver cirrhosis; MSM, men who have sex with men; OR, odds ratio; PCR, polymerase chain reaction.

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*These authors made an equal contribution.

(HCC).[3] Many studies report a poor response rate to interferon treatment and the ineffectiveness of nucleoside/nucleotide analog treatment.[4] Accurate estimation of updated prevalence and risk factors is important to identify risk groups to screen and make an effective policy to control the spread of HDV.

Approximately 15-20 million people are infected with HDV worldwide; however, its prevalence varies in different geographic regions.[4,5] In the past three decades, several longitudinal studies show that the prevalence of HDV is decreasing in formerly highly endemic areas, such as Italy, Spain, Turkey, and Taiwan.[6,7] However, HDV infection has recently reemerged with clustered outbreaks of HDV superinfection among high-risk populations in Venezuela, Ecuador, Mongolia, Greenland, Samara (Russia), Okinawa (Japan), Central Africa, and the Amazon basin, as well as in the immigrant population from endemic areas in Europe.[4]

Taiwan is an endemic area of HBV infection. Before the implementation of a nation-wide HBV vaccination program, the prevalence rate of HBV infection was 15%-20% in the general population. The vaccination program was launched in July 1984 to include newborns of high-risk, HBsAg-positive mothers and extended to all newborns after July 1986.[8] Thereafter, the rate of superinfection with HDV in patients with chronic hepatitis B (CHB) with acute exacerbations decreased from 23.7% in 1983 to 4.2% in 1995.[7] A similar decline in HDV prevalence of injection drug users (IDUs) and prostitutes in Taiwan was observed in 2002, falling to a rate of 14% and 5%, respectively.[9] Smaller studies among IDUs with and without human immunodeficiency virus (HIV) infection reported a varying prevalence of HDV infection from 10% to 91%.[10-16] This decline may be attributed to the successful implementation of the nation-wide HBV vaccination program,[8] as well as sustained educational efforts to the general public.

Between 2003 and 2006, an outbreak of HIV and hepatitis C virus (HCV) coinfection, originating from a geographically large transmission network from China, occurred among IDUs in Taiwan.[17,18] In this outbreak, our group reported an extremely high prevalence of HCV coinfection (up to 98%) and discovered the introduction of several novel HCV genotypes into Taiwan.[17] We hypothesized that this outbreak may have also led to a major change in the prevalence and genotype of HDV infections among IDUs and HIV-infected individuals in Taiwan. The identification of risk factors causing HDV infections in different populations is crucial for public health measures to control HDV infections. The current study aims to investigate the current prevalence, genotype, and risk factors causing HDV infections in various populations in Taiwan in an era of 30 years after a national HBV vaccination program.

Patients and Methods

Study Population. A multicenter, prospective, longitudinal, cohort study of HBsAg-positive individuals was conducted from 2001 through 2012. Six referral hospitals designated for hepatitis and HIV/acquired immune deficiency syndrome care in Taiwan participated in this study, including Taipei Veterans General Hospital (Taipei, Taiwan), Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan), Kaohsiung Medical University Hospital (Kaohsiung, Taiwan), National Cheng Kung University Hospital (Tainan, Taiwan), Taipei Municipal Venereal Disease Control Institute (Taipei, Taiwan), and E-Da Hospital (Kaohsiung, Taiwan). A total of 2,562 individuals were identified to be serologically positive for HBsAg, including 2,029 HBsAg-positive subjects who were followed up at outpatient clinics (304 diagnosed with HCC and 1,725 without HCC) from the general population, 369 individuals with HIV infection (263 IDUs, 70 men who have sex with men [MSM], and 36 heterosexuals), and 164 HIV-uninfected IDUs from the methadone outpatient clinic (Fig. 1). The HIV cohort of 369 HIV-infected individuals with serum HBsAg positivity was recruited from 1,662 HIV-infected individuals taken care of at the HIV outpatient clinic. The HIV-uninfected IDU cohort consisted of 164 of 218 HBsAg-positive individuals and was recruited from 1,157 IDUs attending the methadone outpatient clinics. The seroprevalence rate of HBsAg in the community was derived from a

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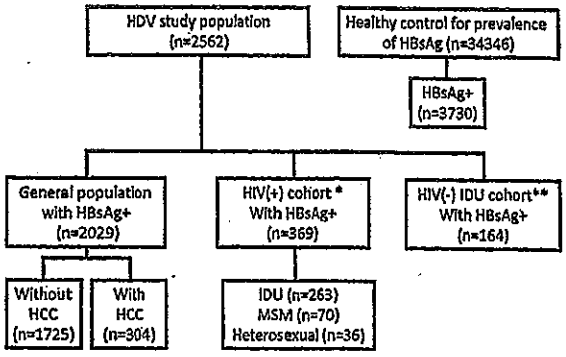

Fig. 1. Flowchart of study subjects who entered the study. *HIV(+) cohort (n = 1661); **HIV(−) IDU cohort (n = 1157); HDV: hepatitis delta virus; HBsAg: hepatitis B virus surface antigen; IDU: injection drug users; MSM: men who have sex with men.

control group of 34,346 healthy, non-IDUs and HIV-uninfected individuals undergoing routine health checkup during the study period.[19] The clinical status of the patients was defined as follows: (1) inactive carrier state: patients who are asymptomatic, have normal alanine transaminase (ALT) and aspartate transaminase (AST) levels, normal sonography, and either negative for hepatitis B e antigen (HBeAg) with an HBV-DNA load less than 2,000 IU/mL or HBeAg positive and HBV-DNA load <20,000 IU/mL; (2) chronic active hepatitis (CAH): patients who had either elevated ALT and AST levels without cirrhosis on sonography, or HBeAg positive with HBV DNA >20,000 IU/mL or HBeAg negative with HBV-DNA load >2,000 IU/mL; and (3) HBV-related cirrhosis: any marker of portal hypertension or ultrasonographic finding of small and coarse echogenicity of liver with round edges. Superinfection with HDV was defined as seroconversion of anti-HDV or low anti-HDV titer ≤100 dilution at acute exacerbation of hepatitis (ALT level ≧400 IU/L) in CHB carriers.[20]

Laboratory Test. Serum antibodies (Abs) to HCV and HIV, HBeAg, the antibody to hepatitis B core antigen (anti-HBc), HBsAg, and quantitative HBsAg levels were assessed by using the Abbott Architect system kits (Abbott Laboratories, Sligo, Ireland). Anti-HDV immunoglobulin G Ab was determined using the ANTI-HDV enzyme-linked immunosorbent assay kit (DiaSorin, Saluggia, Italy). Quantification of HBV DNA was tested using the Cobas TaqMan with a lower limit of detection of 6 IU/mL (Roche Diagnostics, Mannheim, Germany). Genotyping of HBV was performed by polymerase chain reaction (PCR) restriction fragment-length polymorphism of the surface gene of HBV.[21] Serum HDV RNA was detected using in-house real-time PCR, as previously described.[22] Sensitivity of the real-time PCR method assay to detect HDV RNA was 400 copies/mL and the linearity of quantification ranged from 2×10^3 to 2×10^9 copies/mL. HIV plasma viral load was determined using a Cobas Amplicor HIV-1 Monitor Test (version 1.5; Roche Diagnostics) or the HIV-1 RNA 3.0 Assay (bDNA; Siemens, Tarrytown, NY), according to the manufacturers' protocols. Demographic characteristics and behavioral information were collected during interviews. The study protocol was approved by the local institutional review boards.

HDV Genotype. To determine HDV genotype, viral RNA was extracted from 140 μL of plasma using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), and nested reverse-transcription PCR was performed to amplify the HDV delta-gene fragment (nt856-1275 relative to HDV reference strain JA-M27), as described elsewhere.[23] The first primer pairs used were HDV850 (5′-CGG ATG CCC AGG TCG GAC C-3′) and HDV1380 (5'-GGA GCW CCC CCG GCG AAG A-3′). The second primer pairs used were HDV-856 (5′-AGG TGG AGA TGC CAT GCC GAC-3′) and HDV-1275 (5′-GGA YCA CCG AAG AAG GAA GGC C-3′). After purification with a QIA quick PCR Purification kit (Qiagen), samples were screened with XhoI restriction fragment-length polymorphism analysis and then sequenced using an automatic sequencer (3100 Avent Genetic Analyzer, ABI; Applied Biosystems, Foster City, CA).[22] Phylogenetic analysis of a 419-base-pair fragment covering the HDV delta gene fragment was used to determine the HDV genotypes. Sequences were compiled using the BioEdit program (version 7.2.5; http://www.mbio.ncsu.edu/bioedit/bioedit.html), MEGA6 (molecular evolutionary genetics analysis, version 6.0), and CLUSTAL_X. To eliminate potential contamination, all of the sequences obtained were subjected to an HDV BLAST search to compare them with related reference sequences in the HDV database from the Gene Bank of the National Center for Biotechnology Information (Bethesda, MD). Genotypes were assigned after alignment with reference sequences. The following controls were used to construct a tree: HDV genotype I: X85253, X77627, M92448; HDV genotype II: TW2476, X60193; HDV genotype IIb-M: AF309420; HDV genotype III: AB037948; HDV genotype IV: AF209859, TWD62 (AF018077), AY452981; HDV genotype V: AM183326: HDV genotype VI: AM183329; HDV genotype VII: AM183333; and HDV genotype VIII: AX741169. The genetic distance of the HDV sequences analyzed was calculated using the two-parameter model used by Kimura. Phylogenetic

Table 1. Demographic Characteristics of the HBV Carriers in General Population and Various Risk Groups (n = 2,562)

		General Population of HBsAg (+)			HIV Negative	HIV-Positive Patients (n = 369)			
	Total	All	HCC(-)	HCC(+)	IDUs	IDUs	MSM	Heterosexual	
Characteristic	(n = 2,562)	(n = 2,029)	(n = 1,725)	(n = 304)	(n = 164)	(n = 263)	(n = 70)	(n = 36)	P Value
Age, years, mean, SD.	46.6, 13.3	48.6, 13.7	47.2, 13.5	56.5, 12.3	39.5, 8.0	37.9, 7.2	38.0, 7.8	46.2, 12.4	<0.0001
(range)	(9.0-101.0)	(9.0-101.0)	(9.0-101.0)	(28.0-89.0)	(26.0-68.0)	(24.0-64.0)	(23.0-69.0)	(26.0-74.0)	
Sex, male, no. (%)	1,994 (77.8)	1,496 (73.7)	1,247 (72.3)	248 (81.6)	145 (88.4)	251 (95.4)	70 (100.0)	33 (91.7)	<0.001
AST >38 IU/L, no. (%)	1,273 (59.2)	1,055 (63.2)	908 (63.7)	146 (59.8)	63 (38.4)	127 (52.7)	21 (38.9)	8 (33.3)	<0.001
ALT >40 IU/L, no. (%)	1,528 (60.9)	1,279 (63.2)	1,118 (65.0)	160 (52.6)	77 (47.0)	137 (56.9)	25 (44.6)	11 (45.8)	<0.001
ALT ≥ 400 IU/L, no. (%)	246 (9.8)	243 (12.0)	230 (13.4)	13 (4.3)	0 (0.0)	2 (0.8)	0 (0.0)	1 (4.2)	<0.001
Inactive carrier, no. (%)	551 (21.5)	312 (15.4)			78 (47.6)	108 (41.4)	36 (51.4)	17 (47.2)	<0.001
CAH, no. (%)	1,525 (59.5)	1,245 (61.3)			80 (48.8)	150 (57.0)	34 (48.6)	16 (44.4)	<0.001
Cirrhosis, no. (%)	324 (12.6)	313 (15.4)	168 (9.7)	145 (47.7)	3 (1.8)	5 (1.9)	0 (0.0)	3 (8.3)	<0.001
HCV seropositivity, no. (%)	451 (19.3)	73 (4.0)	58 (3.8)	15 (5.3)	132 (81.5)	234 (98.3)	8 (11.4)	4 (11.1)	<0.001
HBsAg level ≧250 IU/mL	1,576 (74.7)	1,255 (76.3)	1,058 (76.5)	196 (75.7)	106 (66.3)	168 (71.5)	34 (68.0)	14 (60.9)	0.02
HBeAg	568 (33.7)	520 (34.8)	487 (37.4)	33 (17.3)	1 (25.0)	30 (22.1)	12 (35.3)	5 (25.0)	<0.001
HBV viral load >100,000 IU/mL	1,091 (49.5)	1,041 (53.4)	924 (54.7)	117 (44.7)	3 (21.4)	25 (14.9)	9 (19.2)	13 (50.0)	<0.001
HBV genotype									
B	730 (65.1)	694 (65.0)	632 (65.6)	62 (60.2)	0 (0.0)	12 (57.1)	14 (82.4)	10 (62.5)	0.33
C	386 (34.4)	370 (34.6)	328 (34.0)	41 (39.8)	0 (0.0)	8 (38.1)	3 (17.7)	6 (37.5)	
Others*	5 (0.4)	4 (0.4)	4 (0.4)	0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)	0 (0.0)	

*Data were 3 of genotypes B and C recombination, 1 of genotype A, and 1 of genotype D.

trees were generated using the neighbor-joining method implemented in the CLUSTAL_X 1.81 program. The branch significance was analyzed by bootstrap with 1,000 replicates. The trees were printed using TreeView software (version 1.6.6). SIMPLOT and BOOTSCAN of the SIMPLOT 3.5.1 program (http://sray.med.som.jhmi.edu/SCRoftware/simplot/) were used to determine potential intergenotypic recombination.[24]

Statistical Analysis. Results were analyzed using Stata software (v10.0; StataCorp LP, College Station, TX). Categorical variables were analyzed using Pearson's chi-square test or Fisher's exact test, as appropriate. The chi-squared test for trend was used to analyze the trend of proportions; 95% confidence interval (CI) was calculated for proportions. Continuous variables were analyzed using the Student *t* test. All tests were two-tailed and a *P* value <0.05 was considered significant. Logistic regression was used to analyze the risk factors for acquiring HDV infection. All variables with $P < 0.10$ in the univariate analysis were considered for inclusion in the multivariate model. Forward selection, using the likelihood ratio test, was used to select the final multivariate model for risk factors for acquiring HDV infection.

Results

Demographic Characteristics of Study Participants. A total of 2,562 HBsAg-positive individuals were investigated in this study, and the demographic characteristics are shown in Table 1. There were 1,994 (77.8%) males and 568 (22.2%) females, with a mean age of 46.6 years (range, 9-101). Age, sex, clinical status (inactive carrier, CAH, and presence of liver cirrhosis [LC]), HCV seropositivity, HBsAg titer, HBeAg, and HBV viral load were significantly different between the study groups. Among HBsAg-positive individuals attending outpatient clinics from the general population, 312 of 2,029 (15.4%) were inactive carriers for HBV, 1,245 of 2,029 (61.3%) had CAH, 313 (15.4%) suffered from LC (of which 145 had HCC), and 159 had HCC without LC. HCV seroprevalence was highest (98.3%) among HIV-infected IDUs and lowest in the general population of HBsAg-positive subjects (4%). HCV prevalence among the general population of HBsAg-positive subjects living in northern and southern Taiwan differed significantly (9 of 818 [1.1%] vs. 64 of 1,010 [6.3%]; $P < 0.001$).

Comparison of HBV Seroprevalence Rates Between the Health Checkup Control Group, HIV, and HIV-Uninfected IDU Cohorts. The prevalence rates of HBsAg in the HIV cohort (22.2%; 369 of 1,662) and HIV-uninfected IDU cohort (18.8%; 218 of 1,157) were both significantly higher than the control group (10.9%; 3,730 of 34,346; $P < 0.001$). The seroprevalence of antibody to hepatitis B surface antigen (anti-HBs), anti-HBc, and isolated anti-HBc among the HIV cohort and HIV-uninfected IDUs was 57.9%, 82.5%, and 19.8% and 60.6%, 85%, and 20.9%, respectively. For individuals born after 1987, there was a significantly higher HBsAg seroprevalence in the HIV cohort, compared to the control group (8.1% [3 of 37] vs. 0% [0 of 97]; $P = 0.02$). In the

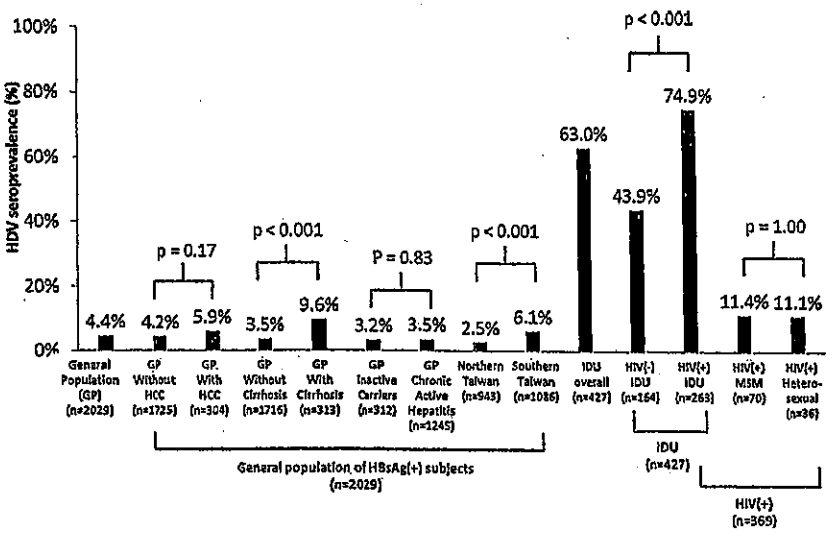

Fig. 2. Seroprevalence of HDV infection among various groups (n = 2,562).

HIV cohort born after 1987 who were HBsAg negative, over half (52.9%) had anti-HBs levels of <10 mIU/mL.

Prevalence of HDV Infection in Risk Groups and HBsAg-Positive Subjects From the General Population. The overall prevalence of HDV infection in HBsAg-positive individuals was 14.5% (371 of 2,562). However, there were distinct differences in prevalence rates among the different groups. The seroprevalence rates of HDV were 74.9%, 43.9%, 11.4%, 11.1%, and 4.4% among the HIV-infected IDUs, HIV-uninfected IDUs, HIV-infected MSM, HIV-infected heterosexuals, and HBsAg-positive subjects attending outpatient clinics from the general population, respectively (Fig. 2). The overall HDV prevalence among IDUs was 63% and was higher in HIV-infected IDUs than non-HIV-infected IDUs (74.9% vs. 43.9%; $P < 0.001$). HIV-infected IDUs had the highest risk for HDV infection (adjusted odds ratio [OR] = 76.61; 95% CI: 28.78-231.45). Among HBsAg-positive subjects attending outpatient clinics from the general population, HDV prevalence rates were 3.2%, 3.4%, 5.9%, and 9.6% among inactive carriers, CAH, HCC, and LC, respectively. A higher HDV prevalence was observed in those who had LC, compared to those without LC (9.6% vs. 3.5%; $P < 0.001$) and in those subjects living in southern Taiwan, compared to those in northern Taiwan (6.1% vs. 2.5%; $P < 0.001$).

Secular Change of HDV Prevalence in Different Subgroups. The trend of HDV prevalence in HIV-infected IDUs revealed a significant increase between 2001 and 2008 from 38.5% in the period 2001-2004 to 89.8% in 2009-2012 (OR = 3.06; 95% CI: 1.68-5.56; $P = 0.0002$, by the chi-squared test for trend; Fig. 3). No differences were observed in the other groups.

Incidence of Acute HDV Superinfection in the HBsAg-Positive Subjects From the General Population. Among HBsAg-positive subjects with acute exacerbations with an ALT level ≧400 IU/L from the general population who showed seroconversion of anti-HDV or low anti-HDV titer ≤100 dilution at acute exacerbation of hepatitis, defined as HDV superinfection, was 3.4% (8 of 237). This demonstrated a significant decrease in the incidence of HDV superinfection, compared with previous studies, reporting incidence rates of 14.6% (77 of 527; $P < 0.001$) in 1997 and 15% (9 of 60; $P = 0.002$) in 1999.[7,25] When analysis was stratified by HBeAg, the rate of HDV superinfection was 1.6% (2 of 126) for HBeAg-positive and 5.4% (6 of 111) for HBeAg-negative individuals.

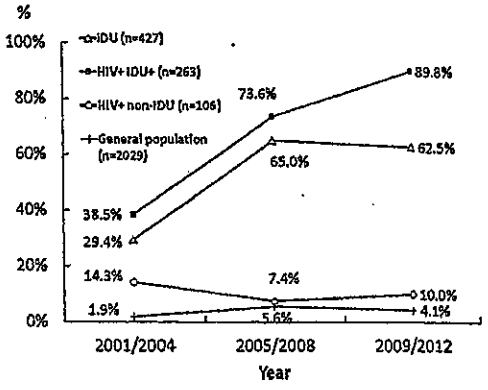

Fig. 3. Trend of HDV seroprevalence among different HBsAg-positive groups (n = 2,562) by 4-year period revealed a significant increase in HIV-infected IDUs from 38.5% in 2001-2004 to 89.8% in 2009-2012 (OR = 3.06; 95% CI: 1.68-5.56; $P = 0.0002$, by the chi-square test for trend) and no differences among the other groups.

Table 2. Multivariate Logistic Regression Analysis for the Risk Factors of HDV Infection (n = 2,562)

Factor	Crude OR	(95% CI)	P Value	Adjusted OR*	(95%CI)	P Value
Age, years						
<40	1.00			1.00		
40-49	0.54	(0.41-0.71)	<0.001	0.93	(0.64-1.37)	0.73
≥50	0.33	(0.25-0.44)	<0.001	1.62	(1.09-2.41)	0.02
Sex, male	2.89	(2.03-4.11)	<0.001			
HIV infection	16.38	(12.6-21.3)	<0.001	2.81	(1.94-4.05)	<0.001
HCV infection	25.16	(19.0-33.3)	<0.001	3.84	(2.34-6.31)	<0.001
HBsAg titer ≧250 IU/mL	1.26	(0.95-1.67)	0.11	2.39	(1.63-3.51)	<0.001
HBeAg positivity	0.49	(0.34-0.71)	<0.001			
HBV genotype						
B	1.00					
C	0.71	(0.37-1.37)	0.31			
AST >38 IU/L	0.78	(0.62-0.99)	0.04			
ALT >40 IU/L	0.86	(0.69-1.09)	0.21			
Injection drug use	33.93	(25.66-44.88)	<0.001	7.18	(4.25-12.14)	<0.001
Duration of drug use, years (n = 232)						
<5	1.00			1.00		
5-9	2.09	(1.04-4.18)	0.04	2.00	(0.92-4.39)	0.08
≧10	2.09	(1.11-3.92)	0.02	2.31	(1.11-4.80)	0.03

*The final multivariate model included age, HIV infection, HCV seropositivity, and IDU status.

Multivariate Analysis of Risk Factors Associated With HDV Infection. In multivariate logistic regression analysis adjusted for age, HIV infection, HCV seropositivity, and IDU status, major risk factors associated with HDV infection were injection drug use, HCV infection, HIV infection, serum HBsAg level ≧250 IU/mL, duration of drug use ≥10 years, and age ≥50 years (Table 2). There was a significantly increasing trend in the HDV prevalence with age in the non-IDU population (OR = 1.32; 95% CI: 1.09-1.60; $P = 0.005$, by the chi-squared test for trend; Fig. 4), but not among the IDUs (OR = 0.85; 95% CI: 0.67-1.08; $P = 0.18$). A significantly increasing trend in the cumulative HDV prevalence was observed in 232 IDUs with each year of injection drug use, in those using drugs for 15 years and less (OR = 1.14; 95% CI: 1.05-1.23; $P = 0.001$; Fig. 5).

Comparison of Patients With and Without HDV Viremia. HDV RNA was detectable in 148 of 342 samples from the anti-HDV-positive individuals (43.3%). Age, gender, transmission routes, HCV seropositive rate, and HIV viral loads were not different between patients with and without HDV viremia (Table 3). HBV viral factors, serum HBV-DNA levels, and HBeAg status were also similar between these two groups. However, HDV viremic individuals had a higher frequency of elevated liver transaminase levels and HBsAg levels of ≧250 IU/mL.

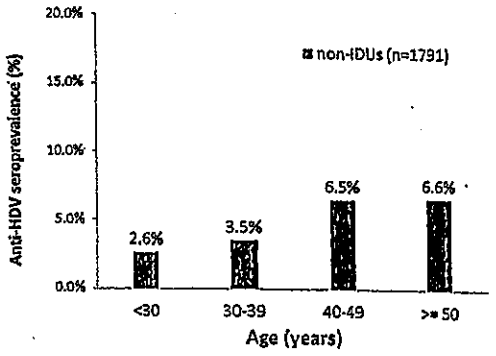

Fig. 4. Trend of HDV prevalence associated with age in the non-IDU population revealed a significant increase associated with age in the non-IDU population (n = 1,791; OR = 1.32; 95% CI: 1.09-1.60; $P = 0.005$, by the chi-squared test for trend).

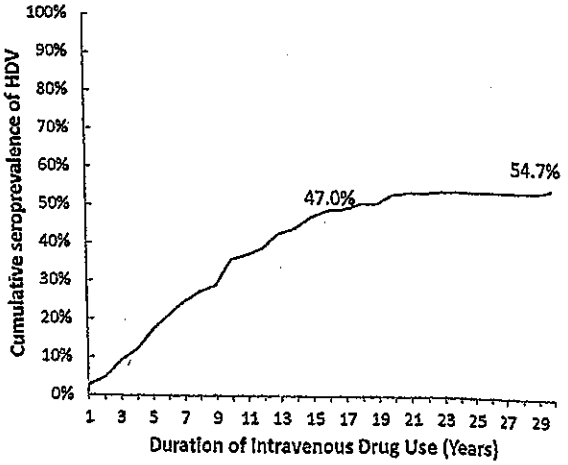

Fig. 5. Cumulative prevalence of HDV infection among the 232 IDUs revealed a significant increase associated with increasing duration of injection drug use (OR = 1.14; 95% CI: 1.05-1.23; $P = 0.001$).

Table 3. Basic Characteristics of the Patients With HDV Seropositivity, With and Without Detectable HDV RNA (n = 342)

Characteristic	N	HDV Seropositive (n = 342)	HDV-RNA Positive (n = 148)	HDV-RNA Negative (n = 194)	P Value
Age (mean, SD, range)	342	40, 10.5 (16.7-80.8)	38.3 , 9.6 (16.7-66.0)	41.4 , 10.8 (21.9-80.8)	0.20
Sex, male, n (%)	342	317 (92.7)	135 (91.2)	182 (93.8)	0.36
Risk factor for HIV infection	182	8 (4.1)	5 (6.1)	3 (2.6)	0.51
MSM		8 (4.1)	5 (6.1)	3 (2.6)	0.51
Heterosexual		4 (2.0)	2 (2.4)	2 (1.8)	
IDU		184 (93.9)	75 (91.5)	109 (95.6)	
CD4 cell count, cells/mm^3	141				
<200		5 (3.6)	2 (3.3)	3 (3.8)	0.95
200-349		34 (24.1)	14 (23.0)	20 (25.0)	
≥350		102 (72.3)	45 (73.7)	57 (71.2)	
HIV viral load, copies/mL	143				
<10,000		114 (79.7)	50 (82.0)	64 (78.1)	0.44
10,000-99,999		24 (16.8)	8 (13.1)	16 (19.5)	
≥100,000		5 (3.5)	3 (4.9)	2 (2.4)	
HCV seropositivity	305	217 (71.1)	92 (68.7)	125 (73.1)	0.40
Liver function tests	328				
AST >38 IU/L		167 (50.9)	79 (44.8)	88 (47.6)	0.18
ALT >40 IU/L		213 (64.9)	104 (72.7)	109 (58.9)	0.009
AST, IU/L, median (IQR) (range)		43 (31-68) (14-2010)	47 (33-77) (14-1660)	40 (29-65) (18-2010)	0.02
ALT, IU/L, median (IQR) (range)		53 (33-91) (11-1850)	57 (35-99) (12-1850)	47 (31-83) (11-953)	0.03
HBeAg positivity	161	25 (15.5)	10 (14.3)	15 (16.5)	0.70
HBsAg titer ≧250 IU/mL	304	234 (77.0)	118 (87.4)	116 (68.6)	<0.001
Serum HBV-DNA positive (%)	342	228 (66.7)	102 (68.9)	126 (65.0)	0.44
Median HBV DNA, IU/mL	228	441.5 (24-46,969)	619.5 (32-29,258)	357 (19-60,697)	0.54
HBV DNA ≧10^4		71 (31.1)	30 (29.4)	41 (32.5)	0.61

Distribution of HDV Genotypes in Risk Groups and HBsAg-Positive Subjects From the General Population. HDV genotypes were determined in 153 of 342 samples from HDV-positive individuals. Distribution of genotypes based on phylogenetic analysis is shown in Fig. 6 and summarized in Table 4. The main circulating HDV genotypes in our study were genotype IV (56.6%), genotype II (34.9%), and genotype I (8.6%). Genotype IV was the major prevalent HDV genotype circulating among the IDUs ($P < 0.001$), even when stratified by HIV status (Table 4). The main HDV genotype circulating in non-IDUs was genotype II. HDV genotype mix or recombination was not detected.

Discussion

Our study showed that there were distinct differences in the prevalence of HDV infection among different populations in an HBV endemic area, in the era of 30 years after a national HBV vaccination program. The prevalence of HDV infection among the HBsAg-positive subjects from the general population remained low in this study (4.4%); however, there was a significant decrease in the incidence of acute HDV superinfection in the general population.[7,25] In contrast, the burden of HDV in high-risk populations was exceptionally high. We demonstrated an extremely high prevalence of HDV infection among IDUs, particularly in those with HIV infection. A significantly increasing trend in the prevalence of HDV infection in HIV-infected IDUs was found from 2001 to 2012. This increasing trend may be explained by a higher prevalence of HBV infection in this population and the consequence of an explosive outbreak of HIV and HCV infection occurring in Taiwan just before the conduction of the study.[17,18]

IDUs who shares needles have the highest prevalence of HDV infection worldwide, with rates varying from 8% to more than 90%.[4,26] Our study revealed that injection drug use was a major risk factor for HDV infection, and that the cumulative HDV seroprevalence increased significantly with increasing years of injection drug use in those who had been users for 15 years or less. The association between the duration of injection drug use and prevalence of hepatitis B and C and HIV infection has been described.[27] However, there is scant literature on the association between HDV infection and the duration of injection drug use. Our study is the first to show a significant, positive trend in the cumulative HDV seroprevalence per year of injection drug use.

HCV infection is the second-strongest risk factor for HDV infection in our study, because it shares the same route of transmission as HDV. Likewise, many studies have reported an association between HDV

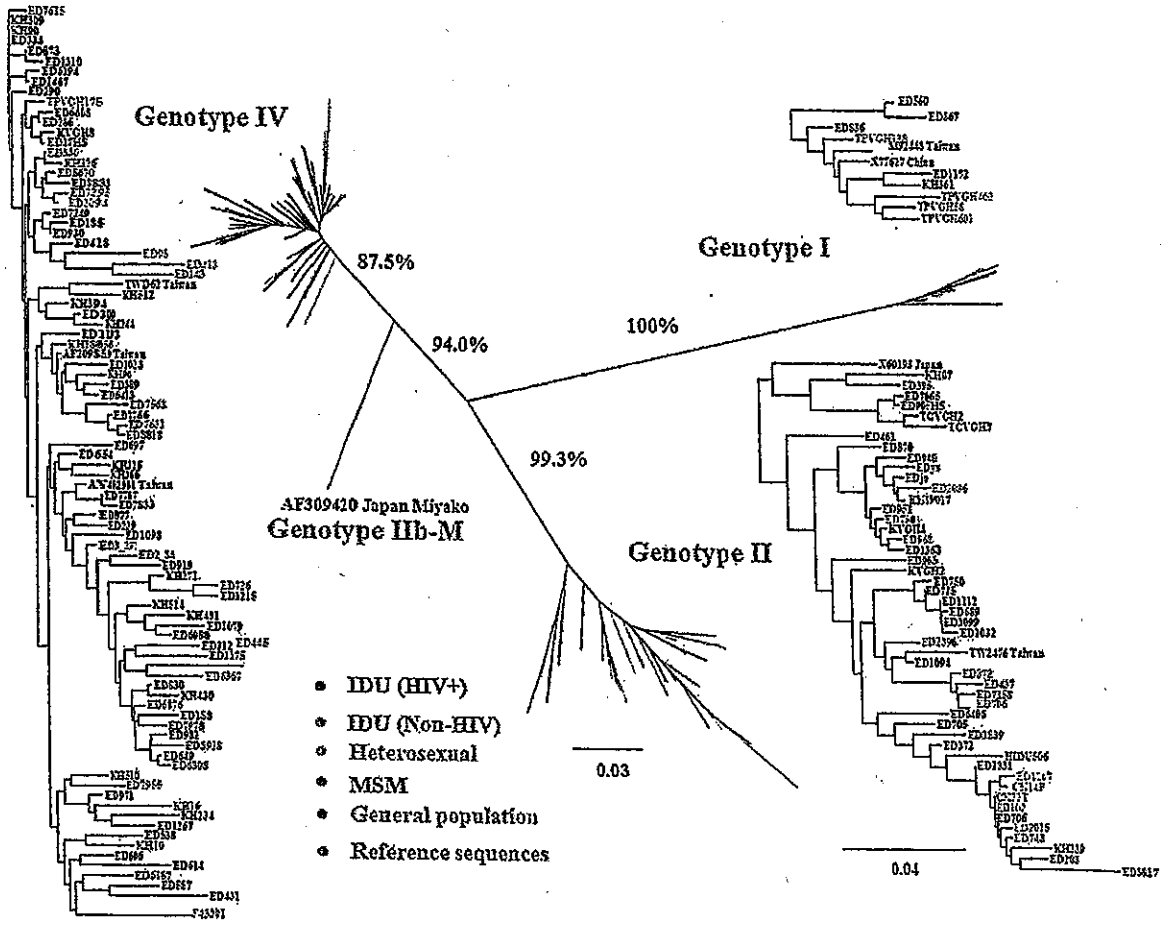

Fig. 6. Phylogenetic analysis based on HDV delta-gene sequences from the study population (n = 153). The horizontal branch was drawn in accord with the relative genetic distance. A number of commonly used reference delta-gene sequences for classifying HDV genotypes were also included and are indicated by accession numbers.

and HCV seropositivity. A large cohort study conducted in Central Europe showed that HCV coinfection is a frequent phenomenon, and approximately one third of patients with HDV infection tested positive for anti-HCV.[28] In a recent study from Northern California, approximately half of the HDV-infected individuals were also HCV infected.[29] Our study also showed that the geographical variation in the prevalence of HDV infection among the HBsAg-positive subjects from the general population paralleled the epidemiology of HCV infection, with a higher prevalence of HDV (6.1% vs. 2.5%; $P < 0.001$) and HCV (6.3% vs. 1.1%; $P < 0.001$) in southern Taiwan, compared to those in northern Taiwan. Geographic difference of HCV prevalence between southern and northern Taiwan has been reported before, and iatrogenic medical injections with reused, contaminated syringes were found to be the major risk factor.[15,30]

Table 4. Distribution of HDV Genotypes According to Risk Factors (n = 153)

Risk Factors (N = 153)	HDV Genotype, n (%) I	II	IV	P Value
IDU vs. non-IDU				
Non-IDU (n = 45)	4 (8.9)	33 (73.3)	8 (17.8)	<0.001
IDU (n = 108)	9 (8.3)	21 (19.4)	78 (72.2)	
HIV infected vs. uninfected				
HIV uninfected (n = 70)	4 (5.7)	32 (45.7)	34 (48.6)	0.04
HIV infected (n = 83)	9 (10.8)	22 (26.5)	52 (62.7)	
IDU vs. non-IDU stratified by HIV status				
HIV positive (n = 83)				
IDUs (n = 76)	9 (11.8)	16 (21.1)	51 (67.1)	0.004
non-IDUs (n = 7)	0 (0.00)	6 (85.7)	1 (14.3)	
HIV negative (n = 70)				
IDU (n = 32)	0 (0.00)	5 (15.6)	27 (84.4)	<0.001
non-IDU (n = 38)	4 (10.5)	27 (71.1)	7 (18.4)	

Older age (≥50 years) was demonstrated to be a significant risk factor for HDV infection, and a significant increasing trend of HDV prevalence was found with age in the non-IDU group. This was consistent with previous studies.[31,32] Among non-IDUs, HIV-infected individuals, both MSM and heterosexuals, had a higher prevalence of HDV infection than the HBsAg-positive subjects from the general population. This finding suggested that people with high-risk sexual behavior are at an increased risk for HDV infection. HDV prevalence in HIV-infected persons did not differ significantly between MSM and heterosexuals in our study.

Importantly, our study found a higher HBV carrier rate in the vaccinated, HIV cohort, compared to the general population, born after nation-wide HBV vaccination. We also found that anti-HBs levels were below the level of protection in more than half of HIV and IDU cohorts who were not HBV carriers. The increased risk of acquiring HBV and HDV infection as well as the high rate of chronicity were most likely owing to both their immunocompromised status and the high-risk behavior leading to repeated exposures to HDV. This high-risk group may become a reservoir for HBV and HDV. Therefore, we suggest that an HBV vaccination booster may be indicated for HIV-infected persons who are HBsAg negative with low levels of anti-HBs (<10 mIU/mL), even if they had received HBV vaccination at birth. This is concordant with the recommendation for booster vaccination in immunocompromized patients by the European Consensus Group on Hepatitis B Immunity.[33] However, vaccine efficacy in HIV-infected individuals requires further study.

There are varied geographical distribution of different HDV genotypes[4]; however, whether the distribution of HDV genotype varies by risk group remains uncertain. The distribution of HIV and HCV genotypes has been reported to vary both geographically and by risk group.[34] In this study, we demonstrated that the HDV genotype circulating among the IDUs was distinct from those circulating among the HBsAg-positive subjects from the general population. Three genotypes of HDV have been reported in Taiwan previously, with a predominance of genotype II, varying from 85.4% in 1995, 82.8% in 1998, to 55.6% in 2006,[35-37] whereas genotype IV (genotype IIb in the old nomenclature) accounted for only 8.6% in 1998 and 13.1% in 2006. A small-scale study of 31 IDUs with HDV infection conducted in 2002 found a predominance of genotype II (58.0%) and genotype I (35.5%), but did not find any cases with genotype IV.[10] A more recent study describing the HDV genotypes among IDUs showed that genotypes II and IV were the two major genotypes.[12] Our study further demonstrated the changing molecular epidemiology of HDV infection in Taiwan, with a shift in the main circulating HDV genotypes to genotype IV (56.6%), followed by genotype II (34.9%) and genotype I (8.6%). Genotype IV was the major prevalent HDV genotype circulating among IDUs, even when stratified by HIV status. However, the main HDV genotype circulating in non-IDUs was genotype II.

Another interesting and novel finding in this study is the association of HDV infection and viremia with a serum HBsAg level ≧250 IU/mL. A cut-off value of 250 IU/mL was arbitrarily used because this value is the upper limit of the quantitative test used in our routine clinical practice. Recently, quantitative HBsAg has been used as a new maker to monitor the natural history and complement HBV-DNA levels to optimize the management of CHB patients.[38] A large, central European, cohort study revealed that the mean HBsAg levels did not differ significantly between HBV-monoinfected patients and individuals with delta hepatitis.[28] Our previous study revealed that the secretion of genotypes I, II, or IV generally correlated with HBsAg levels, but not with HBV genotypes or HBV-DNA levels.[23] The finding that high HBsAg levels was a risk factor for HDV infection and HDV-RNA viremia in patients with and without HIV coinfection can be explained by the fact that the assembly of HDV requires only HBsAg, and not HBV DNA.[1,2] It also implies that the suppression of HBsAg levels may be helpful in controlling HDV infection and viremia. An international study reported that serum levels of HBsAg showed a weak correlation with the histological activity of disease in patients with HDV infection.[39] However, further studies are needed to elucidate the role of serum levels of HBsAg in HDV infection.

In summary, IDUs, especially HIV-infected IDUs, have become the most important risk group in HDV infection and a reservoir for HDV, even after the implementation of a nation-wide HBV vaccination program for 30 years. The dominant HDV genotype in IDUs is genotype IV, in contrast to genotype II in the general population. Effective strategies, such as methadone maintenance therapy and clean syringe exchange programs, and new policies are needed to prevent injection drug use and educate IDUs on the avoidance of practices that may lead to infection with HDV.

Acknowledgment: The authors are greatly indebted to the study patients for their participation.

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No. 12

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
				2015. 5. 19	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		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		Conrad C, Bradley HM, Broz D, Buddha S,et al ; Centers for Disease Control and Prevention (CDC). MMWR Morb Mortal Wkly Rep. 2015 May 1;64(16):443-4.	米国

研究報告の概要

○オキシモルホンの注射器による使用と関連したHIV感染の地域アウトブレイク — インディアナ州、2015年
2015年1月23日に、インディアナ州保健当局(ISDH)は、州の疾患介入専門家から州の南東部の地方から発生したヒト免疫不全ウイルス(HIV)感染症の確診11例の報告を受け、調査を開始している。これまで、この地方におけるHIV感染は年間で5例未満であった。
症例の大半は同じ地域に居住し、処方されたオピオイド・オキシモルホン(強力な経口半合成オピオイド系鎮痛薬)の注射器を回し打ちするパートナーとつながりがあった。4月21日現在、ISDHは4,200人のコミュニティで、135人をHIV感染症と診断した(確診129例、迅速検査で陽性6例)。症例の年齢は18～57歳(平均35歳)、男性74例(54.8%)であった。症例の中には少数の妊婦が含まれていた。108例(80.0%)は注射薬物の使用(IDU)を報告したが、4例はIDUではなく、23例のIDU状況は不明であった。IDUを報告した全例がオキシモルホン錠剤を溶解して使用していた。女性10例(7.4%)は風俗店従業員であった。C型肝炎の重感染を114例(84.4%)で認めた。症例のインタビューから、注射器共有のパートナー、性交渉パートナー、その他のHIV感染の危険のある社会的接触は、平均9名報告された。4月21日までに、373例の接触者が判明し、所在が明らかな247例のうち230例で検査した結果、109例(47.4%)がHIV陽性であった。所在が不明の128例のうち、74例は注射器共有または性交渉パートナー、54例はHIV感染の危険が高い社会的行動者であった。
3月26日に、行政命令による公衆衛生緊急事態が宣言され、一般への教育啓蒙、現場指揮および地域への働き掛けのためのセンターの設立、注射器交換の短期間の許可、HIV感染およびC型肝炎の包括的医療、薬物乱用の治療およびカウンセリングの支援が実施された。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
インディアナ州にて、注射器を回し打ちした結果、4か月間でコミュニティ4,200人中135名がHIVに感染したとの報告である。	日本赤十字社では、化学発光酵素免疫測定法(CLEIA)によりHIV抗体検査を実施することに加えて、全検体に対し個別検体によるNAT(個別NAT)スクリーニングを実施し、陽性血液を排除している。HIV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。

Morbidity and Mortality Weekly Report

Community Outbreak of HIV Infection Linked to Injection Drug Use of Oxymorphone — Indiana, 2015

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On April 24, 2015, this report was posted as an MMWR *Early Release on the* MMWR *website (http://www.cdc.gov/mmwr).*

On January 23, 2015, the Indiana State Department of Health (ISDH) began an ongoing investigation of an outbreak of human immunodeficiency virus (HIV) infection, after Indiana disease intervention specialists reported 11 confirmed HIV cases traced to a rural county in southeastern Indiana. Historically, fewer than five cases of HIV infection have been reported annually in this county. The majority of cases were in residents of the same community and were linked to syringe-sharing partners injecting the prescription opioid oxymorphone (a powerful oral semi-synthetic opioid analgesic). As of April 21, ISDH had diagnosed HIV infection in 135 persons (129 with confirmed HIV infection and six with preliminarily positive results from rapid HIV testing that were pending confirmatory testing) in a community of 4,200 persons (*1*).

The age range of the 135 patients is 18–57 years (mean = 35 years; median = 32 years); 74 (54.8%) are male. A small number of pregnant women were diagnosed with HIV infection and started on antiretroviral therapy during pregnancy. As of April 21, no infants had tested positive for HIV. Of the 135 persons with diagnosed HIV infection, 108 (80.0%) have reported injection drug use (IDU), four (3.0%) have reported no IDU, and 23 (17.0%) have not been interviewed to determine IDU status. Among the 108 who have reported IDU, all reported dissolving and injecting tablets of oxymorphone as their drug of choice. Some reported injecting other drugs, including methamphetamine and heroin. Ten (7.4%) female patients have been identified as commercial sex workers. Coinfection with hepatitis C virus has been diagnosed in 114 (84.4%) patients.

The patients were interviewed about syringe-sharing and sex partners, as well as any social contacts who also might have engaged in high risk behaviors. Those interviewed reported an average of nine syringe-sharing partners, sex partners, or other social contacts who might be at risk for HIV infection. Of the 373 contacts named as of April 21, a total of 247 (66.2%) had been located, 230 (61.7%) were tested, and 17 (4.6%) either declined testing or were not able to be tested. Of the 230 contacts who were tested, test results for 109 (47.4%) were HIV positive, and 121 (52.6%) were HIV negative. Of the 128 contacts who have not yet been located, 74 (57.8%) have been identified as syringe-sharing or sex partners, and 54 (42.2%) are social contacts regarded as at high risk for HIV infection.

Injection drug use in this community is a multi-generational activity, with as many as three generations of a family and multiple community members injecting together. IDU practices include crushing and cooking extended-release oxymorphone, most frequently 40 mg tablets not designed to resist crushing or dissolving. Syringes and drug preparation equipment are frequently shared (e.g., the drug is dissolved in nonsterile water and drawn up into an insulin syringe that is usually shared with others). The reported daily numbers of injections ranged from four to 15, with the reported number of injection partners ranging from one to six per injection event.

Like many other rural counties in the United States, the county has substantial unemployment (8.9%), a high proportion of adults who have not completed high school (21.3%), a substantial proportion of the population living in poverty (19%), and limited access to health care (*1*). This county consistently ranks among the lowest in the state for health indicators and life expectancy (*2*).

ISDH worked with the only health care provider in the immediate community, local health officials, law enforcement, community partners, regional health care providers and CDC to launch a comprehensive response to this outbreak. A public health emergency was declared on March 26 by executive order (*3*). The response has included a public education campaign, establishment of an incident command center and a community outreach center, short-term authorization of syringe exchange, and support for comprehensive medical care including HIV and hepatitis C virus care and treatment as well as substance abuse counseling and treatment. State and local health departments and academic partners, with the assistance of CDC, are working to implement and improve the community outreach programs supported by the executive order and to interrupt IDU-related HIV and hepatitis C virus transmission. Contact tracing by state and CDC disease intervention specialists continues to identify those potentially exposed.

This HIV outbreak involves a rural population, historically at low risk for HIV, in which HIV infection spread rapidly within a large network of persons who injected prescription opioids. The Indiana public health response includes implementing programs to contain the spread of HIV and hepatitis C virus,

curb injection drug use, and concurrently build social resilience in the community. The outbreak highlights the vulnerability of many rural, resource-poor populations to drug use, misuse, and addiction, in the context of a high prevalence of unaddressed comorbid conditions (*4*). The outbreak also demonstrates the importance of timely HIV and Hepatitis C surveillance activities and rapid response to interrupt disease transmission. Finally, the outbreak points to the need for expanded mental health and substance use treatment programs in medically underserved rural areas (*5*).

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No. 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
				2015. 4. 20	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		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		佐山勇輔, 三浦左千夫, 松本千恵子, 佐竹正博. 第89回日本感染症学会学術講演会; 2015年4月16日-17日; 京都府京都市.	日本

研究報告の概要

○日本国内におけるシャーガス病疑い例の検査経験

目的: シャーガス病はラテンアメリカ(LA)で流行し、*Trypanosoma cruzi*(*T.cruzi*)により引き起こされる原虫疾患である。慢性期の感染者(キャリア)では、末梢血や臓器に原虫が存在するため、輸血や移植などによって患者に伝播する可能性がある。日本国内には、流行地出身者が多数滞在しており、キャリアの献血による輸血感染が危惧されている。検査法の評価と陽性検体の確保を兼ねてシャーガス病疑い例を対象に検査を行った。

方法: 2012年8月から2014年10月の間に日本の医療機関、NGOなどから連絡のあったシャーガス病疑い例から血液を採取した。検体は血清学、PCR法、血液培養による検査を行った。また、提供者とサシガメとの接触歴などを調査した。

結果: LA出身者10名、日本人10名から検体が得られた。LA出身者7名が抗体陽性、その内6名がPCR陽性、3名から*T.cruzi*が分離された。サシガメとの接触歴が不明であった提供者からも、*T.cruzi*感染者が確認された。

結論: 提供者の中から*T.cruzi*感染者が認められた。国内でも医療関係者におけるシャーガス病の認知度を上げる必要がある。また、検査を施行し、診断・治療できる体制の整備が必要であることが考えられた。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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

2012年8月～2014年10月に日本の医療機関、NGOなどから連絡のあったシャーガス病疑い例(LA出身者10名、日本人10名)の血液を採取し検査を行ったところ、LA出身者7名が抗体陽性となり、その内6名がPCR陽性、3名から*T.cruzi*が分離されたとの報告である。

今後の対応

日本赤十字社では、輸血感染症対策として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。また、中南米出身者(母親が出身を含む)、通算4週間以上の中南米滞在歴を有する献血者からの血液は、血漿分画製剤の原料にのみ使用する対策を実施している。今後も引き続き情報の収集に努める。

別紙3

MedDRA/J Ver.18.1J

C

4月17日(金)

JRC2015T-017

第8会場

14時50分～15時30分

一般演題（口演）　原虫・寄生虫

司会：有吉 紅也(長崎大学熱帯医学研究所)

O2-076. 日本国内におけるシャーガス病疑い例の検査経験

○佐山 勇輔[1] 、三浦 左千夫[1] 、松本 千惠子[1] 、佐竹 正博[1]

日本赤十字社血液事業本部中央血液研究所[1]

【目的】シャーガス病はラテンアメリカ（LA）で流行し，*Trypanosoma cruzi*（*T.cruzi*）により引き起こされる原虫疾患である．慢性期の感染者（キャリア）では，末梢血や臓器に原虫が存在するため，輸血や移植などによって患者に感染が伝播する可能性がある．日本国内には，流行地出身者が多数滞在しており，キャリアの献血による輸血感染が危惧されている．日本赤十字社（日赤）では，献血者における感染状況を把握するため調査を行っている．それに先立ち，検査法の評価と陽性検体の確保を兼ねてシャーガス病疑い例を対象に検査を行ったので報告する．なお，現在日赤では輸血用血液による*T.cruzi*感染を防ぐため製造制限を設け，安全対策を行っている．

【方法】2012年8月から2014年10月の間に医療機関，NGOなどから連絡のあったシャーガス病疑い例から血液を採取した．検体は血清学，PCR法，血液培養による検査を行った．また，提供者からサシガメとの接触歴などを調査した．

【結果】LA出身者10名，日本人10名から検体が得られた．LA出身者7名が抗体陽性，その内6名がPCR陽性，3名から*T.cruzi*が分離された．サシガメとの接触歴が不明であった提供者からも，*T.cruzi*感染者が確認された．

【結論】提供者の中から*T.cruzi*感染者が認められた．国内でも医療関係者におけるシャーガス病の認知度を上げる必要がある．また，検査を施行し，診断・治療できる体制の整備が必要であることが考えられた．

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2015 年 06 月 09 日	新医薬品等の区分	厚生労働省処理欄

一般的名称	①②③⑩⑪pH4 処理酸性人免疫グロブリン ④⑤⑥⑦⑧⑨ポリエチレングリコール処理人免疫グロブリン ⑫⑬人免疫グロブリン	研究報告の公表状況	japanese.yonhapnews.co.kr/society/2015/06/09/0800000000AJP20150609000400882.HTML/2015/06/09	公表国 韓国
販売名 （企業名）	①献血ポリグロビン N5%静注 0.5g/10mL （日本血液製剤機構） ②献血ポリグロビン N5%静注 2.5g/50mL （日本血液製剤機構） ③献血ポリグロビン N5%静注 5g/100mL （日本血液製剤機構） ④献血ヴェノグロブリン IH5%静注 0.5g/10mL （日本血液製剤機構） ⑤献血ヴェノグロブリン IH5%静注 1g/20mL （日本血液製剤機構） ⑥献血ヴェノグロブリン IH5%静注 2.5g/50mL （日本血液製剤機構） ⑦献血ヴェノグロブリン IH5%静注 5g/100mL （日本血液製剤機構） ⑧献血ヴェノグロブリン IH5%静注 10g/200mL （日本血液製剤機構） ⑨献血ヴェノグロブリン-IH ヨシトミ （日本血液製剤機構） ⑩献血ポリグロビン N10%静注 5g/50mL （日本血液製剤機構） ⑪献血ポリグロビン N10%静注 10g/100mL （日本血液製剤機構） ⑫グロブリン筋注 450mg/3mL「JB」 （日本血液製剤機構） ⑬グロブリン筋注 1500mg/10mL「JB」 （日本血液製剤機構）			

研究報告の概要

【ソウル聯合ニュース】
韓国保健福祉部は 9 日、中東呼吸器症候群（MERS）コロナウイルスの感染者が新たに 8 人増え、計 95 人になったと発表した。また、感染者のうち 1 人が死亡し、韓国国内の死者は 7 人に増えた。
新たな感染者のうち 3 人は、先月 27～29 日にソウルのサムスンソウル病院の救急室で 2 次感染者から感染した。同病院から発生した患者は 37 人に増えたが、増加の勢いは衰えた。
また 1 人は、これまで 7 人の患者が発生している大田市の建陽大病院で感染した。残り 4 人は三つの医療機関での感染者だ。これら病院は MERS コロナウイルス患者が来院したことはあったが、感染者は確認されていなかった。
また、感染者のうち 1 人が隔離前に地方の 3 病院を来院していたことが確認され、これらの病院を訪れた人もすべて自宅、または病院に隔離された。
一方、7 人目の死者は 60 代の女性で、弁膜に疾患があった。先月 27～28 日に呼吸困難を起こしサムスンソウル病院の救急室に運ばれた際、感染者に接触した。陽性判定が出た後、隔離され治療を受けていたが、容体が悪化し死亡した。
保健福祉部は「サムスンソウル病院の救急室を訪れた患者らによる第 2 次の流行は収まりつつあり、そのほかの医療機関で発生したケースは散発的な様相となっている。今週が感染拡大を断つためのヤマ場になると判断し、対応に総力を挙げる」と話した。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL 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献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 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人免疫グロブリンを濃

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化粧品

報告企業の意見	今後の対応	
「Middle east respiratory syndrome coronavirus：MERS-CoV（マーズコロナウイルス）」と命名された新種のコロナウイルスである。コロナウイルスは、コロナウイルス科（Coronaviridae）コロナウイルス亜科（Coronavirinae）に属する直径 80～220nm の円形或いは楕円形のエンベロープを有する 1 本鎖 RNA ウイルスで、万一原料血漿にコロナウイルスが混入したとしても、モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	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。	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、ウイルス除去膜によるろ過処理及び pH3.9～4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

YONHAP NEWS AGENCY
聯合ニュース

MERS感染者8人増の95人 死者は7人に＝韓国

2015/06/09 09:04

【ソウル聯合ニュース】韓国保健福祉部は9日、中東呼吸器症候群（MERS）コロナウイルスの感染者が新たに8人増え、計95人になったと発表した。また、感染者のうち1人が死亡し、韓国国内の死者は7人に増えた。

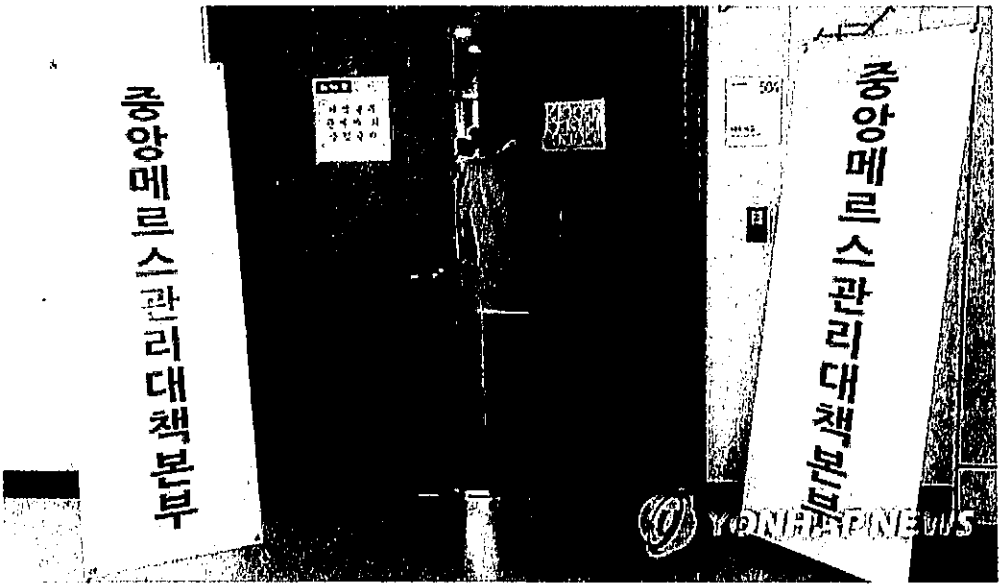
新たな感染者のうち3人は、先月27～29日にソウルのサムスンソウル病院の救急室で2次感染者から感染した。同病院から発生した患者は37人に増えたが、増加の勢いは衰えた。

また1人は、これまで7人の患者が発生している大田市の建陽大病院で感染した。残り4人は三つの医療機関での感染者だ。これら病院はMERSコロナウイルス患者が来院したことはあったが、感染者は確認されていなかった。

また、感染者のうち1人が隔離前に地方の3病院を来院していたことが確認され、これらの病院を訪れた人もすべて自宅、または病院に隔離された。

一方、7人目の死者は60代の女性で、弁膜に疾患があった。先月27～28日に呼吸困難を起こしサムスンソウル病院の救急室に運ばれた際、感染者に接触した。陽性判定が出た後、隔離され治療を受けていたが、容体が悪化し死亡した。

保健福祉部は「サムスンソウル病院の救急室を訪れた患者らによる第2次の流行は収まりつつあり、そのほかの医療機関で発生したケースは散発的な様相となっている。今週が感染拡大を断つためのヤマ場になると判断し、対応に総力を挙げる」と話した。



保健福祉部の中央MERS管理対策本部＝（聯合ニュース）

別紙様式第2-1

No. 7

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
				2015. 7. 10	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	Euro Surveill. 2015;20(25):pii=21163. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21163	公表国 韓国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			

研究報告の概要

○2015年5月から6月の韓国における中東呼吸器症候群(MERS)コロナウイルス感染症のアウトブレイクについての予備疫学的評価

アラビア半島以外で最大の、MERSコロナウイルス感染症アウトブレイクが韓国において発生している。2015年6月19日までに、24名の死亡者を含む166名の感染が検査により確認された。このうち回復した30名は退院したが、112名は依然として入院しており、16名は危篤状態に陥っている。我々の推定では平均潜伏期間は6.7日、感染源の発症から2次感染者の発症までの平均発症間隔は12.6日であった。感染性が発症に先行する可能性は低いことが判明した。現在入手可能なデータによると、全体的な致死リスクは21%であると予測する。

初発患者は、最終的に当該感染症の診断を受け隔離される前に、複数の医療機関を受診しており、当該患者に対する認識が遅れたことが韓国において進行しているアウトブレイクの発生につながった。

人獣共通ウイルスであるMERSコロナウイルスの既知の保有宿主が韓国に存在していないこと、中東を除く地域において今日までに認められたMERSの症例はごくわずかであるため、更なる感染者が国外から入国した確率は低いと考えられること、並びに感染性が発症に先行する可能性は低いことを踏まえ、現在の流行をコントロールする鍵は、隔離者への接触を厳重に追跡し、隔離者に対し厳密な医学的調査を実施することにより、常に新たな症例を迅速に認識し、感染者を隔離することであると考える。

報告企業の意見

アラビア半島以外で最大の、中東呼吸器症候群(MERS)コロナウイルス感染症アウトブレイクが韓国において発生していること、同感染症の感染性は発症に先行する可能性は低いことが判明したという報告である。

今後の対応

日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としているほか、発熱などの体調不良者を献血不適としている。また、中東呼吸器症候群(MERS)が指定感染症として定められたことから、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

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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

RAPID COMMUNICATIONS

Preliminary epidemiological assessment of MERS-CoV outbreak in South Korea, May to June 2015

B J Cowling[1,2], M Park[1,2], V J Fang[1], P Wu[1], G M Leung[1], J T Wu (joewu@hku.hk)[1]
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2. These authors contributed equally to this work

Citation style for this article:
Cowling BJ, Park M, Fang VJ, Wu P, Leung GM, Wu JT. Preliminary epidemiological assessment of MERS-CoV outbreak in South Korea, May to June 2015. Euro Surveill. 2015;20(25):pii=21163. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21163

Article submitted on 15 June 2015/ published on 25 June 2015

South Korea is experiencing the largest outbreak of Middle East respiratory syndrome coronavirus infections outside the Arabian Peninsula, with 166 laboratory-confirmed cases, including 24 deaths up to 19 June 2015. We estimated that the mean incubation period was 6.7 days and the mean serial interval 12.6 days. We found it unlikely that infectiousness precedes symptom onset. Based on currently available data, we predict an overall case fatality risk of 21% (95% credible interval: 14–31).

South Korea is experiencing the largest outbreak of Middle East respiratory syndrome coronavirus (MERS-CoV) infections outside the Arabian Peninsula. Up to 19 June 2015, there have been 166 laboratory-confirmed cases, including 24 deaths, 30 recovered individuals discharged from hospital, and 112 still remaining in hospital [1]. The aim of our study was to conduct a preliminary epidemiological assessment of the MERS-CoV outbreak in South Korea in order to further describe and update key epidemiological determinants of MERS-CoV outbreaks.

Primary case

The ongoing outbreak in South Korea began when the primary case developed respiratory illness on 11 May after returning on 4 May from Bahrain (18 April–2 May) via Qatar (2–3 May). Further epidemiological investigation showed that the primary case had also travelled to the United Arab Emirates (29–30 April) and Saudi Arabia (1–2 May) during their stay in Bahrain [2]. Feeling unwell after returning to South Korea, the primary case visited a local clinic (Hospital A) in Pyeongtaek, Gyeonggi province on 12, 14 and 15 May and was hospitalised in Hospital B from 15 to 17 May*. However, this patient did not initially report their recent travel in the Middle East. Upon discharge from Hospital B, the patient visited another clinic (Hospital C) and was admitted to a general hospital (Hospital D) in Seoul on 17 May, where the patient was later diagnosed with MERS-CoV on 20 May. Since then, the patient has been isolated and treated in another hospital designated by the Korean government to treat MERS patients.

Sources of data

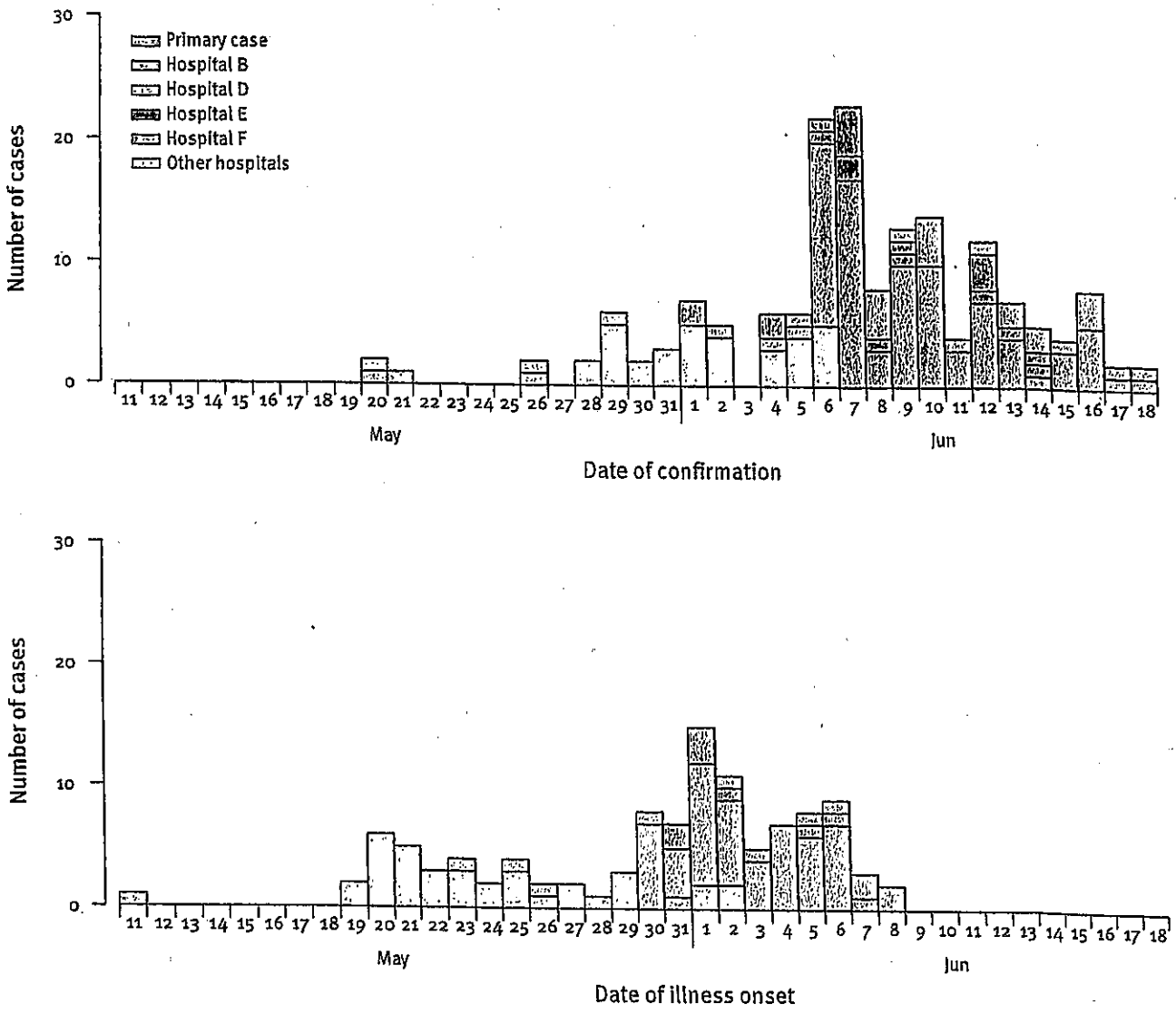
We retrieved publicly available data from multiple sources, including the Korea Centers for Disease Control and Prevention (Korea CDC), the Korean Ministry of Health and Welfare (MoH), the WHO and local Korean news reports to compile a line list of all confirmed cases reported by 19 June 2015. In case of any data discrepancy between the different sources, we used the most up-to-date information from official reports published by the Korea CDC and MoH on a daily basis during the outbreak. The official reports were only available in Korean language and included a brief description of each confirmed case, including demographic characteristics (e.g. age and sex), date of exposure and onset of symptoms, as well as possible linkage with confirmed cases and the associated hospital cluster (e.g. Hospital A to P).

Statistical analysis

We fitted parametric distributions to the time intervals (i) from infection to onset (i.e. the incubation period) and (ii) from illness onset to case confirmation. We also fitted a nonparametric distribution on the incubation period. The exact dates of infection were not known for most cases, but exposure windows were available, and we accounted for the consequent interval censoring in the likelihood function [9] and the possibility of infectiousness before illness onset (details on the methodology are available from the corresponding author on request). We used survival models to fit alternative parametric distributions including log-normal, Weibull and gamma distributions, and compared the goodness of fit of these parametric distributions using the Bayesian information criterion. We observed that the delay from illness onset to confirmation shortened as the epidemic progressed, so we fitted two separate survival curves for onset before and after 28 May. We used the same approach to estimate the serial interval

FIGURE 1

Epidemic curve of MERS-CoV infections, South Korea, 11 May–19 June 2015 (n = 166)



MERS-CoV: Middle East respiratory syndrome coronavirus.

Data up to 19 June 2015. Colours indicate the primary case (light green) and the hospital associated with a confirmed case. We selected the four hospitals (B, D, E and F) with the largest number of either secondary (yellow) or tertiary infections (all other colours).

A: By date of laboratory confirmation.

B: By date of illness onset for 110 of 166 confirmed cases with available onset data.

distribution, based on data on illness onset times for linked cases. We calculated the 95% credible interval (CrI) by bootstrapping.

To estimate the case fatality risk (CFR) allowing for the uncertain clinical outcomes of those who remained in hospital on the date of analysis (19 June 2015), we used the methods proposed by Garske et al. which adjusts the fatality risk based on the time-to-death distribution [10]. We assumed that the time from onset to death followed a log-normal distribution, and used Markov chain Monte Carlo methods to estimate the parameters in a Bayesian framework, setting an informative prior for the time from onset to death with a mean of 14 days [11], and non-informative priors for the other parameters. All statistical analyses were conducted in R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Outbreak description

The number of laboratory-confirmed cases increased rapidly until 7 June, when 23 cases were confirmed on a single day but appears to have subsided since then (Figure 1A). Figure 1B shows the epidemic curve by date of illness onset for 110 cases with available data. It should be recognised that while the outbreak has not

TABLE 1

Demographic characteristics of confirmed cases of MERS-CoV infection, South Korea, 11 May–19 June 2015 (n = 166)

Characteristics	All cases (n = 166)	Fatal cases (n = 24)*
Age group		
0–18 years	1 (1%)	0 (0%)
19–39 years	31 (19%)	0 (0%)
40–59 years	64 (39%)	5 (21%)
60–79 years	61 (37%)	16 (67%)
≥ 80 years	9 (5%)	3 (13%)
Sex		
Male	101 (61%)	17 (71%)
Female	65 (39%)	7 (29%)
Occupation		
Healthcare personnel	30 (18%)	0 (0%)
Not healthcare personnel	136 (82%)	24 (100%)

MERS-CoV: Middle East respiratory syndrome coronavirus.

yet ended, our preliminary assessment shows that the epidemic to date may have peaked on 1 June when 15 cases reported illness onset. Median age of the 166 cases was 56 years, 101 of 166 (61%) were male, and 30 of 166 (18%) were healthcare personnel (Table 1).

Transmission chains

Figure 2 shows a summary sketch of the transmission chain (additional material** showing the detailed chains is available at: http://sph.hku.hk/bcowling/eurosurveillance2015appendix.zip). 119 cases were identified by Korea CDC as having had contact with a confirmed case in the period before their illness onset, and three of these cases had contact with more than one confirmed case. A total of 27 secondary cases in a single hospital have been traced back to the primary case (excluding six cases with an unclear linkage), and two of these, Cases 14 and 16, led the second wave of the outbreak by infecting at least 73 and 24 tertiary cases, respectively, following the initial outbreak generated by the primary case in Hospital B (Figure 2). In particular, Case 14 infected at least 70 cases between 27 and 29 May while being treated in the emergency room in Hospital D, one of the five largest hospitals located in Seoul with 3,980 healthcare professionals and more than 8,000 outpatient visits per day [12]. According to the press conference given at Hospital D on 7 June, at least 893 patients and visitors were potentially exposed to the virus during this period [13], which explains a significant increase in the number of cases confirmed and notified between 6 and 11 June. Since 12 June, when the first fourth-generation case was confirmed, 10 more potential fourth-generation cases have been reported. Because of the marked heterogeneity in transmissibility, with the vast majority of cases associated with just these three superspreading events in the nosocomial setting, it would be misleading to summarily characterise the transmissibility of the virus in this ongoing outbreak with a single average value of the reproductive number [14]. The mean serial interval was 12 to 13 days in each of four epidemiological clusters associated with Cases 1, 14, 15 and 16.

Epidemiological parameters

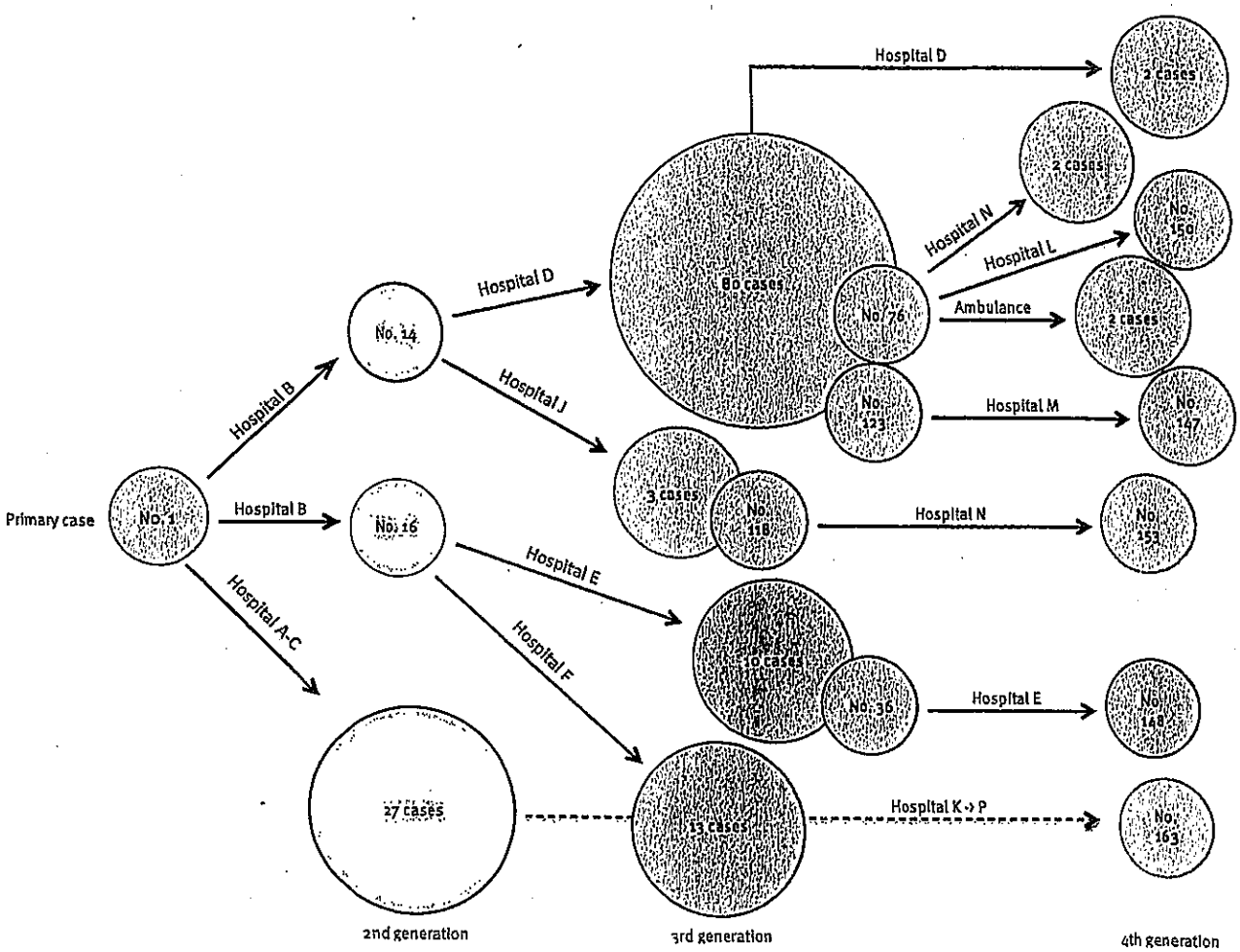
We found that a gamma distribution had the best fit to the incubation period distribution and was very similar to the nonparametric estimate (Figure 3A). The fitted gamma distribution had a median of 6.3 days (95% CrI: 5.7–6.8), a mean of 6.7 days (95% CrI: 6.1–7.3) and a 95th percentile of 12.1 days (95% CrI: 10.9–13.3). Using data on 99 cases with single identified infectors, we found that a gamma distribution with a mean of 12.6 days (95% CI: 12.1–13.1) and standard deviation of 2.8 days (95% CI: 2.4–3.1) provided best fit to the serial interval distribution (Figure 3B). The mean duration of illness onset to laboratory confirmation was 8.1 days for cases with illness onset before May 28, and substantially shorter (mean: 4.4 days) for cases with illness onset after that date (Figure 3C). We used a log-normal regression model for the time from illness onset to laboratory confirmation to estimate that healthcare worker status was not significantly associated with time to confirmation (beta = – 0.05; 95% CI: – 0.34 to 0.25), with the point estimate signifying a 5% reduction in time to confirmation in healthcare workers.

Presymptomatic infectiousness

It appeared that a small number of cases might have been infected before their infectors became symptomatic. Furthermore, Cases 37 and 39 were epidemiologically linked to multiple confirmed cases. To account for the possibility of presymptomatic infectiousness and the uncertainty of who infected Cases 37 and 39 when estimating the incubation period, we (i) simultaneously inferred the incubation period of the infector of Case 37, (ii) assumed that Case 39 was equally likely to be infected by all cases to whom he had been epidemiologically linked, namely Cases 9, 11, 12 and 14 (because the infector of Case 39 was not statistically identifiable), and (iii) introduced a parameter Y to represent the time interval between onset of symptoms and onset of infectiousness For example, if cases become infectious two days before onset of symptoms, then $Y = 2$ days. For a given value of Y, the dates of exposure of a case must not precede the date of symptom onset of the case's infector by more than Y days. The data were adjusted accordingly during the estimation of the incubation period. Furthermore, we excluded Case 40 when performing the estimation because their exposure and onset date were the same, which was implausible. We used Markov chain Monte Carlo methods to estimate the parameters of this model in a Bayesian framework.

FIGURE 2

Simplified transmission diagram illustrating the superspreading events associated with Cases 1, 14, 16 and fourth-generation infections of MERS-CoV, South Korea, 11 May–19 June 2015 (n = 166)



MERS-CoV: Middle East respiratory syndrome coronavirus.

In this modelling analysis of presymptomatic infectiousness, our model suggested that infectiousness might begin 0.4 days (95% CrI: − 1.2 to 2.4) before illness onset, which corresponded to a very small (right) shift from the prior distribution. Hence, there was no evidence that infectiousness preceded symptom onset. The same conclusion remained when the standard deviation of the prior was halved or doubled.

Severity of infections

Up to 19 June 2015, 24 cases have died while 30 have recovered and been discharged; the other 112 cases remain in hospital and 16 are in critical condition. Among the 24 fatal cases to date, none of which were in healthcare workers, the median age was 68.5 years (range: 49–83 years). We predicted the final CFR to be 21% (95% CrI: 14–31), allowing for the uncertain outcomes of cases that remained in hospital on the date of analysis.

Comparative epidemiology of MERS and SARS

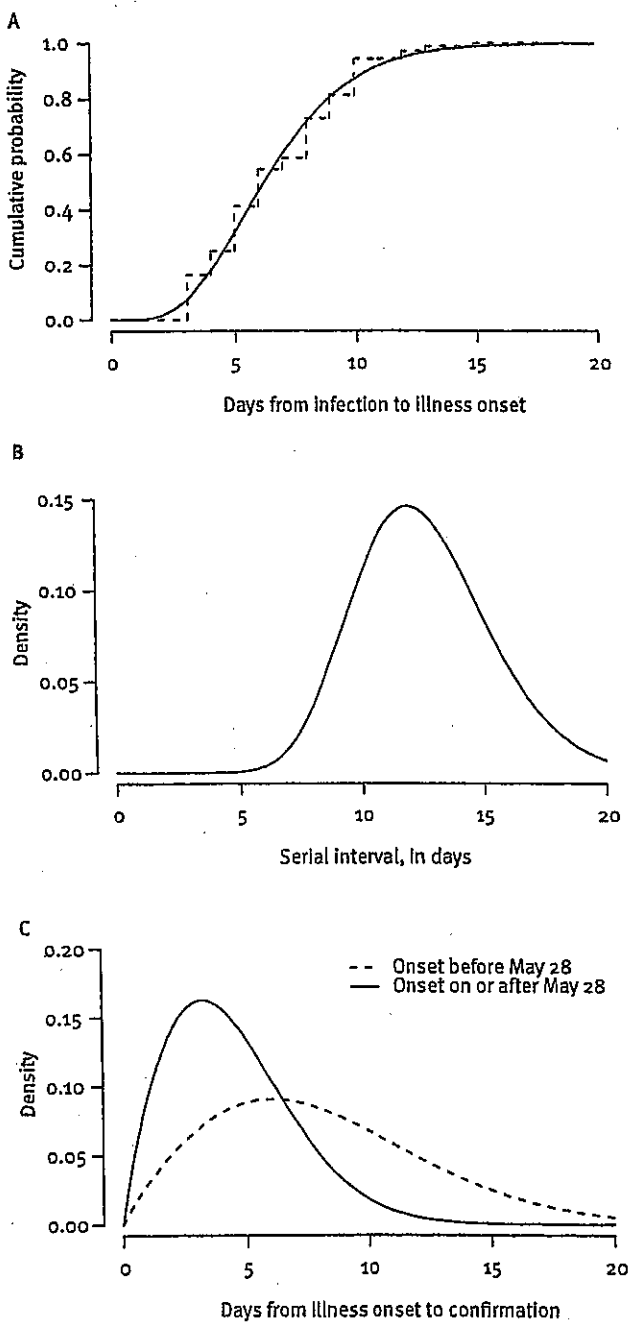
Table 2 compares key features of the MERS outbreak in South Korea with the features of MERS epidemiology in previous outbreaks in other countries as well as the 2003 outbreak of severe acute respiratory syndrome (SARS) [7,9,11,15-18]. In all MERS outbreaks, current and previous, men were more likely to be cases than women, and the mean age of the cases was around 56 years. There was a marked similarity in the incubation periods and serial intervals and in the case fatality risk.

Discussion

MERS is a relatively new disease, with the first confirmed case reported in Saudi Arabia in 2012 [2,3]. Globally, a total of 1,321 laboratory-confirmed cases of MERS-CoV infection, including 466 deaths, have been reported to the World Health Organization (WHO) to date, of which more than 1,000 occurred in Saudi Arabia [2,4]. One of the major challenges in countering

FIGURE 3

Estimates of key epidemiological distributions, MERS-CoV outbreak, South Korea, 11 May–19 June 2015 (n = 166)



MERS-CoV: Middle East respiratory syndrome coronavirus.

A: Incubation period distribution i.e. the time from infection to illness onset based on 105 cases with available data on potential infection times, accounting for interval censoring. Dashed line: nonparametric estimate of the distribution; solid line: fitted gamma distribution.

B: Distribution of serial intervals.

C: Distribution of times from illness onset to laboratory confirmation. Dashed line: cases with illness onset before 28 May 2015; solid line: cases with illness onset on or after 28 May 2015.

the spread of MERS-CoV is the limited understanding of the transmissibility and transmission patterns of the virus, in part because MERS-CoV is a novel pathogen and the experience to date remains mostly confined to cases in Saudi Arabia [4]. However, the outbreak of MERS-CoV in Jeddah, Saudi Arabia in 2014 highlighted an increased transmissibility for secondary human-to-human transmission in healthcare settings [5].

Our findings confirm that the epidemiology of MERS in South Korea is similar to that observed in the Middle East [7] and in fact closely resembles that of the 2002–03 outbreak of SARS [17]. The epidemic thus far has undergone four generations of infection events (Figure 2) arising from delayed recognition of the primary patient who sought care at multiple healthcare facilities before finally being diagnosed and isolated. The Korean outbreak is remarkable in that 148 of 166 transmission events (89%), or 125 of 166 (75%) if those who were epidemiologically linked to a cluster but not any infector are excluded, can be attributed to just three clusters of nosocomial superspreading events (Figure 2). Importantly, there has not been any evidence of community transmission thus far.

Given that (i) there is no known zoonotic reservoir of MERS-CoV in South Korea, (ii) the probability of further foreign importation of infected cases appears to be low because very few MERS cases have been identified outside of the Middle East to date and (iii) infectiousness is unlikely to precede symptom onset, the key to controlling the present epidemic remains prompt recognition and isolation of further cases through rigorous contact tracing and close medical surveillance of those quarantined. This also applies to other outbreaks of MERS that may occur in the future. We estimated that the incubation period had a 95th percentile of 12.1 days, which supports the quarantine period of two weeks currently recommended by public health authorities.

Previous studies based on several outbreaks in the Arabian Peninsula estimated the basic reproductive number (R_0) to be between 0.6 and 0.8 overall [6,7,19,20], although with apparent heterogeneity leading to sporadic outbreaks in which R_0 exceeded 1 [21]. In our analysis described here we felt that it would not be appropriate to estimate an average reproductive number because of the heterogeneity in transmissibility associated with the three superspreading events. However, it is clear that apart from those three events, the MERS-CoV had low transmissibility in this outbreak.

The CFR of 21% (95% CrI: 14–31) estimated here is substantially lower than the overall CFR in a previous analysis of cases most of whom were from the Middle East (444/1,163; 38%) [2], but the same as the CFR reported by Cauchemez et al. for secondary cases excluding sporadic cases identified after presenting with serious disease (21%) [7], and very similar to the CFR of SARS in Hong Kong in 2003 (Table 2) [17]. While

TABLE 2

Comparison of epidemiological features of the MERS outbreak in South Korea in 2015 with other outbreaks of MERS, and with SARS in Asia in 2003

	MERS South Korea (2015)	MERS [7,11,15] Global (2012–13)	SARS [9,16–18] Hong Kong (2003)
Mean incubation period	6.7 days	5.2 days	4.4 days
Mean serial interval	12.6 days	7 - 12 days	8.4 days[a]
Case fatality risk	21%	21%[b]	17%
Mean age (range)	55.4 years (16–87)	56 years (15–94)[c]	43.5 years (0–100)
Male	61%	77%[c]	44%
Healthcare personnel	18%	31%[d]	23%

MERS: Middle East respiratory syndrome; SARS: severe acute respiratory syndrome.

[a] Singapore.

[b] Secondary cases only; includes cases from Europe and the Middle East.

[c] Saudi Arabia.

[d] Jeddah.

our estimate of the CFR accounts for uncertainty in the final outcomes of patients that remain in hospital, it is challenging to have accurate estimates of the CFR early in the course of an outbreak [10,22]. If the CFR in this outbreak remained below 25% once the final outcome for all cases has been ascertained, it would indicate a lower severity of MERS-CoV than in some previous and contemporary reports. A lower CFR would be consistent with the much lower severity observed among secondary cases in the Middle East that were identified through contact tracing, presumably owing to a combination of earlier supportive treatment and/or a lower infective dose and/or lower clinical severity due to other host factors [23]. Greater severity in the sporadic cases could be an artefact of surveillance biased towards infections associated with serious illnesses. Consistent with previous reports, older age was associated with greater risk of severe disease [15,24,25]. We did not have data on underlying medical conditions, but it is known from other outbreaks of MERS that a history of chronic disease is another risk factor for disease progression and mortality [11,15,25,26].

Our epidemiological characterisation relied on the assumption that the transmission network as ascertained by the MoH was accurate. Specifically, the network essentially comprised secondary cases of three superspreading events (namely infections caused by Case 1, 14 and 16). The serial interval and incubation period of the secondary cases generated by these three superspreading events were similar, which supports the validity of the network ascertained by the MoH. Nonetheless, infected people with apparently longer incubation periods in the data might have been tertiary instead of secondary cases, in which case we would have overestimated the incubation period. On the other hand, because the outbreak in South Korea is still ongoing and driven by superspreading events, cases with very long incubation periods and/or long serial intervals may not have been identified yet and we may have underestimated the incubation period and serial interval distributions.

This outbreak demonstrates the potential for clusters of emerging infectious diseases to have very substantial societal and economic impact. In South Korea with a population of 50 million, 166 cases of MERS caused major reductions in tourism, nationwide school closures, and some preliminary forecasts for a growth in annual gross domestic product reduced by at least 0.1% [27]. As this outbreak appears to be coming to an end, focus of public health authorities may shift from the immediate control efforts towards a detailed investigation of the mechanisms and causes that led to the superspreading events. The parallels with superspreading events driving the spread of SARS in 2003 in Hong Kong and Singapore emphasise the importance of understanding these events and of determining the measures that could be taken to reduce the risk of similar incidents happening in the future.

* Author's correction

On request of the authors, the travel dates of the primary case in this sentence were corrected April to May. This change was made on 26 June 2015.

** Note

Additional material made available by the authors on an independent website is not edited by *Eurosurveillance*, and *Eurosurveillance* is not responsible for the content. The material can be accessed at: http://sph.hku.hk/bcowling/eurosurveillance2015appendix.zip.

Acknowledgments

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Conflict of interest

BJC reports receipt of research funding from Medimmune Inc. and Sanofi Pasteur and consults for Crucell NV. The authors report no other potential conflicts of interest.

Authors' contributions

GML and JTW conceived the study. MP collected the data. BJC, MP, VJF and JTW analysed the data. All authors interpreted the results. All authors wrote the manuscript.

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識別番号・報告回数			報告日	第一報入手日 2015年08月03日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②抗HBs人免疫グロブリン ③④乾燥抗HBs人免疫グロブリン ⑤　ポリエチレングリコール処理抗HBs人免疫グロブリン	研究報告の公表状況	Emerging Infectious Diseases 2015; 21(7): 1267-1269	公表国 中国
販売名 (企業名)	①抗HBs人免疫グロブリン筋注200単位/1mL「JB」（日本血液製剤機構） ②抗HBs人免疫グロブリン筋注1000単位/5mL「JB」（日本血液製剤機構） ③ヘブスブリン筋注用200単位　（日本血液製剤機構） ④ヘブスブリン筋注用1000単位　（日本血液製剤機構） ⑤ヘブスブリンIH静注1000単位（日本血液製剤機構）			

研究報告の概要

編集者へ：
インフルエンザウイルスの亜型H6は、米国において1965年に七面鳥から最初に分離され、その後世界各地で発見された。過去数十年にわたり、H6ウイルスの陽性率は野鳥および家禽において劇的に増加している。中国では、高病原性インフルエンザA（H5N1）、低病原性インフルエンザ（H9N2）、およびH6は家禽の間で最も流行している鳥インフルエンザウイルスである。ヒトにおけるH6ウイルス感染の1例のみが世界中で報告されているが、H6ウイルスの幾つかの生物学的特性は、それらが哺乳動物に対して非常に易感染性であることを示している。中国で流行しているH6ウイルスの約34%は、ヒト様受容体（α-2, 6 NeuAcGal）との親和性を高めている。H6ウイルスはまた、事前の馴化なしにマウスに感染することができ、幾つかのH6ウイルスはモルモット間で効率的に伝播することができる。ヒトへのH6ウイルスの潜在的な脅威を評価するために、我々は職業的にH6ウイルスに暴露した集団の組織的な血清学的研究を行った。
2009年から2011年の間に、合計15,689の血清サンプルは、中国本土22省の家禽市場の労働者、小規模養鶏農家、大規模養鶏農家、家禽屠殺工場労働者、および野鳥生息地の労働者から収集した。中国本土の支配的なH6ウイルスの代表的な分離株、A/トリ/Y94/広東/2011(H6N2)は、血清学的検査のために使用した（オンライン技術付録　表1、図. 1, 2、http://wwwnc.cdc.gov/EID/article/21/7/15-0135-Techappl.pdf）。世界保健機関のガイドラインによって示されるように、赤血球凝集阻害（HI）分析法は全ての血清サンプルについて行い、HI力価≥20のサンプルはマイクロ中和（MN）分析法により確認した。≥20のMN結果は陽性と考えた。
HIの結果は15,689検体の内298でH6N2ウイルスについて≥20であった、そしてとMNの結果は298検体の内63で陽性であった（全体的な血清陽性範囲 20～320, 平均32.7, 0.4%）（オンライン技術付録 表2）。血清陽性であった割合は、職業的暴露に応じて明らかに違っていた（p=0.0125）。血清陽性は家禽市場の労働者の間で最も高く、小規模養鶏農家、そして野鳥生息地の労働者であった（それぞれ、0.66%、0.42%、そして0.51%）（表）。カイ二乗検定結果によると、家禽市場の労働者の間の血清陽性は、大規模養鶏農家の間のそれよりも明らかに高かった（p=0.015、調整後α=0.005）。無条件ロジスティック回帰モデルによる分析は、家禽市場への曝露は鳥インフルエンザH6ウイルスのヒト感染の危険因子であることを示した（オッズ比2.1, 95%CI 1.27～3.47）。
血清陽性は、検査した男女の間で有意差はなかった（p=0.08）（表）。小児はH6N2陽性ではなかった。他の年齢層では、血清陽性は0.25%から0.45%の範囲であったが、違いはなかった（P> 0.05）（表）。
血清検体が収集された22省の内、北部11省、南部11省であった。陽性検体は、南部全ての省で検出された。中国北部の河南省、遼寧省、吉林省では検出されなかった。カイ二乗検定結果によると、中国南部の血清陽性は、中国北部における血清陽性よりもかなり高かった（p=0.0375）（表）。
中国本土におけるインフルエンザH6ウイルスのヒト感染は報告されていないが、我々の研究で検査した63の血清検体はH6ウイルスに陽性であった。血清陽性のこのレベルは、我々が検査した2検体だけが陽性であった（データは示さず）ため、高病原性鳥インフルエンザA

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリンIH静注1000単位の記載を示す。
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抗体陰性であることを確認している。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ウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

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（H5N1）ウイルスのためのそれよりもはるかに高いが、低病原性鳥インフルエンザ A（H9N2）ウイルスの血清陽性レベルよりはるかに低い；検査したサンプルの 3.4%は、A/トリ/香港/G9/1997（H9N2）一様ウイルスに陽性であった（データは示さず）。
以前の米国の研究は、獣医師において H6N2 抗体陽性を報告していた。我々の結果および獣医師の研究は、H6N2 ウイルスがヒトに感染する可能性があることを示している。
我々の研究では、陽性サンプルは 22 省の内 19 省で、そして全ての検査した労働者集団で検出され、H6 ウイルスが中国の鳥類で広く流行していることを示唆している。家禽市場の曝露は、鳥インフルエンザ H6 ウイルスのヒト感染の主要な危険因子である。この研究の限界は、抗原の選択は H6 ウイルスの異なるサブタイプのための中和抗体を正確に検出しない可能性があることにある。鳥類における H6 ウイルスおよび職業的暴露集団の監視は、パンデミック対策のために強化されるべきである。

報告企業の意見	今後の対応
インフルエンザウイルス（influenza virus）は、オルトミクソウイルス科（Orthomyxoviridae）に属する A 型インフルエンザウイルス（influenzavirus A）、B 型インフルエンザウイルス（influenzavirus B）、C 型インフルエンザウイルス（influenzavirus C）の 3 属を指す。A 型と B 型のウイルス粒子表面にはヘマグルチニン（HA）とノイラミニダーゼ（NA）の糖蛋白があり、これらが感染防御免疫の標的抗原となっている。特に A 型では、16 種類の HA と 9 種類の NA の組み合わせにより様々なウイルスが、ヒト以外にもブタやトリなどその他の宿主に広く分布している。ウイルスの大きさは直径 80～120nm の球形粒子で、エンベロープを有する 1 本鎖 RNA ウイルスで、万一原料血漿にインフルエンザウイルスが混入したとしても、各種モデルウイルスのウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	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。

抗 HBs

Table. Prevalence of *Bartonella* spp. in bats from 2 sites in Dong Nai, Vietnam, 2013

Bat species	No. *Bartonella* spp.–positive bats/no. bats trapped (%)		
	Cat Tien National Park	Dong Nai Nature Reserve	Total
*Cynopterus sphinx**	0/0	0/14	0/14 (0)
Hipposideros armiger†	2/6	0/0	2/6 (33.3)
Hipposideros larvatus†	3/5	0/0	3/5 (60)
*Megaerops niphanae**	0/0	½	1/2 (50)
Megaderma spasma†	0/0	1/2	1/2 (50)
Megaderma lyra‡	1/1	0/0	1/1 (100)
Rhinolophus acuminatus†	0/0	9/17	9/17 (52.9)
Rhinolophus chaseli†	2/5	0/0	2/5 (40)
Rhinolophus sinicus†	0/3	2/4	2/7 (28.6)
Rhinolophus luctus†	0/1	0/0	0/1 (0)
Total	8/21 (38.1)	13/39 (33.3)	21/60 (35)

*Fruit-eating.
†Insectivorous.
‡Carnivorous.

caused by crowded roosting areas and sharing of roosts by multiple species. This behavior provides opportunities for transmission of *Bartonella* bacteria or exchange of infected ectoparasites, such as *Cyclopodia* spp. (*8*), although the precise roles of these 2 processes are unknown.

Although no human cases of *Bartonella* spp. infection have been reported in Vietnam, *Bartonella* spp. have been identified in febrile humans elsewhere in Southeast Asia (*9*) and are also common in rats in southern Vietnam (*10*). Because close contact with bats (i.e., through manure farming and consumption of bat meat) and potential arthropod vectors (i.e., through handling and consumption of fruit) is common in parts of Vietnam, targeted screening of bats and their human contacts might improve our understanding of the zoonotic potential of these bacteria and their potential effect on public health.

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Seropositivity for Avian Influenza H6 Virus among Humans, China

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To the Editor: Influenza virus subtype H6 was first isolated from a turkey in 1965 in the United States (*1*) and was subsequently found in other parts of the world (*2*). Over the past several decades, the prevalence of H6 virus has dramatically increased in wild and domestic birds (*2–4*). In China, highly pathogenic influenza A(H5N1), low pathogenicity influenza (H9N2), and H6 are the most prevalent avian influenza viruses among poultry (*5*). Although only 1 case of H6 virus infection in a human has been reported worldwide (*6*), several biological characteristics of H6 viruses indicate that they are highly infectious to mammals. Approximately 34% of H6 viruses circulating in China have enhanced affinity to human-like receptors (α-2,6 NeuAcGal) (*2*). H6 viruses can also infect mice without prior adaptation (*2,7*), and some H6 viruses can be transmitted efficiently among guinea pigs (*2*). To evaluate the potential threat of H6 viruses to human health, we conducted a systematic serologic study in populations occupationally exposed to H6 viruses.

During 2009–2011, a total of 15,689 serum samples were collected from live poultry market workers, backyard poultry farmers, large-scale poultry farmers, poultry-slaughter factory workers, and wild bird habitat workers in 22 provinces in mainland China. A/chicken/Y94/Guangdong/2011 (H6N2), a representative isolate of predominant H6 viruses in mainland China, was used for the serologic testing (online Technical Appendix Table 1, Figures 1, 2, http://wwwnc.cdc.gov/EID/article/21/7/15-0135-Techapp1.pdf). Hemagglutination inhibition (HI) assay was performed for all serum samples, and samples with an HI titer ≥20 were verified by a microneutralization (MN) assay, as indicated by World Health Organization guidelines (*8*). An MN result of ≥20 was considered positive.

The HI result was ≥20 for H6N2 virus in 298 of the 15,689 specimens, and the MN result was positive in 63 of the 298 specimens (overall seropositivity range 20–320, mean 32.7, 0.4%) (online Technical Appendix Table 2). The proportion of group members who were seropositive differed significantly according to occupational exposure ($p = 0.0125$). Seropositivity was highest among workers in live poultry markets, backyard poultry farmers, and workers in wild bird habitats (0.66%, 0.42%, and 0.51%, respectively) (Table). According to χ^2 test results, seropositivity among workers in live poultry markets was significantly higher than that among large-scale poultry farmers ($p = 0.0015$, adjusted $\alpha = 0.005$. Analysis by unconditional logistic regression model showed that exposure to live poultry markets was a risk factor for human infection with avian influenza H6 virus (odds ratio 2.1, 95% CI 1.27–3.47).

Seropositivity did not differ significantly among male and female persons tested ($p = 0.08$) (Table). No children were positive for the H6N2 virus. For other age groups, seropositivity ranged from 0.25% to 0.45%, but differences were not significant ($p>0.05$) (Table).

Of the 22 provinces from which serum specimens were collected, 11 were northern provinces and 11 were southern provinces. Positive specimens were detected in all southern provinces. In northern China, no seropositive results were detected in Henan, Liaoning, or Jilin Provinces. According to χ^2 test results, seropositivity in southern China was significantly higher than seropositivity in northern China ($p = 0.0375$) (Table).

Human infection with influenza H6 virus in mainland China has not been reported, but 63 serum specimens tested in our study were positive for the H6 virus. This level of seropositivity is much higher than that for highly pathogenic

Table. Seropositivity of occupationally exposed populations for the influenza (H6N2) virus, China, 2009–2011*

Population	Total no. serum samples	Mean titer for MN ≥20	No. serum samples with MN ≥20	Seropositivity (95% CI)	Odds ratio† (95% CI)
Total	15,689	32.70	63	0.40 (0.40–0.41)	
Occupation					
Live poultry market	3,950	43.08	26	0.66 (0.64–0.68)	2.10 (1.27–3.47)
Poultry farm	3,762	25.71	7	0.19 (0.18–0.19)	0.40 (0.18–0.87)
Backyard poultry farm	4,324	26.67	18	0.42 (0.40–0.43)	1.05 (0.61–1.82)
Poultry slaughter factory	1,235	30.00	2	0.16 (0.15–0.17)	0.38 (0.09–1.57)
Wild bird habitat	788	20.00	4	0.51 (0.47–0.54)	1.28 (0.47–3.54)
Other	1,630	23.33	6	0.37 (0.35–0.39)	0.91 (0.39–2.11)
Sex					
F	7,620	24.29	28	0.37 (0.36–0.38)	Reference
M	8,069	39.39	35	0.43 (0.42–0.44)	1.18 (0.72–1.94)
Age group, y					
Children, ≤14	74	–	0	0	0 (0)
Youth, 15–24	1,168	20.00	3	0.26 (0.24–0.27)	0.75 (0.19–3.00)
Adult, 25–59	1,2450	34.07	54	0.43 (0.43–0.44)	1.27 (0.54–2.94)
Elderly, ≥60	1,748	13.33	6	0.34 (0.33–0.36)	Reference
No age record	249	–	0	0	–
Geographic distribution					
South	10,522	32.00	50	0.48 (0.47–0.48)	Reference
North	5,167	35.38	13	0.25 (0.24–0.26)	0.59 (0.30–1.15)

*MN, microneutralization; –, not applicable.
†Odds ratios were calculated by using unconditional logistic regression model (SPSS 17.0, Armonk, NY, USA).

avian influenza A(H5N1) virus, for which only 2 of the serum specimens we tested were positive (data not shown), but much lower than the seropositivity level for low pathogenicity avian influenza A(H9N2) virus; 3.4% of the samples tested were positive for A/Chicken/Hong Kong/G9/1997(H9N2)–like virus (data not shown). A previous US study has reported H6N2-positive antibodies in veterinarians (*9*). Our results and the veterinarian study indicate that the H6N2 virus could infect humans.

In our study, positive samples were detected in 19 of 22 provinces and in all tested worker populations, suggesting that the H6 virus has been broadly circulating in birds in China. Live poultry market exposure is the major risk factor for human infection with avian influenza H6 virus. The limitation of this study is that antigen selection may not accurately detect neutralization antibodies for different subtypes of H6 viruses. Surveillance of the H6 virus in birds and occupationally exposed populations should be strengthened for pandemic preparedness.

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Absence of MERS-Coronavirus in Bactrian Camels, Southern Mongolia, November 2014

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To the Editor: Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified among humans in 2012 in Saudi Arabia (*1*). As of February 5, 2015, a total of 971 MERS cases and 356 associated deaths had been confirmed (*2*). Because MERS is a zoonotic disease, it is essential that the animal reservoirs and hosts that sustain virus circulation in nature be identified.

Seroepidemiologic and virologic studies have demonstrated evidence of MERS-CoV infection in dromedary camels (*Camelus dromedarius*) in the Arabian Peninsula (*3*), and viruses isolated from dromedaries appear capable of infecting the human respiratory tract (*4*). In some instances, MERS-CoV infection in dromedaries has preceded infection in humans (*5*), indicating that dromedaries are a natural host for MERS-CoV and a possible source of human infection. Thus, it is important to define the geographic range of MERS-CoV infection in camels and the species of camelids that are infected by MERS-CoV in nature.

Two species of camels exist: 1-hump dromedaries (*C. dromedarius*) and 2-hump Bactrian camels (*C. bactrianus*).

[1]These authors contributed equally to the article.

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販売名(企業名)	－					

研究報告の概要

重症熱性血小板減少症候群(SFTS)は、2011 年に中国で初めて明らかとなった新規のブニアウイルス科の SFTS ウイルス(SFTSV)によるマダニ媒介感染症であり、死亡率が高いことや有効な治療法やワクチンがないことから社会的な問題となっている。
本邦では 2013 年 1 月に初めて患者が報告され、2015 年 1 月までに 102 例が報告されている。死亡率は約 3 割と高い。
また、本邦では報告されていないが、中国では患者の血液、体液を介した二次感染事例も報告されている。
診断については PCR による診断が確立されているが、今後は簡便で迅速な病原体診断法の開発と抗体検査法の普及が望まれる。
治療については現在のところ根本的な治療法やワクチンはなく、全身管理による対症療法となるが、早期診断による臨床現場での迅速で適切な対応が求められる。そのためには、一般住民への啓発、注意喚起も重要である。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
［患者への説明］
本剤の投与又は処方にあたっては、疾病の治療における本剤の必要性とともに、本剤の製造に際し感染症の伝播を防止するための安全対策が講じられているが、ヒト血液を原料としていることに由来する感染症伝播のリスクを完全に排除することができないことを、患者に対して説明し、理解を得るよう努めること。

報告企業の意見	今後の対応
重症熱性血小板減少症候群(SFTS)は、SFTSV によって引き起こされるダニ媒介性感染症で、国内においても 2013 年 1 月に患者が確認され、中国においては血液、体液を介した二次感染事例の報告についても記載されている。 現時点まで血漿分画製剤から伝播が疑われた報告はないが、今後の情報に注目していきたい。	今後とも SFTSV に関する情報等に留意していく。

㈱シンポジウム 1
X1580007

S1-3

重症熱性血小板減少症候群 (SFTS) の実態と対応

岸本　寿男
岡山県環境保健センター

重症熱性血小板減少症候群 (SFTS) は、2011年に中国で初めて明らかとなった新規のブニア ウイルス科のSFTSウイルス (SFTSV) によるマダニ媒介感染症である。死亡率が高いことや、有効な治療法やワクチンがないことから、社会的な問題となっている。

わが国では2013年1月に初めての患者が報告されて以来、検査法の確立と検査体制の整備、四類感染症の指定、また研究班の組織などの体制づくりが迅速になされてきた。また全国でのマダニや動物の疫学調査が進められ、徐々に実態が明らかにされつつあるが、まだ不明な点も多く、臨床面での課題や今後感染リスク、重症化リスク等の軽減をはかるために解決すべき課題も多い。

2013年1月から2015年1月までに102例が報告されているが、月別発生患者数は春から秋までが最も多く、西日本を中心に発生している。患者は高齢者が多く、重症例、死亡例も60-80歳代の高齢者に多い傾向があり、死亡率も約3割と高い。また、わが国では報告されていないが中国では患者の血液、体液を介した二次感染事例も報告されており、院内感染対策も重要である。マダニや動物の疫学調査の成績からは、広域で多種のマダニがSFTSVを保有し、動物も抗体を保有していることから、現在西日本を中心に報告されている症例が、さらに広がる可能性が示唆されている。わが国で分離されたSFTSVの遺伝子解析によると、中国の株とは異なり、土着の株がほとんどを占めることが明らかとなっている。診断については、現在PCRによる診断が確立されているが、今後は簡便で迅速な病原体診断法の開発と抗体検査法の普及が望まれる。治療については、現在のところ、根本的な治療法やワクチンはない。全身管理による対症療法となるが、早期診断による臨床現場での迅速で適切な対応が求められる。そのためには、一般住民への啓発、注意喚起も重要である。

別紙様式第2-1

No. 3

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
				2015. 6. 4	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	Kim WY, Choi W, Park SW, Wang EB, Lee WJ, Jee Y, Lim KS, Lee HJ, Kim SM, Lee SO, Choi SH, Kim YS, Woo JH, Kim SH. Clin Infect Dis. 2015 Jun 1; 60(11):1681-3. doi: 10.1093/cid/civ128. Epub 2015 Feb 18.	公表国 韓国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社).			

研究報告の概要

○韓国における重症熱性血小板減少症候群の院内感染
韓国で重症熱性血小板減少症候群(SFTS)患者のケアを行った27名の医療従事者のうち、心肺機能蘇生を担当した4名が発熱を訴え、セロコンバージョンを起こしSFTSと診断された。呼吸器分泌物、血液、体液に汚れたガウンが、医療従事者のSFTS感染と有意に関連していた。
我々の報告並びにこれまでの研究は、SFTSVを院内感染病原体の候補リストに加える必要性を示唆している。
医療従事者は、患者、特にウイルス性出血熱またはダニ媒介性リケッチア症の疑いを有する患者との接触時において、血液および体液に関する所定の注意事項を厳守する必要がある。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
韓国で重症熱性血小板減少症候群(SFTS)患者のケアを行った4名の医療従事者が、SFTSに院内感染したとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また国内症例については、発熱などの体調不良者を献血不適とすることで対応している。今後も引き続き情報の収集に努める。

Nosocomial Transmission of Severe Fever With Thrombocytopenia Syndrome in Korea

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Of the 27 healthcare workers (HCWs) who had contact with a fatally ill patient with severe thrombocytopenia syndrome in Korea (SFTS), 4 who were involved in cardiopulmonary resuscitation complained of fever and were diagnosed with SFTS via seroconversion. Exposure to respiratory secretions, blood, or gowns soiled by body fluids was significantly associated with infection of HCWs.

Keywords. SFTS; nosocomial transmission; healthcare worker.

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease, first reported in China [1] and recently reported in Korea and Japan [2, 3]. The causative agent is a novel bunyavirus, designated SFTS virus (SFTSV) [1]. Although SFTS is thought to be transmitted by ticks such as *Haemaphysalis longicornis* [4], the exact mode of transmission remains unclear. Previous studies have identified several clusters of SFTSV infections in family members that appear to have been transmitted by human contact [4–9]. However, only 2 studies mention possible transmission from index patients to healthcare workers (HCWs) [4, 6], and data on the details of nosocomial transmission of SFTSV are limited in terms of attack rates and risk factors for transmission to HCWs. We report the results of an investigation of apparent cases of nosocomial transmission of SFTSV to HCWs.

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SUBJECTS AND METHODS

Epidemiologic Investigation

The cluster involving suspected nosocomial transmission occurred in a tertiary care hospital in Seoul, South Korea. On 15 September 2014, a doctor working in the emergency department was admitted with fever to the infectious disease ward. During history taking, his contact on 4 September 2014 with the index patient with suspected fatal scrub typhus was noted. On 18 September 2014, we received a report from the Korea Centers for Disease Control and Prevention that the index patient was positive for reverse transcription polymerase chain reaction (RT-PCR) for SFTSV. At that point, we suspected possible nosocomial transmission from the index patient. An epidemiological investigation of all HCWs who had been in contact with the index patient was immediately initiated. A standardized questionnaire was used to collect demographic information, symptoms, details of exposure to the index patient, and history of outdoor activity. We collected paired sera from all the HCWs between 19 and 25 September (about 3 weeks after exposure to the index patient) and 13–17 October (about 6 weeks after exposure to the index patient).

Laboratory Testing

An immunofluorescence assay (IFA) was used to detect anti-SFTSV immunoglobulin G (IgG), and RT-PCR was performed to detect SFTSV RNA. RNA was extracted from the serum using a viral RNA extraction kit (iNtRON Biotechnology, Gyeonggi, South Korea) according to the manufacturer's instructions. To detect SFTSV RNA, the one-step RT-PCR was performed using a DiaStar 2X OneStep RT-PCR Pre-Mix kit (SolGent, Daejeon, South Korea) with the primers MF3 (5′-GATGAGATGGTCCATGCTGATTCT-3′) and MR2 (5′-CTCATGGGGTGGAATGTCCTCAC-3′) under the following condition: 30 minutes at 50°C for reverse transcription and 15 minutes at 95°C for denaturation as initial step, followed by 35 cycles of 20 seconds at 95°C, 40 seconds at 58°C, and 30 seconds at 72°C, and a final extension step of 5 minutes of 72°C. Virus was isolated by incubating sera into 2 wells of Vero E6 cells. For IFA, Vero E6 cells infected with SFTSV were incubated at 37°C in a 5% CO_2 incubator. Cells were harvested, inoculated,

Table 1. Clinical, Laboratory, and Serological Findings for the Index Patient and 4 Healthcare Workers With Severe Fever With Thrombocytopenia Syndrome

		Nurses		Doctors	
Characteristic	Index	1	2	1	2
From contact to symptom onset	NA	5 d	12 d	7 d	9 d
Clinical findings					
Fever	Yes	Yes	Yes	Yes	Yes
Myalgia	Yes	Yes	Yes	Yes	Yes
Malaise	Yes	Yes	Yes	Yes	Yes
Bleeding	Yes	No	No	No	No
Rash	Yes	No	No	No	No
Personal protective device use					
Mask	NA	Yes	Yes	Yes	Yes
Glove	NA	No	No	Yes	Yes
Facial shield or goggle	NA	No	No	No	No
White blood cell count ($\times 10^9$/mL)	10 000	2900	2600	6000	2100
Platelet count ($\times 10^9$/mL)	52	151	123	221	101
IFA (IgG)					
Acute	<1:32	1:512	<1:32	1:64	<1:32
Convalescence	NA	1:1024	1:512	1:1024	1:512
RT-PCR	(+)	(–)	(–)	(–)	(–)
Viral culture	(+)	ND	ND	ND	ND

Abbreviations: (+), positive results; (–), negative results; IFA, immunofluorescence assay; IgG, immunoglobulin G; NA, not applicable; ND, not done; RT-PCR, reverse transcription polymerase chain reaction.

and fixed with acetone on Teflon-coated well slides. IFA was carried out using the patient's serum as the primary antibody and fluorescein-labeled antihuman IgG secondary antibodies (Thermo Fisher Scientific). A monoclonal anti-SFTSV N antibody (manufactured in our laboratory) was used as the positive control.

Statistical Analysis

Categorical variables were compared using the χ^2 test or Fisher exact test, as appropriate. All tests of significance were 2-tailed and a P value of <.05 was considered to indicate statistical significance. Calculations were performed using the SPSS for Windows software package, version 21 (SPSS Inc, Chicago, Illinois).

RESULTS

Index Patient

The index patient was a 68-year-old woman who lived in a rural area 50 km from Seoul and frequently worked in a kitchen garden. She was admitted to hospital with altered mental status on 4 September 2014. Physical exam revealed an eschar on her arm. Laboratory testing performed on admission revealed leukopenia (white blood cell count, 10.0×10^9/L) and thrombocytopenia (platelet count, 52×10^9/L). Scrub typhus, which is endemic in South Korea, was suspected initially and doxycycline was administrated. Seizure with respiratory arrest occurred 9 hours after admission, and cardiopulmonary resuscitation (CPR) was performed. Despite this, the patient died 12 hours after admission. The final diagnosis was available on 18 September 2014: RT-PCR for SFTSV was positive and the viral titer was 3.7×10^8 copies/mL. Culture was positive for SFTSV, but IgG against SFTSV was <1:32 (Table 1).

Nosocomial Cases of SFTS and Contact Investigation

A total of 27 HCWs contacted the index patient in the emergency department and isolation ward. Of these, 7 were actively involved in CPR of the index patient, of whom 4 complained of fever (Table 1). The median time from contact to symptom onset was 8 days (range, 5–12 days). At the time of the epidemiologic investigation, only 1 of the HCWs, a doctor, had fever. PCR using sera from these 4 HCWs about 3 weeks after the exposure gave negative results. IFA using paired sera obtained from all the HCWs at about 3 weeks and again about 6 weeks after the exposure revealed seroconversion in 3 of the symptomatic HCWs and a 2-fold increase in titer in the remaining symptomatic HCW (Table 1). One HCW without any symptoms had IgG titers of 1:256 both 3 and 6 weeks after the exposure. He had no recent history of outdoor activity. The remaining 22 HCWs all exhibited IgG titers of <1:32 approximately 3 weeks and 6 weeks after the exposure. There was no evidence by serology of subclinical infection. The overall attack rate was 15% (95% confidence interval [CI], 4%–34%), but in the subgroup of 7 HCWs who were actively involved in CPR, the attack rate was 57% (95% CI, 18%–90%). HCWs who were exposed to respiratory secretions (3 of 7 HCWs) and blood (4 of 13 HCWs) demonstrated more symptomatic infection than those who were not exposed to respiratory secretions (1 of 20 HCWs; $P = .02$) or blood (0 of 14 HCWs; $P = .04$). In addition, HCWs who had gowns soiled with body fluid (3 of 5 HCWs) exhibited more symptomatic infection than those who did not (1 of 22 HCWs; $P = .01$). None of the HCWs used a face shield or goggles as personal protective equipment (PPE). Only 9 HCWs wore a surgical mask, 5 wore gloves, and 3 wore a surgical mask and gloves. Four of 11 HCWs who had used PPE (ie, surgical mask, gloves) had symptomatic infection vs 0 of 16 HCWs who had not used PPE ($P = .02$). Four of 9 HCWs who wore surgical mask had symptomatic infection vs 0 of 17 HCWs who did not ($P = .007$), and 2 of 5 HCWs who wore gloves had symptomatic infection vs 2 of 22 HCWs who did not ($P = .14$).

DISCUSSION

The previous studies mentioned possible transmission from the index patient to HCWs [4, 6]. Our report, together with

previous studies, indicates that SFTSV should be listed among possible nosocomially transmissible pathogens. Indeed, the HCWs who contacted the index patient did not wear appropriate PPE (as they would have been unlikely to do in a developed country) because the initial presumptive diagnosis was scrub typhus, in which no human-to-human transmission has been reported. We found that wearing PPE such as mask or gloves was associated with an increased risk of transmission. Possible explanations could be that the PPE was a proxy for the risk procedures in this study; on the other hand, inappropriate use of PPE (ie, only 3 HCWs wore both surgical mask and gloves and there was no facial shield or goggle usage) might not protect against transmission of SFTSV. The strict adherence to routine blood and body fluid precautions is necessary when HCWs are in contact with any patient, especially with anyone with suspected viral hemorrhagic fever or a tick-borne rickettsial disease.

There was no evidence of subclinical infection in any of the HCWs who were in contact with the index patient. Data on subclinical infection with SFTSV are limited. One study reported 1 symptomatic and 1 asymptomatic infection with SFTSV among 6 family members who were contacts of a fatally ill patient with SFTS [9]. Further studies are needed on this issue.

Our study has several limitations. First, infection in 1 of the symptomatic HCWs was not confirmed by IFA because there was only a 2-fold increase in antibody titer between the paired samples. However, those symptoms developed 5 days after contact with the index patient. The first serum was taken 2 weeks after symptom onset, and the IFA titer was high (1:512). We therefore assume that we were unable to document a 4-fold rise in titer because we failed to get an acute-stage serum sample. Second, because all HCWs breached the universal precautions, we could not evaluate what type of breaches of standard precautions were associated with SFTS transmission.

In conclusion, we have demonstrated transmission of SFTSV from a fatally ill patient to HCWs, possibly by blood or respiratory secretions. Standard strict precautions are needed with suspected patients with SFTS.

Notes

Acknowledgments. We thank the subjects who volunteered for this study. We also thank the members in the Office for Infection Control, Asan Medical Center, Seoul, South Korea.

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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別紙様式第2-1

No. 2

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
				2015. 5. 15	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		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		ProMED 20150513.3357776	オーストラリア

研究報告の概要

○ロスリバーウイルス－オーストラリア

[1]西オーストラリア州

西オーストラリア保健サービスは、同州の中西部でロスリバーウイルス感染症が増加していることを受け、警報を発した。2015年4月中には16症例が報告されたが、2014年の同時期には0件だった。同疾患は蚊の刺咬により媒介され、関節痛、筋肉痛、疲労、発熱、頭痛及び発疹を引き起こすことがある。保健サービスは、蚊に刺されるのを防ぐよう、住民に呼びかけている。

[2]クイーンズランド州

ブリズベンで発生した暴風雨により蚊が大量発生し、ロスリバーウイルスアウトブレイクが長期化している。季節が変わっても蚊による疾患拡大が止まらず、1週間で80人以上が感染した。2015年1月以降、同州の感染者は4,645人を超え、過去20年間で最悪となっている。

ブリズベン市では、南東部の広範囲で洪水が発生した5月1日の暴風雨の後、大規模な殺虫剤散布を行った。

クイーンズランド保健当局は、同アウトブレイクは周期的なものであり、蚊の抑制措置と季節の変化によってブリズベン地区の全域で蚊の数が減少傾向に転じており、ピークは過ぎたと述べている。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
オーストラリアの西オーストラリア州及びクイーンズランド州でロスリバーウイルス感染症のアウトブレイクが起こり、クイーンズランド州では長期化したことから過去20年で最悪となっているとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

JRC2015T-016

Published Date: 2015-05-13 11:57:29
Subject: PRO/AH/EDR> Ross River virus - Australia (08): (WA, QL) alert
Archive Number: 20150513.3357776

ROSS RIVER VIRUS - AUSTRALIA (08): (WESTERN AUSTRALIA, QUEENSLAND) ALERT
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A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

In this update:
[1] Western Australia
[2] Queensland

******
[1] Western Australia
Date: Sun 10 May 2015
Source: ABC (Australian Broadcasting Corporation) [edited]
http://www.abc.net.au/news/2015-05-11/rise-in-mid-west-wa-ross-river-cases-prompts/6460570

A jump in Ross River virus cases.in midwest Western Australia has prompted warnings from the WA [Western Australia] Country Health Service.

In April this year [2015], 16 cases were reported, compared to none during the same period last year [2014].

The disease is transmitted through mosquito bite.

Dr Marisa Gilles is urging locals and visitors to take precautions. She said mosquito numbers could be reduced by removing stagnant water around the home or campsites.

The health service said Ross River virus and Barmah Forest virus could cause painful joints, aching muscles, tiredness, fever, headache, and skin rashes. These symptoms could last for several weeks and sometimes months.

The health service said people should try to avoid mosquito bites by:
- Avoiding outdoor exposure around dawn and dusk, especially the 1st few hours after dark.
- Wearing protective, long, loose-fitting, light-coloured clothing when outdoors.
- Applying a personal repellent containing diethyltoluamide [DEET] or picaridin to exposed skin or clothing. The most effective and long-lasting formulations are lotions or gels. Natural or organic repellents are generally not as effective as DEET or picaridin or need to be reapplied more frequently.
- Using mosquito coils and mosquito lanterns and applying barrier sprays containing bifenthrin in patio and outdoor areas around houses.
- Ensuring insect screens are installed and in good condition on houses and caravans.
- Using mosquito nets and mosquito-proof tents when camping.
- Ensuring infants and children are adequately protected against mosquito bites, preferably with suitable clothing, bed nets or other forms of insect screening.

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<promed@promedmail.org>

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[2] Queensland
Date: Tue 12 May 2015
Source: Courier Mail [edited]
http://www.couriermail.com.au/news/queensland/southeast-queenslands-freak-storm-prolongs-ross-river-virus-outbreak/story-fnn8dlfs-1227350878606

Brisbane's freak storm has unleashed another wave of mosquitoes and prolonged the outbreak of Ross River virus. The onset of cooler weather has done little to stop mozzies spreading the disease [virus], which infects more than 80 people a week.

Brisbane City Council has carried out more mass insect spraying following the disastrous 1 May [2015] storm that caused widespread flooding in the southeast.

Nigel Beebe, a vector biologist from the University of Queensland and CSIRO, said saltmarsh mosquitoes, which transmit Ross River virus, had this year [2015] been blessed with perfect breeding conditions. "We've just had ideal rainfall," he said. "It's allowed mosquito populations to lift to a new level. It's been an unusual year for the Ross River virus [transmission]."

Dr Beebe said the mosquito explosion over summer and early autumn had coincided with high populations of other insects, such as butterflies and grasshoppers. "It's been a very good season for insects," he said.

Ross River virus has struck down more than 4645 people since 1 Jan [2015], which makes it the worst outbreak in almost 20 years.

A Queensland Health spokesman said the outbreak had been "cyclic" and had already peaked. "Due to the storm, some breeding sites in the Brisbane area have produced more mosquitoes," a spokesman said. "However, overall numbers of Ross River virus mosquitoes are reducing across the greater Brisbane area due to ongoing control measures and the onset of cooler weather."

The initial outbreak was triggered at the start of the year [2015] by torrential rain and kingtides, which flushed out saltmarshes along the Queensland coast.

Symptoms of Ross River virus include headaches, fevers, joint or muscle pain, rash and fatigue. The debilitating viral illness can cause patients to be bedridden for weeks. The effects of Ross River virus [infection], named after the river in northern Queensland where it was 1st identified, can continue to last for years.

[Byline: Damon Guppy]

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[Ross River virus (RRV) infections occur sporadically across Australia. This has been a very active year for RRV transmission, with 6404 cases reported as of 25 Apr 2015 (see ProMED-mail archive no 20150426.3322934). Especially hard hit has been Queensland state, where cases have been mounting, approaching 4000 (see ProMED-mail archive no 20150328.3262332) and now reaching 4645 cases reported above. Cases are occurring currently across the country in Western Australia, as indicated in the report above.

Ross River virus is a zoonotic alphavirus transmitted by a wide range of mosquitoes, including _Aedes_ and _Culex_ species. The recommendation to avoid mosquito bites is prudent and should be adopted by individuals living in or visiting these hot spots where transmission is occurring.

Some useful references about RRVD provided in an earlier post by Steve Berger include:
1. Berger S: Infectious diseases of Australia, 2015. 616 pages, 165 graphs, 3941 references. Gideon e-books, http://www.gideononline.com/ebooks/country/infectious-diseases-of-australia/
2. Berger S: Australo-Pacific arboviruses: global status, 2015.
http://www.gideononline.com/ebooks/disease/australo-pacific-arboviruses-global-status/
3. http://www.gideononline.com/wp/wp-content/uploads/Gideon-Graphs.pps

Maps of Australia can be seen at http://www.ezilon.com/maps/images/oceania/australia-political-map.gif and http://healthmap.org/promed/p/289. - Mod.TY]

See Also

Ross River virus - Australia (07) 20150426.3322934
Ross River virus - Australia (06): (QL) 20150328.3262332
Ross River virus - Australia (05): (QL) 20150314.3230696
Ross River virus - Australia (04): (QL) alert 20150225.3192415
Ross River virus - Australia (03): (QL) background 20150219.3176519
Ross River virus - Australia (02): (QL) 20150218.3167404
Ross river virus - Australia: (NS) 20150126.3121230
.....sb/jw/ty/mj/msp/jw

別紙様式第2-1

No. 21

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
				2015. 8. 5	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		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		International Conference on Emerging Infectious Diseases, Georgia, from August 24 to 26, 2015	米国

研究報告の概要

○ミネソタ州において新たに出現したヒト疾患の原因となるジェームズタウンキャニオンウイルス
背景:ジェームズタウンキャニオンウイルス(JCV)は北米全域にわたり広く分布している蚊媒介性ウイルスであるが、ヒト疾患の原因として報告されることは稀である。JCVはブニヤウイルス科カリフォルニア血清群のウイルスであり、他のアルボウイルスと同様に典型的な症状は発熱、頭痛、疲労であり、重篤症例の場合は髄膜炎または脳炎が認められる。ミネソタ州の蚊およびオジロジカから当該ウイルスが同定され、2013年に最初のヒト感染症例が確認された。
方法:ミネソタ保健局衛生試験所は他のアルボウイルスの検査用に提出された血清検体および脳脊髄液検体を対象として、EIA法によるJCV IgM抗体検査を実施した。
結果:2014年5月から2014年10月までの期間に、61名の患者から採取した84検体を対象とした。84検体のうち、7名の患者から採取した10検体(11.9%)がJCV仮陽性となった。plaque reduction neutralization testにより、3名の患者から採取された4検体(40%)についてはJCV感染が確認され、2名の患者がラクロス脳炎であることが確認され、患者1名は全てのアルボウイルスが陰性となり、1名は結果保留となった。JCV感染が確認された患者の年齢幅は15歳から62歳であり、3名のうち2名が男性であった。発症時期は5月下旬から8月初旬であった。臨床症状は発熱、疲労、筋肉痛、関節痛、および頭痛であり、1名は脳炎を発症した。3名全員がミネソタ州において当該ウイルスに曝露されていた可能性が高いと考えられた。
結論:アルボウイルス検査用検体からのJCV感染の検出件数は上昇しており、当該ウイルスがヒト疾患の原因であることの認識の上昇に伴い、JCV症例の同定が続くことが予想される。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
ジェームズタウンキャニオンウイルス(JCV)は北米全域にわたり広く分布している蚊媒介性のブニヤウイルス科カリフォルニア血清群のウイルスであり、JCVの認識の拡大に伴い、JCV症例の同定が続くことが予想されるという報告である。	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。

JRC2015T-029

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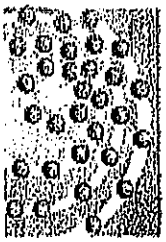

ICEID 2015

International Conference on Emerging Infectious Diseases

August 24 – 26, 2015 • Hyatt Regency Atlanta • Atlanta, Georgia

Presentation Abstract

Title: Jamestown Canyon Virus as an Emerging Cause of Human Disease in Minnesota

Category: +Novel agents of public health importance

Presentation Time Start: 8/25/2015 3:30:00 PM

Presentation Time End: 8/25/2015 3:45:00 PM

Author Block: **E. Schiffman**, D. Neitzel, J. Palm; Minnesota Dept. of Hlth., St. Paul, MN

Abstract Body: **Background:** Jamestown Canyon virus (JCV) is a mosquito-borne virus that is widely distributed throughout North America, but rarely reported as a cause of human disease. JCV is a member of the California serogroup of bunyaviruses and is likely transmitted to humans through the bite of an infected *Aedes* sp. mosquito. The clinical spectrum of human disease is not known but the typical presentation, which includes fever, headache, fatigue, and in severe cases, meningitis or encephalitis, is similar to other arboviruses. Previous studies have identified the virus in Minnesota in mosquitoes and white-tailed deer, although the enzootic maintenance cycle has not been fully described. Minnesota detected its first human case in 2013. **Methods:** In 2014, the Minnesota Department of Health Public Health Laboratory (PHL) began using an internally developed EIA assay specific to JCV on serum and CSF specimens submitted for other arboviral testing. It was suspected that prior to the development of this assay positive results were being missed due to the relatively poor sensitivity of a broader California group IFA in detecting JCV. **Results:** From May through October of 2014, the PHL performed the JCV IgM EIA on 84 samples from 61 unique patients. Of these 84, 10 (11.9%) specimens from seven patients were preliminary positives for JCV. Confirmatory testing using plaque reduction neutralization (PRNT) was performed, with four specimens (40%) from three patients confirming as JCV and four specimens (40%) from two patients confirming as La Crosse encephalitis. One patient was negative for all arboviruses by PRNT, and one is pending. The confirmed JCV patients range in age from 15 to 62, and two of the three were male. Illness onsets were from late May to early August. Clinical presentation included fever (3/3), fatigue (3/3), myalgia/arthralgia (3/3), and headache (2/3). One patient developed encephalitis. All three patients were likely exposed in Minnesota. **Conclusions:** Detection of JCV virus infections in humans has increased with the implementation of routine testing of arboviral specimens submitted to the PHL, providing further evidence of the virus as an emerging cause of human disease in Minnesota. It is expected that with continued testing and increased awareness of JCV as a cause of human disease, cases will continue to be identified.

医薬品 研究報告 調査報告書

識別番号・報告回数		回	報告日 年 月 日	第一報入手日 2015年 7月 21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	Eghrari et al., First Human Case of Fungal Keratitis Caused by a Putatively Novel Species of Lophotrichus, Journal of Clinical Microbiology. 2015;:. doi: 10.1128/JCM.00471-15.	公表国 米国	
販売名(企業名)						

研究報告の概要

問題点：米国においてLophotrichus属の新種と推定される菌類による侵襲性真菌性角膜炎を呈した初めてのヒト症例が確認された。

【概要】新たにヒトにおいて感染することが認められた感染症に関する情報。

［症例報告］
本症例は、就寝中に飼い犬に右眼を踏まれ、5日後（2011年4月）に救急科を受診した米国メリーランド州在住の50歳女性であった。受傷後すぐに異物感および霧視を呈し、受傷4日目に急激な視力低下が発現した。救急科受診時には犬に踏まれた右眼は明暗視のみ可能であった。コンタクトレンズの使用歴はなく、受診１年前に白内障手術（両眼）を受けており、読書以外では眼鏡を使用していなかった。受傷5日目の理学的検査では右眼光覚あり（裸眼）、左眼分数視力20/25（視力0.8）、求心性瞳孔反射異常は認められなかった。右眼の細隙灯顕微鏡検査にて上下眼瞼に紅斑および浮腫を認め、結膜充血（3＋）を呈し、角膜には黄緑色の分泌物を伴う直径6mmの大型の中心部潰瘍が生じていた。前眼房に水晶体または後眼房が目視できないほど著明な線維化を認め、バンコマイシンおよびトブラマイシン点眼液の投与（右眼）を開始した。角膜潰瘍の擦過物は培養試験に供試された。受傷6日目に経口ドキシサイクリンおよびシプロフロキサシン軟膏による治療を追加した。

超音波検査では脈絡膜剥離または網膜剥離は認められなかったが、受傷10日目に重度の疼痛を訴えたため点眼の回数を増やしたものの、顕著な血管新生形成と菲薄化を伴い、角膜浸潤および角膜潰瘍は改善しなかった。眼圧触診検査にて眼球の硬さは柔らかく、全周（360度）に浅い脈絡膜剥離を認め、角膜の微穿孔と一致した。

角膜の潰瘍の擦過物培養にて糸状菌類が確認された。バンコマイシンおよびトブラマイシン治療に加えてボリコナゾール1%の点眼投与を開始した。膜生検を行い、眼表面の羊膜移植を施行したところ、受傷17日目に角膜浸潤は消失し始め、角膜は部分的に上皮化した。角右視力は光覚弁であった。受傷31日目に右眼の角膜は上皮化したが、充血した虹彩血管を伴う3.5mmの前房出血を呈し、既往のヘルペス感染の経過と一致したことから、経口アシクロビルおよびプレドニゾロン酢酸1%点眼液の投与を開始した。バンコマイシンおよびトブラマイシンは投与期間6週間で中止し、ボリコナゾール点眼薬は術後１年で中止した。角膜下部に大きな傍中心瘢痕と顕著な虹彩角膜癒着が残り、眼圧調整のためにドルゾラミド/チモロール点眼液を、ヘルペス感染の再発を防ぐために経口アシクロビルを継続して投与した。右眼は新たな潰瘍や湿潤を呈することなく、外見上、一年間安定した状態を保った。

［結果］
・病理組織学
角膜生検は角膜上部から得られた。損傷のない上皮が残存する深部角膜実質部位に壊死が生じていた。HE染色、PAS染色、GMS染色、Gram-Weigert染色およびBrown-Hopps法を用いた細菌類、真菌類またはアカントアメーバの染色はすべて陰性であった。
・菌学および分子生物学的同定
ITS領域におけるBLAST検索の結果、潰瘍擦過物培養で検出された糸状菌類の塩基配列はLophotrichus fimeti基準株と98%一致した。本菌は形態観察によってLophotrichus属種と同定された。基準株を含むLophotrichus属の系統解析により、本菌はLophotrichusクレードに含まれることが高く支持されたが、既知の6種（L. ampullus、L. bartlettii、L. fimeti、L. macrosporus、L. martinii、 L. plumbescens）とは支持されなかったため、本菌はLophotrichus属の新種と考えられた。

［考察］
本症例はLophotrichus属の新種と推定される菌類に起因する侵襲性真菌性角膜炎を呈した初めてのヒト感染例であり、ボリコナゾールで回復した。角膜穿孔の急速な進行はin vitroでのLophotrichus属でみられる急激な菌糸成長と一致した。潰瘍の大きさおよび重症度から第一選択薬として強化広域スペクトル抗生物質が投与されたが、それら局所抗生物質の頻回投与に対して反応不良、抗真菌剤治療で良好であったことから、潰瘍の主因は細菌感染ではないことが示唆された。真菌性角膜炎は角膜上皮欠損によって角膜への病原菌侵入が促進されることから、本症例においては犬の足に踏まれて生じた外傷によって角膜実質に病原菌が直接植菌されたか、あるいはヘルペス性角膜上皮感染により 病原菌が侵入したと考えられた。

使用上の注意記載状況・その他参考事項等

BYL-2015-0432

Eghrari et al., First Human Case of Fungal Keratitis Caused by a Putatively Novel Species of Lophotrichus, Journal of Clinical Microbiology. 2015;:. doi: 10.1128/JCM.00471-15.

報告企業の意見	今後の対応
本件は、米国においてLophotrichus属の新種と推定される菌類による侵襲性真菌性角膜炎を呈した初めてのヒト症例が確認された報告である。 コージネイトFSの製造工程における病原体除去・不活化処理は、ウイルス、細菌及び真菌に対して有効であることが報告されている。なお、2007年4月以降、コージネイトFSの販売は行っていない。	現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も、新規人畜共通感染症や新たな感染症に関する情報収集に努める。

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JCM Accepted Manuscript Posted Online 24 June 2015
J. Clin. Microbiol. doi:10.1128/JCM.00471-15

BYL-2015-0432

Revision

First Human Case of Fungal Keratitis Caused by a Putatively Novel Species of *Lophotrichus*

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Abstract

We report an aggressive fungal keratitis caused by a putatively novel species of *Lophotrichus* in a patient with traumatic injury to the cornea from a dog paw. The organism was isolated from the patient's necrotic cornea, which perforated despite coverage with hourly fortified, broad-spectrum topical antibiotic therapy. This report represents the first case of human infection caused by this species.

Key words: *Lophotrichus*, fungal keratitis, corneal ulcer, corneal perforation, ITS, beta tubulin, LSU

CASE REPORT

A 50-year-old woman from rural Maryland, USA presented to the Johns Hopkins Emergency Department in April 2011 five days after her dog stepped on her right eye during sleep. She experienced foreign body sensation and blurry vision at the time but experienced a rapid decrease in vision on Day 4. When she presented on Day 5, she could only see light and dark in the right eye. She had no known history of contact lens use. She had undergone cataract surgery in both eyes one year prior to presentation and did not wear glasses except for reading.

Physical examination conducted five days after the injury revealed uncorrected visual acuity of light perception in the right eye and 20/25 at distance in the left eye. There was no afferent pupillary defect present. Slit-lamp examination of the right eye revealed upper and lower eyelid erythema and edema. Conjunctiva was 3+ injected and cornea demonstrated large central ulceration measuring 6 mm in diameter with yellow-green discharge (Figure 1A). The anterior chamber demonstrated a robust fibrinous reaction with no view of the lens or posterior segment. The patient immediately started fortified, topical 25 mg/mL vancomycin and 14 mg/mL tobramycin ophthalmic solution hourly to the right eye. Corneal scrapings were obtained and inoculated onto three culture media: blood agar containing 5% sheep blood, chocolate agar, and Sabouraud dextrose agar with gentamicin.

On Day 6 after the injury, the patient added oral doxycycline 100 mg twice daily and ciprofloxacin ointment nightly to her regimen. Ultrasonography revealed no choroidal or retinal detachment. When the patient returned on Day 10, she complained of severe pain. Despite frequent drop use, the infiltrate and ulceration persisted with

formation of significant corneal neovascularization and inferior thinning. The eye was soft to palpation and 360 degree shallow choroidal detachments were noted, consistent with microperforation of the cornea.

At this point, four days after corneal scrapings and cultures, preliminary results demonstrated the presence of a filamentous fungus (on the chocolate agar) and very light growth (first quadrant of the culture plate) of *Corynebacterium macginleyi*. Topical voriconazole 1% was started hourly in the right eye in addition to continuing fortified vancomycin and tobramycin. She was taken to the operating room where a corneal biopsy was conducted and an Ambio5 (IOP Ophthalmics, Costa Mesa, CA, USA) amniotic membrane patch was grafted onto the ocular surface.

On Day 17 after the initial injury, the infiltrate began to clear superiorly and the cornea had partially epithelialized, at which point drops were reduced in frequency to four times daily. Visual acuity was light perception in the right eye. On Day 31, the right cornea had epithelialized, but a 3.5 mm hyphema was present with engorged iris vessels, consistent with underlying background herpetic infection. Accordingly, she was started on acyclovir 800 mg five times daily by mouth, and two days later, topical prednisolone acetate 1% ophthalmic suspension four times daily. Vancomycin and tobramycin were discontinued at six weeks, and voriconazole eye drops were discontinued at 1 year after surgery. A large, inferior paracentral scar remained (Figure 1B). Significant iridocorneal adhesions remained and the patient was maintained on dorzolamide-timolol for intraocular pressure control and 800 mg of oral acyclovir daily to prevent recurrence of herpetic infection. The right eye remained stable in appearance, with no new ulceration or infiltrate, at one year.

Histopathology

A total of three partial-thickness corneal biopsy specimens were acquired from the superior cornea; the inferior cornea was avoided due to thinning and microperforation. Histopathological examination revealed necrosis in regions of the deep cornea stroma where an intact epithelium remained (Figure 2). Hematoxylin and eosin (H&E), Periodic Acid Schiff, Gomori methenamine silver (GMS), Gram-Weigert, and Brown & Hopps stains were negative for bacteria, fungi or *Acanthamoeba* organisms.

Mycology and Molecular Identification

Culture from the initial corneal scraping grew a filamentous fungus on the chocolate agar within 4 days at 37°C and the fungus was then sub-cultured onto the potato dextrose agar for sporulation. Lactophenol cotton blue stain revealed an irregular and branched hyphal structure; no sexual structure or sporulation was seen. Growth of this fungus was inhibited by cycloheximide. DNA sequencing was performed to identify the organism using methods described previously (1). Briefly, after DNA extraction, the internal transcribed spacer (ITS) region was amplified and sequenced, and the results were analyzed by SmartGene (SmartGene, Inc., Raleigh, NC, USA) and used as a BLASTn query of NCBI database. The ITS region showed 98% identity to *Lophotrichus fimeti* type strain CBS 129.78 (GenBank AY879799.1). Since *Lophotrichus* is very rarely isolated from clinical samples and the ITS sequence result only showed 98% identity, the isolate was sent to two reference laboratories for further investigation.

The fungus was referred to the Fungus Testing Laboratory (FTL) at the University of Texas Health Science Center at San Antonio (UTHSCSA) for identification

by phenotypic characteristics (UTHSCSA DI14-343). A *Lophotrichus* species was confirmed based on morphological features on a variety of media as illustrated in Figure 3.

The fungus was also referred to the University of Alberta Microfungus Collection & Herbarium (UAMH) for species level identification, where it was accessioned as UAMH 11809. Six *Lophotrichus* strains (five of which are type strains) (*L. ampullus* UAMH 9122, *L. bartlettii* UAMH 9287^T, *L. fimeti* UAMH 4257^T, *L. macrosporus* UAMH 9258^T, *L. martinii* UAMH 8692^T and *L. plumbescens* UAMH 8710^T) were included in the phylogenetic analysis. Genomic DNA was extracted from mycelia of all seven isolates, and the ITS, LSU and beta-tubulin (BT2) genes were PCR amplified and sequenced using primer pairs BMB-CR and ITS4 for the ITS region, BMB-CR and LR7 for the LSU region, and BT2a and BT2b for the BT2 region (2-4). Maximum parsimony (MP) analyses were performed individually for each locus. MP and Bayesian analyses were performed on the combined ITS+BT2 sequences using PAUP version 4.0b10 and Mr Bayes 3.1.2 respectively (5, 6). Clade support was assessed using the full heuristic search option for 2000 bootstrap replications (7). Gaps were treated as missing data. Clades with BS value ≥ 70% were considered strongly supported. The Bayesian analysis used the general time-reversible (GTR) substitution model including estimation of invariant sites and assumed a discrete gamma distribution (GTR + I + G) as selected by the Modeltest version 3.7 (8). Four Markov chains were run simultaneously and trees were sampled every 100th generation out of a total of two million, with the first 2000 trees being discarded as "burn-in". Inferences of posterior probabilities (PP) were calculated from 18001 trees and only clades with PP values ≥95% were considered to

be strongly supported. The consensus tree was visualized using PAUP. The topology of the MP trees for individual locus was congruent. The topology of the Bayesian tree was also congruent with the single most parsimonious tree for the concatenated BT2-ITS data set. Results from MP and Bayesian analyses indicated that the fungus was closely related to *Lophotrichus* species (Figure 4). The BT2+ITS tree places UAMH 11809 in the *Lophotrichus* clade and supported by high BS and PP (100/1.00), but there is insufficient support for conspecificity with any of the other *Lophotrichus* species. Thus our case strain was identified as a putatively novel species of *Lophotrichus* and will be described in a separate study. ITS and BT2 sequence data were deposited in GenBank under accession numbers KM580494 and KM609216, respectively.

Antifungal susceptibility testing

Antifungal susceptibility testing on the case strain was performed by broth microdilution according to CLSI methods for filamentous fungi (M38-A2) (9). Minimum inhibitory concentrations (MIC) for amphotericin B, fluconazole, itraconazole, and voriconazole were read as the lowest concentration of each agent that resulted in 100% inhibition of growth compared to the growth control after 48 hours incubation. The MIC results were as follows: amphotericin B, 2 µg/mL; fluconazole, 64 µg/mL; itraconazole, 1 µg/mL; and voriconazole, 0.125 µg/mL.

Discussion

Lophotrichus species (belonging to the family Microascaceae, class Sordariomycetes) have been isolated from soil, leaf litter, and decaying wood as decomposers. Although

members of this genus are ubiquitous in soil, they are rarely isolated from goat and rabbit dung (10).

Here, we describe the first case of fungal keratitis associated with a putatively novel species of *Lophotrichus*, and successful treatment with voriconazole. Filamentous fungi are frequently implicated in fungal keratitis in humans, especially species of *Fusarium* and *Aspergillus* (11). However, numerous organisms have been associated with keratitis, especially with contact lens wear (12). In the present case report, the rapidity of progression to corneal perforation was consistent with rapid growth of *Lophotrichus in vitro* (10). Review of the Johns Hopkins Hospital (JHH) Microbiology Laboratory records revealed only one other putative infection caused by *Lophotrichus*, which was isolated from bronchoalveolar lavage fluid (JHH accession number: 49-3R0949; unpublished data).

In the present case, the patient was initially started on fortified antibiotics due to the size and severity of the ulcer. The poor response to frequent topical antibiotics but good response to antifungal treatment suggests bacterial infection was unlikely the principal cause of her ulcer. *Corynebacterium macginleyi* is typically isolated from conjunctival biota, as in our patient; however, corneal ulceration associated with this organism is notably mild in severity (13).

Lophotrichus was isolated in culture but it was not present in histology, which revealed necrotic tissue. However, a positive histological finding is largely dependent on the location and depth of tissue sampling. In a series of consecutive corneal biopsies conducted for microbial keratitis over 20 years, only 42% identified organisms (14). In

our case, resection of tissue represents a balance between obtaining an adequate sample for diagnostic purposes and maintaining adequate tissue to retain structural integrity of a necrotic cornea (the biopsies were notably acquired from a thicker, less involved region of the cornea in order to avoid requiring a full thickness corneal transplant in a cornea with extreme thinning). As such, a low yield would be expected.

In cases of fungal keratitis, entry of the organism into the cornea often is facilitated by an epithelial defect. Here, trauma from the dog's paw could have directly inoculated the corneal stroma, or a herpetic epithelial infection may have provided an entry point for the fungus to invade the cornea.

Voriconazole was initially selected for its broad antifungal activity. In an *in vitro* study of the susceptibility of 381 filamentous ascomycetes to antifungals, voriconazole was active against the majority of those tested (14). Notably, among all isolates, members of Microascaceae consistently required the highest mean inhibitory concentrations (MICs) of all drugs. Microascaceae tested were more susceptible to voriconazole than to amphotericin B and itraconazole. This is consistent with our in vitro susceptibility analyses, as well as clinical reports in which infections by these organisms show significant resistance to treatment (15).

In summary, we report a case of fungal keratitis associated with a putatively novel species of *Lophotrichus*. A severe ulcer progressed rapidly to perforation on topical fortified antibiotics but healed with the addition of frequent topical voriconazole and amniotic membrane grafting. This is to our knowledge the first report of this species

associated with human infection; this organism should be considered in the differential of fungal keratitis in a rural setting.

223 **Acknowledgements:**

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226 technical assistance.

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241 **Figure Legends**

242 **Figure 1.**

243 Large central corneal ulcer associated with trauma from dog paw. **A**: Day 10 after injury,
244 a 6 mm corneal ulcer progressed to perforation despite hourly fortified broad-spectrum
245 topical antibiotics. **B:** After application of amniotic membrane graft, hourly topical
246 fortified antibiotics and voriconazole, infiltrate resolved and an inferior corneal opacity
247 remained. The eye remained stable in appearance, with no new ulceration or infiltrate,
248 at one year.

249

250 **Figure 2.**

251 Histology from superior corneal biopsy (H&E). Partial-thickness corneal biopsy acquired
252 at the time of patch graft revealed diffuse stromal necrosis in deeper layers, consistent
253 with severe infection. No organisms were observed in this section.

254

255 **Figure 3.**

256 *Lophotrichus* sp. A, B, & C are 60 mm diameter plates. A. Carnation leaf agar (CLA)
257 showing perithecial ascomata developing on and around carnation leaves after 6 weeks
258 incubation at 25°C; B. V-8 juice agar (V8) demonstrating filamentous growth and
259 ascomatal development at the periphery after 3 weeks at 25°C; C. Colonial morphology
260 on potato flakes agar (PFA) after 6 weeks at 25°C; showing only vegetation growth ; D.
261 Immature perithecium with a long neck produced on CLA; E. Crushed ascoma showing

262 released ascospores (arrow) and curved ascomatal hairs; F. Thick and thin ascomatal
263 hairs, and pale brown ellipsoidal ascospores with polar germ pores ; G. Thick
264 ascomatal hairs with hooked ends.

265

266 **Figure 4.**

267 Bayesian tree based on combined sequences of ITS and BT2. Maximum parsimony
268 bootstrap values ≥ 70% (left value) and Bayesian posterior probabilities ≥ 95% (right
269 value) are shown. Maximum parsimony bootstrap values ≤ 70% and Bayesian posterior
270 probabilities ≤ 95% are indicated by -. Sequence of *Petriella setifera* was used as the
271 out group taxon. T = ex type culture.

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Figure 1

A

B

Figure 2

H & E

Figure 3

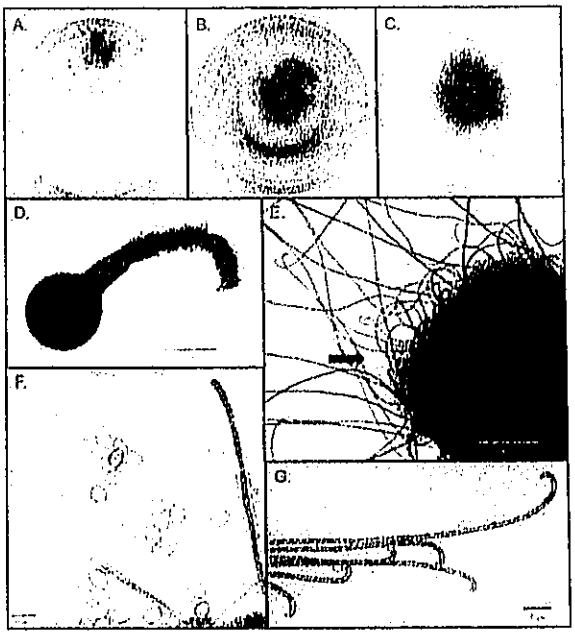
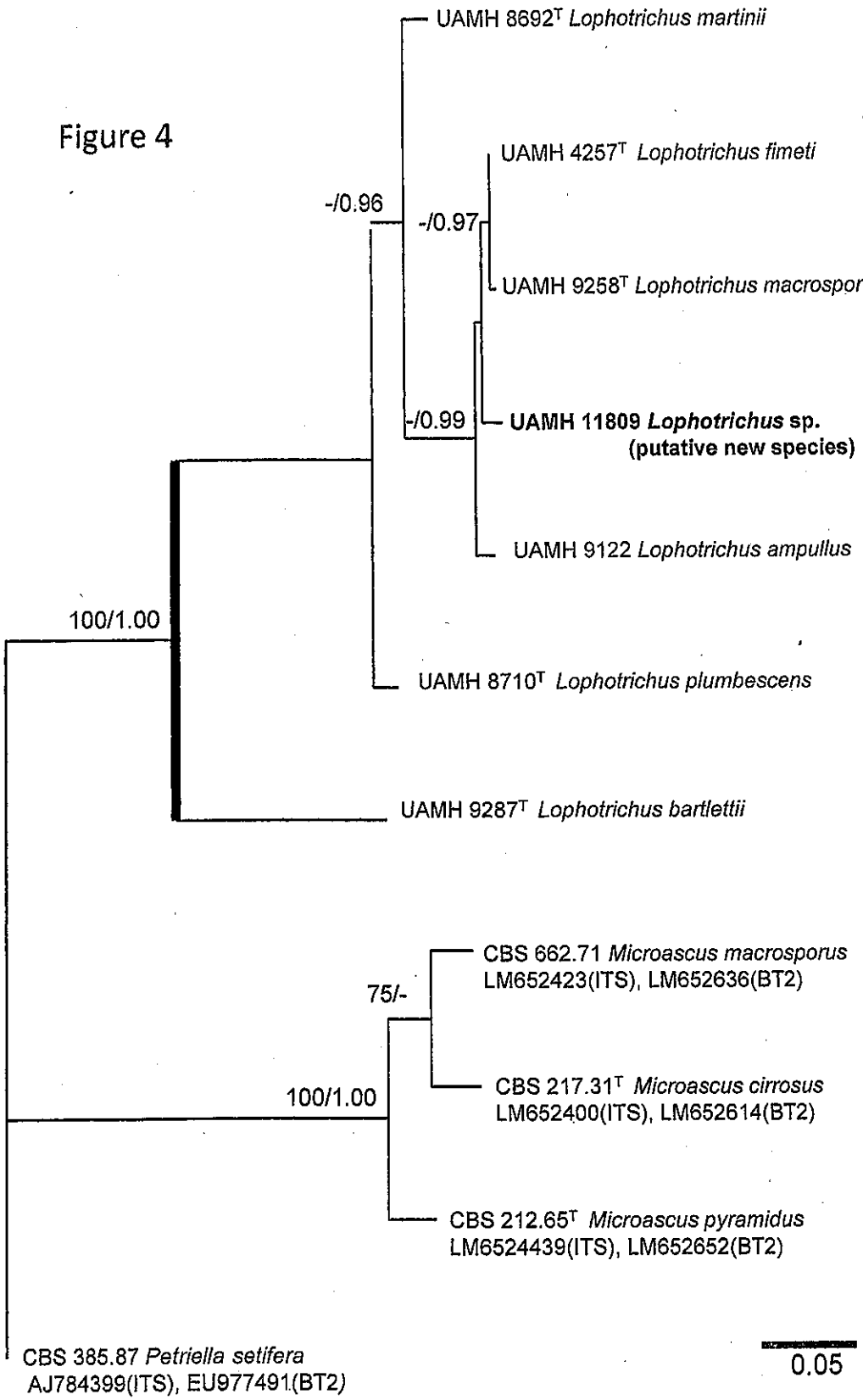


Figure 4



**2005年12月改訂(第6版)
*2005年5月改訂

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本剤は，製造工程中にヒト血漿たん白溶液を使用しており，原料となった血液を採取する際には問診，感染症関連の検査を実施するとともに，製造工程において一定の不活化・除去処理などを実施し，感染症に対する安全対策を講じているが，製品中に残存するヒト血漿たん白による感染症伝播のリスクを完全に排除することはできないため，疾病の治療上の必要性を十分に検討の上，必要最小限の使用にとどめること．(「使用上の注意」の項参照)

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■原則禁忌(次の患者には投与しないことを原則とするが，特に必要とする場合には慎重に投与すること)
本剤の成分に対し過敏症の既往歴のある患者

■ 組成・性状

組成

1瓶中		コージネイトFS 250IU注射用	コージネイトFS 500IU注射用	コージネイトFS 1000IU注射用
有効成分	オクトコグ アルファ(遺伝子組換え)	250 国際単位	500 国際単位	1,000 国際単位
添加物	L-ヒスチジン	8.0mg	8.0mg	8.0mg
	アミノ酢酸	58mg	58mg	58mg
	塩化ナトリウム	4.4mg	4.4mg	4.4mg
	塩化カルシウム	0.7mg	0.7mg	0.7mg
	精製白糖	28mg	28mg	28mg
	ポリソルベート80	200μg	200μg	200μg
添付溶剤	日本薬局方注射用水	2.5mL	2.5mL	2.5mL

本剤の培養工程の培地成分としてヒト血漿たん白溶液(採血国：米国，採血の区別：非献血)を使用している．また，精製工程に用いるマウスモノクローナル抗体産生の培養培地成分としてヒト血清アルブミン(採血国：米国，採血の区別：非献血)及びウシインスリン(膵臓抽出成分)を使用している．[「献血又は非献血の区別の考え方」参照]

製剤の性状

本剤は，白色～淡黄色の凍結乾燥製剤であり，添付の溶剤で溶解したとき，無色の澄明な液剤となる．
pH：6.6～7.0
浸透圧比(生理食塩液に対する比)：約1.6

■ 効能・効果

血液凝固第Ⅷ因子欠乏患者に対し，血漿中の血液凝固第Ⅷ因子を補い，その出血傾向を抑制する．

■ 用法・用量

本剤を添付の溶解液2.5mLで溶解し，緩徐に静脈内注射又は点滴注入する．なお，1分間に5mLを超える注射速度は避けること．用量は，通常，1回体重1kg当たり10～30国際単位を投与するが，症状に応じて適宜増減する．

用法・用量に関連する使用上の注意
輸注速度が速すぎるとチアノーゼ，動悸を起こすことがあるので，1分間に5mLを超えない速度でゆっくり注入すること．

■ 使用上の注意

1. 慎重投与(次の患者には慎重に投与すること)
(1)マウスモノクローナル抗体により精製した製剤又はハムスター腎細胞由来の製剤に過敏症の既往歴のある患者
(2)ヒト血漿由来の第Ⅷ因子製剤に過敏症の既往歴のある患者

**2. 重要な基本的注意
[患者への説明]
本剤の使用にあたっては，疾病の治療における本剤の必要性とともに，本剤の製造に際し感染症の伝播を防止するための安全対策が講じられているが，製品中に残存するヒト血漿たん白に由来する感染症伝播のリスクを完全に排除することができないことを患者に対して説明し，理解を得るよう努めること．
(1)本剤の培養培地にはヒト血漿たん白溶液が使用されている．製品中に残存するヒト血漿たん白の原材料となる血漿については，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．さらに，プールした試験血漿については，HBV-DNA，HCV-RNA及びHIV-RNA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．その後のヒト血漿たん白の製造工程であるコーンの低温エタノール分画法及び60℃，10時間液状加熱処理は，HIVをはじめとする各種ウイルス不活化・除去効果を有することが確認されている．また，本剤の精製工程においてTNBP/ポリソルベート80によるSD処理*を実施している．しかし，現在の製造工程では，ウイルスを完全に不活化・除去することが困難である．
*SD処理：Solvent/Detergent Treated
Solvent[有機溶媒：TNBP(Tri-n-Butyl-Phosphate)]
Detergent[界面活性剤：ポリソルベート80]
(2)アナフィラキシー様症状が起こる可能性があるので，観察を十分に行うこと．
(3)患者の血清中に血液凝固第Ⅷ因子に対するインヒビターが発生するおそれがあるので，観察を十分に行うこと．
(4)大量投与により血管内に凝固による栓塞を起こすおそれがあるので，慎重に投与すること．
**(5)本剤の培養工程には，ヒトインスリン(遺伝子組換え)が添加されている．このヒトインスリン(遺伝子組換え)は，医療用医薬品として承認を受けたものである．ヒトインスリン(遺伝子組換え)のセルバンクの製造において，カナダ及び米国産のウシ由来原料(せき柱骨を含む)からなる成分が用いられている．その成分は，米国医薬食品局

*注)注意—医師等の処方せんにより使用すること

及び欧州医薬品審査庁のすべてのガイダンス，ならびに欧州薬局方に適合している．最終製品にはヒトインスリン(遺伝子組換え)は含まれていない．本剤の精製工程(陰イオン交換クロマトグラフィー)において，伝播性海綿状脳症(TSE)伝播の原因である異常プリオンを低減し得るとの報告がある．また，本剤の投与によるTSE感染の報告はない．したがって，本剤によるTSE伝播リスクはきわめて低いと考えられるが，理論的リスクを完全には否定できないことから，その旨の患者への説明を考慮すること．

3. 副作用

本剤の承認時での調査例数20例中1例(5.0%)にCD4上昇，CD8低下及びCD4/CD8比上昇が認められた．

また，コージネイトでの承認時及び市販後の使用成績調査・特別調査(長期使用症例)での調査症例742例中40例(5.39%)に副作用が認められた(再審査終了時)．

	0.1～5%未満	0.1%未満
過敏症[注]		発疹，蕁麻疹
消化器	嘔気	嘔吐
注射部位	血管痛	

注)このような場合には投与を中止し，適切な処置を行うこと．

4. 高齢者への投与

一般に高齢者では生理機能が低下しているので，患者の状態を観察しながら慎重に投与すること．

5. 妊婦，産婦，授乳婦等への投与

妊婦又は妊娠している可能性のある婦人には，治療上の有益性が危険性を上回ると判断される場合のみ投与すること．[妊娠中の投与に関する安全性は確立していない．]

6. 適用上の注意

(1)調製時：

1)他剤との混合注射を避けること．

2)使用後の残液は細菌汚染のおそれがあるので使用しないこと．[本剤は保存剤が含有されていないため．]

3)溶解した液を注射器に移す場合，ろ過網のあるセットを用いること．

(2)投与時：

1)溶解時に不溶物の認められるもの又は混濁しているものは使用しないこと．

2)一度溶解したものは3時間以内に使用すること．

3)凍結した溶液は使用しないこと．

(3)家庭療法時：

1)子供の手の届かないところへ保管すること．

2)使用済の医療用具等の処理については，主治医の指示に従うこと．

7. その他の注意

本剤はvon Willebrand因子を含んでいない．

■ 薬物動態

重症血友病A患者5例に本剤を単回投与した場合，血漿中第Ⅷ因子凝固活性の消失半減期は13.96±4.18時間，生体内リカバリーは67.9±11.3%であった[1]．

〈参　考〉

分　布

ウサギに^{125}I-rFⅧを0.8μg/kg(0.29IU/kg)を単回静脈内投与した実験では，血液，肝，脾及び遊離ヨウ素の取込みが推定される甲状腺以外，いずれの組織においても放射能の有意な取込みは認められていない[2]．

排　泄

ウサギに^{125}I-rFⅧを0.8μg/kg(0.29IU/kg)を単回静脈内投与した実験では，投与後72時間の尿中排泄率は65%である．尿中排泄は比較的速やかで，投与後24時間以内にその86%以上が排泄される[2]．

■ 臨床成績

血友病A患者20例について実施された臨床試験における治療期間は，24週間から最長84週間(1年間以上のもの5例)である．764出血エピソードにおいて止血効果が検討され，有効率98.0%(749/764エピソード)と満足すべき治療効果が得られている[1]．

出血エピソードごとの止血効果

出血部位	出血回数	有効率
関節	666	98.0% (653/666)
筋肉	39	97.4% (38/ 39)
皮下	19	94.7% (18/ 19)
血尿	6	100.0% (6/ 6)
歯肉	13	100.0% (13/ 13)
その他	11	100.0% (11/ 11)
2ヵ所出血	10	100.0% (10/ 10)
計	764	98.0% (749/764)

■ 薬効薬理

血液凝固第Ⅷ因子欠乏患者に対し，血漿中の血液凝固第Ⅷ因子を補い，その出血傾向を抑制する[3]．

■ 有効成分に関する理化学的知見

一般名：オクトコグ アルファ(遺伝子組換え)
[octocog alfa(genetical recombination)] JAN

本　質：ヒトT細胞ハイブリドーマのmRNAに由来するヒト第Ⅷ因子cDNAの発現により，ベビーハムスター腎細胞で産生される2,332個のアミノ酸残基からなる糖蛋白質(分子量：300,000～350,000)

■ 取扱い上の注意

記録の保存：本剤は特定生物由来製品に該当することから，本剤を投与又は処方した場合は，医薬品名(販売名)，製造番号(ロット番号)，投与又は処方年月日，投与又は処方を受けた患者の氏名，住所等を記録し，少なくとも20年間保存すること．

■ 包　装

注射剤　瓶

250IU×1バイアル[(日本薬局方 注射用水2.5mL)を添付]

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1000IU×1バイアル[(日本薬局方 注射用水2.5mL)を添付]

■ 主要文献

1)バイエル薬品社内資料(1999)

2)Newgren, J. O. ：未発表(1990)

3)Giles, A. R. et al.：Blood, 72(1), 335(1988)

■ 文献請求先

バイエル薬品株式会社・学術情報

〒532-8577 大阪市淀川区宮原三丁目5番36号

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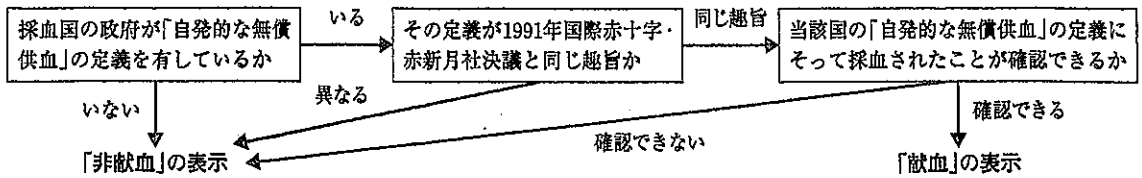

別紙様式第2-1

No. 9

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
				2015. 6. 4	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	Pritzkow S, Morales R, Moda F, Khan U, Telling GC, Hoover E, Soto C. Cell Rep. 2015 May 26;11(8):1168-75. doi: 10.1016/j.celrep.2015.04.036. Epub 2015 May 14	公表国 米国	
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)				

研究報告の概要

○感染性プリオンは草本植物に結合、維持、摂取、伝播される
プリオンは、プリオン病の原因となる感染性蛋白質病原体である。プリオンによる環境汚染が疾患伝播に関与すると考えられている。本稿で我々は、感染性プリオン蛋白(PrP^{Sc})の植物への結合と維持を調べた。希釈された脳ホモジネートあるいは排泄物(尿と糞便)に含まれる少量のPrP^{Sc}がハマムギの根と葉に結合し、野生型のハムスターが、プリオンに汚染されたこれらの植物の摂取によって効率的に感染することが明らかになった。プリオンと植物の相互作用は、慢性消耗性疾患を始めとする様々な発生源由来のプリオンで見られた。さらに、プリオン含有物を噴霧して汚染した葉は、PrP^{Sc}を数週間、生きた植物中で維持した。最後に、植物は汚染された土壌からプリオンを取り込み、地上部(茎と葉)に運ぶことが明らかになった。これらの所見から、植物が感染性プリオンと効率的に結合し感染性を伝播するキャリアとなり、疾患の水平伝播において環境汚染が役割を担う可能性があることが示唆される。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
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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
プリオン病の感染性蛋白質病原体であるPrP^{Sc}による環境汚染が疾患の拡散に関与するという報告である。	今後もプリオン検出法等の技術を含め、CJD等プリオン病に関する新たな知見及び情報の収集に努める。

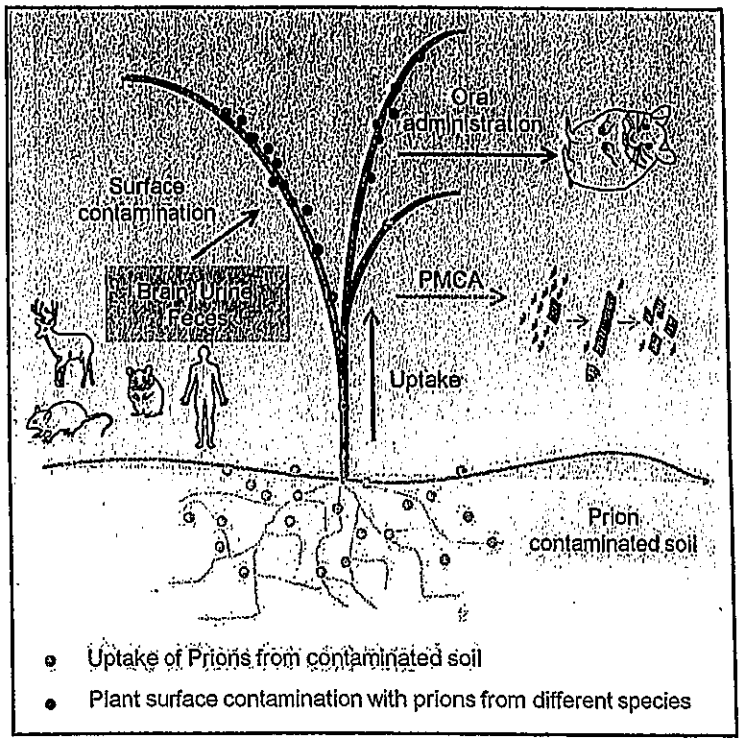
JRC2015T-019

Report

Cell Reports

Grass Plants Bind, Retain, Uptake, and Transport Infectious Prions

Graphical Abstract



Authors

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In Brief

Prions are the proteinaceous infectious agents responsible for prion diseases. Pritzkow et al. report that prions from brain and excreta can bind grass plants and remain attached to living plants for a long time and that contaminated plants can infect animals. In addition, grass plants can uptake and transport prions from infected soil.

Highlights

- Grass plants bind prions from contaminated brain and excreta
- Prions from different strains and species remain bound to living plants
- Hamsters fed with prion-contaminated plant samples develop prion disease
- Stems and leaves from grass plants grown in infected soil contain prions

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Cell Reports
Report

Grass Plants Bind, Retain, Uptake, and Transport Infectious Prions

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SUMMARY

Prions are the protein-based infectious agents responsible for prion diseases. Environmental prion contamination has been implicated in disease transmission. Here, we analyzed the binding and retention of infectious prion protein (PrP^{Sc}) to plants. Small quantities of PrP^{Sc} contained in diluted brain homogenate or in excretory materials (urine and feces) can bind to wheat grass roots and leaves. Wild-type hamsters were efficiently infected by ingestion of prion-contaminated plants. The prion-plant interaction occurs with prions from diverse origins, including chronic wasting disease. Furthermore, leaves contaminated by spraying with a prion-containing preparation retained PrP^{Sc} for several weeks in the living plant. Finally, plants can uptake prions from contaminated soil and transport them to aerial parts of the plant (stem and leaves). These findings demonstrate that plants can efficiently bind infectious prions and act as carriers of infectivity, suggesting a possible role of environmental prion contamination in the horizontal transmission of the disease.

INTRODUCTION

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of fatal, infectious neurodegenerative disorders that affect humans and other mammals (Collinge, 2001; Prusiner, 2001). The most common animal TSE is scrapie, a disorder of sheep and goats that was first recognized almost 200 years ago and has become an endemic problem. However, the most recent and worrisome animal prion outbreaks are bovine spongiform encephalopathy (BSE) affecting cattle and chronic wasting disease (CWD) affecting cervids (deer, elk, moose). BSE, because of its proven transmission to humans, generating a fatal new disease, termed variant Creutzfeldt-Jakob disease (vCJD) (Collinge, 1999) and CWD, due to its uncontrolled spread among wild and captive cervids in North America and its uncertain transmissibility to humans and/or domestic animals (Miller and Williams, 2004; Sigurdson and Aguzzi, 2007; Gilch et al., 2011). The nature of the infectious agent in TSEs has been the center of passionate controversy (Soto and Castilla, 2004). The most accepted hypothesis proposes that the misfolded form of the prion protein (PrP^{Sc}) is the sole component of the infectious agent that replicates in infected individuals by transforming the normal version of the prion protein (PrP^{C}) into the misfolded isoform (Prusiner, 2001; Soto, 2011).

Prion diseases are transmissible between animal-to-animal, animal-to-human, and human-to-human; however, we still do not understand completely the mechanisms, factors, and biological processes that control the transmission of this unique infectious agent. The transmission of some of the naturally acquired forms of TSEs (such as vCJD, kuru, BSE) has been linked to the consumption of meat or meat-derived products from individuals affected by the disease (Collinge, 2001; Prusiner, 2001). On the other hand, some of the most prevalent and horizontally transmissible animal TSEs, including scrapie and CWD, have implicated environmental contamination with prions as a putative mode of transmission (Mathiason et al., 2009; Gough and Maddison, 2010; Bartelt-Hunt and Bartz, 2013). Various studies have shown that infectious prions can enter the environment through saliva, feces, urine, blood, or placenta from infected animals, as well as by decaying carcasses (Mathiason et al., 2006; Haley et al., 2009, 2011; Tamgüney et al., 2009; Maddison et al., 2010; Terry et al., 2011). It has been shown that infectious prions bind tightly to soil and remain infectious for years in this material, suggesting that environmental contamination of soil may play a role in TSE spreading (Johnson et al., 2006, 2007; Seidel et al., 2007). Since the main natural hosts for animal TSEs (sheep, cattle, and cervids) are herbivores, it is surprising that the interaction between prions and plants and the putative role of these organisms as carriers of prion infectivity have not been studied in detail. The main goal of this study was to evaluate whether plants can bind, retain, uptake, and transport prions in an experimental setting. Overall, our findings show that grass plants efficiently interact with prions, suggesting that they may play an important role in natural prion transmission, particularly in wild animals.

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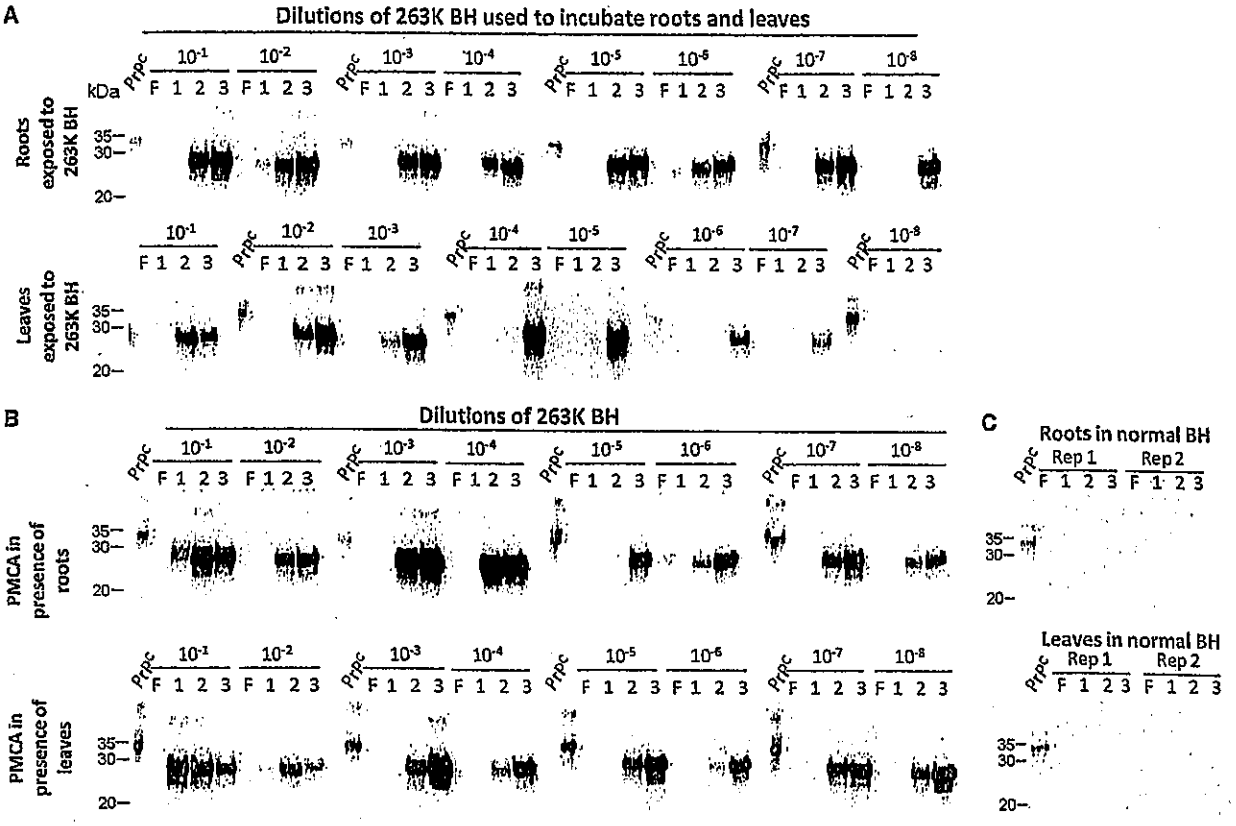

Figure 1. Detection of PrP^{Sc} Bound to Leaves or Roots by PMCA

(A) Serial dilutions of 263K brain homogenate (BH, 10^{-1} to 10^{-8}) done in PBS were incubated with either wheat grass roots (15 mg weight) or leaves (2 cm^2) during 16 hr at room temperature. Thereafter, unbound material was discarded, and leaves and roots were thoroughly washed five times with water and deposited into tubes containing 120 µl of 10% normal hamster brain homogenate. The presence of plant-attached PrP^{Sc} was detected by serial rounds of PMCA, as described in Experimental Procedures. Positive PrP^{Sc} signal was detected by western blot after proteinase K (PK) digestion.

(B) Serial dilutions of 263K brain homogenate (10^{-1} to 10^{-8}) were directly loaded into tubes containing NBH PMCA substrate and wheat grass roots and leaves not previously exposed to PrP^{Sc}. The purpose of this experiment was to study the level of amplification expected for the total amount of PrP^{Sc} contained in each dilution of sick brain homogenate.

(C) To investigate the possible induction of PrP^{Sc} formation by plant material and to rule out cross-contamination, we exposed leaves and roots to 10% normal brain homogenates and subjected the material to several rounds of PMCA as described in (A). The figure shows two replicates of the same experiment (Rep 1 and 2). No PMCA amplification was detected for any of the samples. F, non-amplified control. 1, 2, and 3, number of PMCA rounds performed. Each round consisted of 96 PMCA cycles (2 days). All samples were digested with PK, except the normal brain homogenate (NBH, PrP^{C}) used as a migration control.

RESULTS

Prions Bind to Plants and Bound-PrP^{Sc} Efficiently Sustain Prion Replication

To study whether plants can interact with prions, we exposed wheat grass roots and leaves to brain homogenate from hamsters that have succumbed to prion disease induced by experimental inoculation with the 263K prion strain. The presence of PrP^{Sc} and infectivity attached to the plants was studied in vitro using the protein misfolding cyclic amplification (PMCA) technique and in vivo by infectivity bioassays. For in vitro analyses, the plant tissues (roots and leaves) were incubated for 16 hr with serial dilutions of 263K-brain homogeniate ranging from 10^{-1} to 10^{-8}. Roots and leaves were washed thoroughly and analyzed for the presence of PrP^{Sc} by serial PMCA (Morales et al., 2012). The results show that even highly diluted PrP^{Sc} can bind to roots and leaves and sustain PrP^{C} conversion (Figure 1A). Although a direct comparison cannot be made, because of differences on the effective surface, roots appear to retain PrP^{Sc} better than leaves. However, both roots and leaves capture PrP^{Sc} efficiently, even at very small concentrations, equivalent to those present in biological fluids, such as blood and urine (Chen et al., 2010). By comparing the detection of PrP^{Sc}-bound to plants (Figure 1A) with an experiment in which the same dilutions of 263K brain homogenate were added directly to the tubes containing normal brain homogenate and an equivalent piece of leaves or roots (Figure 1B), we can estimate that a high proportion of PrP^{Sc} present in the sample was attached to the plant tissue. Importantly, no detection of PrP^{Sc} was observed when leaves and roots were

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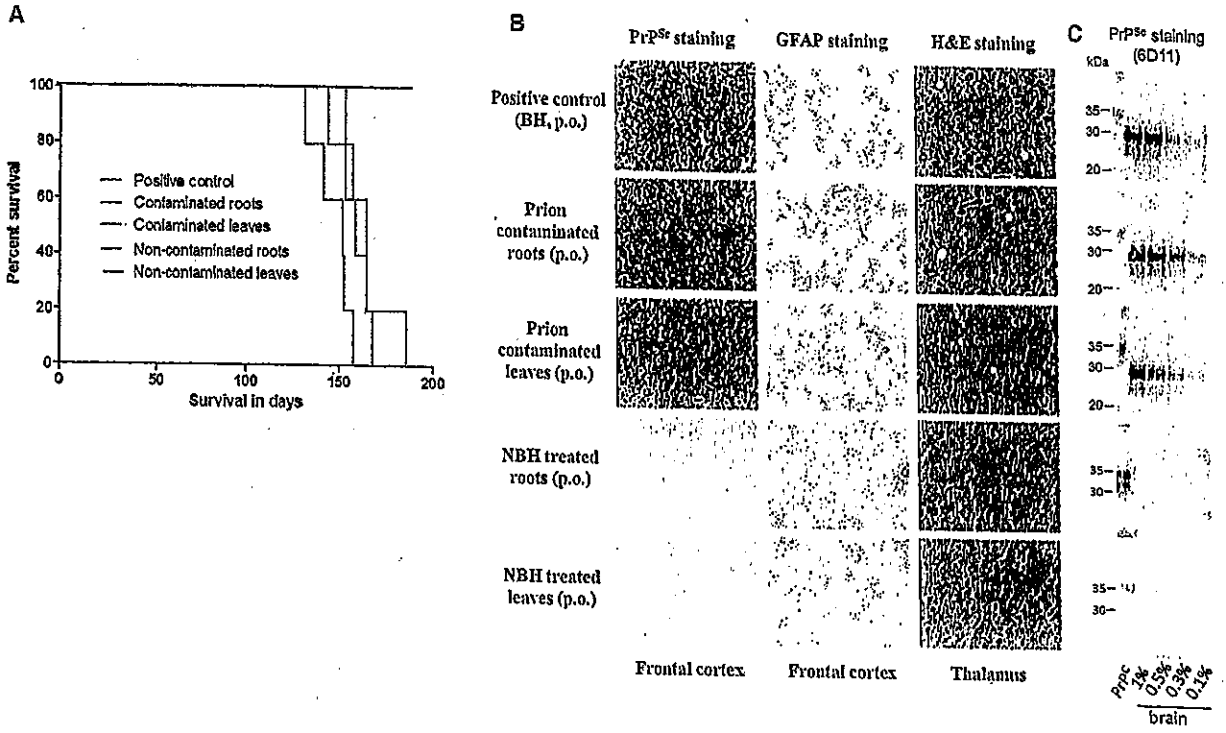

Figure 2. PrP^{Sc} Contaminated Plants Induce Prion Disease by Oral Ingestion

(A) Survival curve of hamsters orally inoculated with leaves or roots exposed to 263K BH. Plant tissue was exposed to prions as described in Figure 1 and in Experimental Procedures. Three units of leaves and roots were used to orally inoculate healthy hamsters. The positive control group consisted on hamsters orally inoculated with 750 µl of 5% 263K BH. Negative control groups were inoculated with leaves and roots incubated with normal brain homogenate. All sick animals exhibited the typical 263K clinical signs, including ataxia, hyperactivity, aggressiveness, and sensitivity to noise, and were sacrificed at the terminal stage of the disease. Hamsters injected with leaves and roots treated with healthy brain homogenates did not show any clinical signs up to 550 days post-inoculation. The differences in the survival curves of animals infected with 263K brain homogenate versus those infected with prion-contaminated leaves or roots were statistically significant ($p = 0.0136$ and 0.047, respectively) as analyzed by the log-rank (Mantel-Cox) test.

(B) Brains from hamsters orally infected with roots and leaves exposed to prions displayed neuropathological alterations typical of prion disease, including characteristic synaptic and diffuse patterns of PK-resistant PrP^{Sc} deposition (antibody 6H4, left panels), astrogliosis (middle panels), and spongiosis (right panels). These alterations were not observed in animals fed with plant tissue exposed to normal brain homogenate. Magnification 20× in all panels.

(C) Biochemical analysis confirmed the presence of PrP^{Sc} accumulation in the brain of all animals showing signs of prion disease. The figure shows a western blot of different brain dilutions from a representative animal per group. All samples were digested with PK, except the normal brain homogenate (PrP^{C}) used as a migration control.

exposed to normal brain homogenate (Figure 1C). However, comparing PMCA amplification in the presence (Figure 1B) or in the absence (Figure S1A) of plant tissue, it is possible to appreciate that plants (both leaves and roots) partially inhibits the PMCA reaction. This explains why in most of the experiments with plants, protease-resistant PrP^{Sc} is only observed after two rounds of PMCA. In our current PMCA settings, no false-positive PrP^{Sc} signals were ever detectable when samples did not contain PrP^{Sc} inoculum (Figure S1B). These results indicate that leaves and roots can efficiently bind PrP^{Sc}, which remains able to catalyze PrP^{C} to PrP^{Sc} conversion, leading to prion replication. In these experiments, plant tissues were incubated with prions for 16 hr, but a similar experiment in which roots and leaves were exposed to a 10^{-5} dilution of 263K brain homogenate for different times, we found that as little as 2 min of incubation was sufficient for the efficient contamination of plants (Figure S2).

Animals Can Be Infected by Oral Administration of Prion-Contaminated Plants

To investigate whether prion-contaminated plants were able to infect animals by ingestion, leaves and roots previously incubated with either 263K-infected or control hamster brain homogenates were orally administered into naive hamsters. After exposure, plants were extensively washed five times with water and animals were fed with dried material. As positive controls, we orally administered 750 µl of 5% 263K brain homogenate (same material used to contaminate plant tissue). All animals that ingested prion contaminated leaves and roots developed typical prion disease. Although the incubation times were significantly longer in animals ingesting prions attached to leaves and roots as compared with those fed directly with the brain material, the differences were not as high as one could have expected (Figure 2A). Indeed, incubation periods were 147 ± 10,

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159 ± 10, and 164 ± 13 days (mean ± SEM) for the groups inoculated with brain homogenate, and prion contaminated roots and leaves, respectively. Prion disease was confirmed by histological study of PrP^{Sc} deposition, astrogliosis, and brain vacuolation (Figure 2B), as well as by biochemical detection of protease-resistant PrP^{Sc} by western blot (Figure 2C). None of the animals inoculated with leaves and roots exposed to normal brain homogenate developed disease up to 550 days post-inoculation. Histological analysis did not show any PrP^{Sc} staining or disease specific alteration in control animals.

Plants Bind Prions from Different Strains and Species

To analyze prion-plant interaction with other species and strains of the prion agent, we performed similar studies as described in Figure 1, by incubating leaves and roots with a preparation containing hamster, murine, cervid, and human prions corresponding to the Hyper, 301C, CWD, and vCJD prion strains, respectively. PrP^{Sc} from these strains and species showed good amplification by PMCA, using homologous substrates (Figure S3A). In all cases, leaves and roots bound prions from these species and retained the ability to replicate in vitro (Figure S3B), indicating that the interaction of PrP^{Sc} with plants is a general feature of infectious prions.

Contamination of Plants with Prions Excreted in Urine and Feces

Under natural conditions, it is likely that the main source of prions in the environment comes from secretory and excretory fluids, such as saliva, urine, and feces. We and others have shown that PrP^{Sc} is released in these fluids and excretions in various animal species (Gonzalez-Romero et al., 2008; Haley et al., 2009, 2011; Maddison et al., 2010; Terry et al., 2011; Moda et al., 2014). It has been estimated that the amount of infectious prions spread by excreta during the animals' lifespan could match or even surpass the quantity present in the brain of a symptomatic individual (Tamgüney et al., 2009). To study whether plant tissue can be contaminated by waste products excreted from prion-infected hamsters and deer, leaves and roots were incubated with samples of urine and feces and the presence of PrP^{Sc} analyzed by serial rounds of PMCA. For these experiments, plant tissues were incubated for 1 hr with urine or feces homogenates obtained either from 263K-infected hamsters or CWD-affected cervids. This time was chosen because longer incubation with these biological fluids affected the integrity of the plant tissue. After being thoroughly washed and dried, PrP^{Sc} attached to leaves and roots was detected by PMCA. The results clearly show that PrP^{Sc} was readily detectable after three or four rounds of PMCA in samples of wheat grass leaves and roots exposed to both urine and feces from 263K sick hamsters (Figure 3A) and CWD-affected cervids (Figure 3B). Comparing these results with studies of the direct detection of PrP^{Sc} in urine and feces (Figures 3A and 3B), it seems that the majority of PrP^{Sc} present in these waste products was effectively attached to leaves and roots. No signal was observed in plant tissue exposed to urine or feces coming from non-infected hamsters.

Prions Bind to Living Plants

To investigate a more natural scenario for prion contamination of living plants, we sprayed the leaves of wheat grass with a preparation containing 1% 263K hamster brain homogenate. Plants were let to grow for different times after exposure, and PrP^{Sc} was detected in the leaves by PMCA in duplicates for each time point. The results show that PrP^{Sc} was able to bind to leaves and remained attached to the living plants for at least 49 days after exposure (Figure 4). Considering that PrP^{Sc} signal was detectable normally in the second or third round of PMCA without obvious trend in relation to time, we conclude that the relative amount of PrP^{Sc} present in leaves did not appear to change substantially over time. These data indicate that PrP^{Sc} can be retained in living plants for at least several weeks after a simple contact with prion contaminated materials, and PrP^{Sc} remains competent to drive prion replication.

Plants Uptake Prions from Contaminated Soil

The experiments described above were done by exposure of the surface of leaves and roots with different solutions containing prions. To evaluate whether living plants can uptake PrP^{Sc} from contaminated soil, we grew barley grass plants on soil that was contaminated by addition of 263K brain homogenate. Plants were grown for 1 or 3 weeks under conditions that carefully prevented any direct contact of the aerial part of the plant with the soil. After this time, pieces of stem and leaves were collected and analyzed for the presence of PrP^{Sc} by PMCA. As shown in Figure 5A, all plants grown for 3 weeks in contaminated soil contained PrP^{Sc} in their stem, albeit in small quantities that required four serial rounds of PMCA for detection. One of the four plants analyzed contained a detectable amount of PrP^{Sc} in the leaves (Figure 5B), indicating that prions were uptaken from the soil and transported into the aerial parts of the plants, far from the soil. These results differ from a recent article reporting that infectious prions were not detectable in above the ground tissues of wheat plants exposed to CWD prions (Rasmussen et al., 2014). The lack of detection in this article is most likely due to the low sensitive techniques (western blots or ELISA) employed to analyze the presence of PrP^{Sc}. Indeed, as we reported previously, PMCA has a power of detection, which is several millions times higher than western blots or ELISA (Saá et al., 2006). In order to estimate the amount of PrP^{Sc} present in stem and leaves coming from contaminated soil, we performed a quantitative PMCA study, as previously described (Chen et al., 2010). Unfortunately, by comparing the PMCA amplification in the absence or the presence of plant tissue, it is possible to conclude that stems and leaves substantially interfered with the PMCA procedure, and thus the calculation cannot be very precise (Figure S4). Indeed, after two rounds of PMCA we cannot detect any protease-resistant PrP^{Sc}, but on the third round we observed the maximum amplification (10^{-9}), presumably because at this round the concentration of PMCA inhibitors has been reduced enough to permit good amplification. At this point, we can estimate that the amount of PrP^{Sc} that reaches the stem and leaves from contaminated soil is equivalent to the PrP^{Sc} concentration present in a 10^{-6} to 10^{-9} dilution of sick brain homogenate. Nevertheless, this result is interesting, because it indicates that the amount of prions uptaken from

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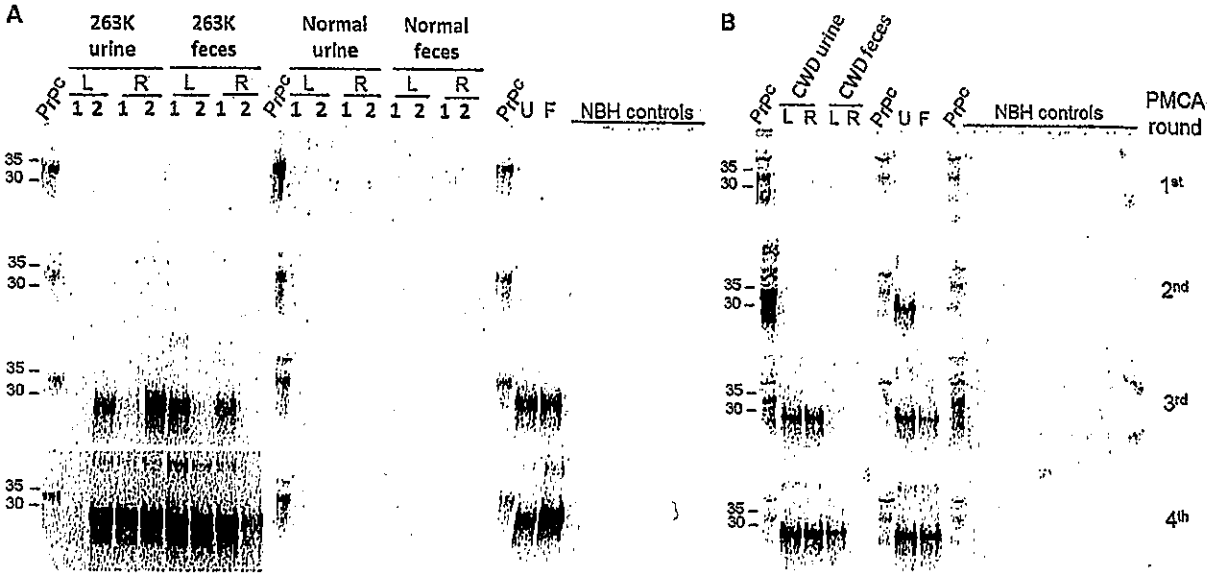

Figure 3. PrP^{Sc} Contained in Urine and Feces of Prion-Infected Animals Binds to Leaves and Roots

(A) Wheat grass roots (R) and leaves (L) were incubated for 1 hr with 1 ml of urine or 1 ml of 20% feces homogenate from sick hamsters experimentally infected with 263K prions. Controls included similar experiments using urine and feces from healthy animals. After exposure, roots and leaves were thoroughly washed five times with water and dried, and the presence of plant-attached PrP^{Sc} was detected by serial rounds of PMCA. The figure shows the results of two replicated experiments (1 and 2). In the right blot of this panel, we show the results of the positive control experiment aiming to directly detect PrP^{Sc} in urine (U) and feces (F) from 263K-infected animals. We also include several negative controls for the PMCA reaction, containing only the normal brain homogenate (NBH) used as substrate, to rule out cross-contamination or de novo formation of PrP^{Sc}.

(B) A similar experiment as described in (A) was done using urine and feces from white-tailed deer clinically affected by CWD. In this case, leaves (L) and roots (R) were incubated in 1:2.5 diluted urine or with 5% feces homogenates. The middle blot shows the positive control experiment in which PrP^{Sc} was detected directly in urine and feces from CWD-affected deer. No PrP^{Sc} signal was detected for various negative controls in which the PMCA reaction was carried out in the absence of infectious samples (right panel). Both (A) and (B) show the results obtained in the first, second, third, and fourth round of PMCA. Each round consisted of 96 PMCA cycles (2 days). All samples were digested with PK, except the normal brain homogenate (PrP^{C}) used as a migration control.

soil and transported to aerial parts of the plant is within the infectious range. Indeed, titration studies showed that the last infectious dilution of a 263K brain homogenate is ~10^{-9} (Gregori et al., 2006).

DISCUSSION

This study shows that plants can efficiently bind prions contained in brain extracts from diverse prion infected animals, including CWD-affected cervids. PrP^{Sc} attached to leaves and roots from wheat grass plants remains capable of seeding prion replication in vitro. Surprisingly, the small quantity of PrP^{Sc} naturally excreted in urine and feces from sick hamster or cervids was enough to efficiently contaminate plant tissue. Indeed, our results suggest that the majority of excreted PrP^{Sc} is efficiently captured by plants' leaves and roots. Moreover, leaves can be contaminated by spraying them with a prion-containing extract, and PrP^{Sc} remains detectable in living plants for as long as the study was performed (several weeks). Remarkably, prion contaminated plants transmit prion disease to animals upon ingestion, producing a 100% attack rate and incubation periods not substantially longer than direct oral administration of sick brain homogenates. Finally, an unexpected but exciting result was that plants were able to uptake prions from contaminated soil and transport them to aerial parts of the plant tissue. Although it may seem farfetched that plants can uptake proteins from the soil and transport it to the parts above the ground, there are already published reports of this phenomenon (McLaren et al., 1960; Jensen and McLaren, 1960; Paungfoo-Lonhienne et al., 2008). The high resistance of prions to degradation and their ability to efficiently cross biological barriers may play a role in this process. The mechanism by which plants bind, retain, uptake, and transport prions is unknown. We are currently studying the way in which prions interact with plants using purified, radioactively labeled PrP^{Sc} to determine specificity of the interaction, association constant, reversibility, saturation, movement, etc.

Epidemiological studies have shown numerous instances of scrapie or CWD recurrence upon reintroduction of animals on pastures previously exposed to prion-infected animals. Indeed, reappearance of scrapie has been documented following fallow periods of up to 16 years (Georgsson et al., 2006), and pastures were shown to retain infectious CWD prions for at least 2 years after exposure (Miller et al., 2004). It is likely that the environmentally mediated transmission of prion diseases depends upon the interaction of prions with diverse elements, including soil, water, environmental surfaces, various invertebrate animals, and plants. However, since plants are such an important component of the environment and also a major source of food for many animal

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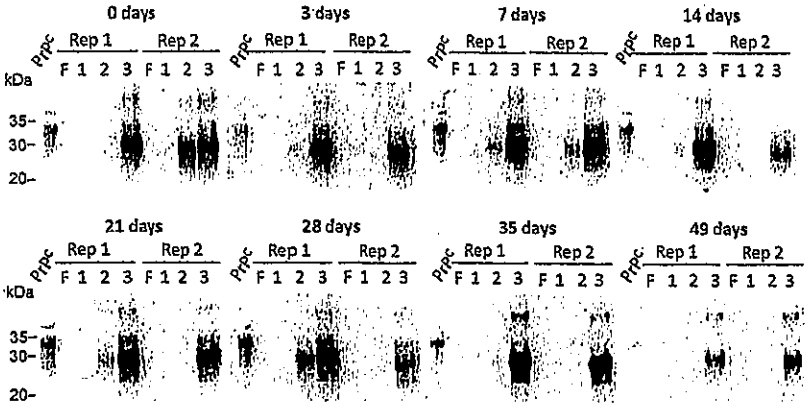

Figure 4. PrP^{Sc} Binds to Living Plants

The leaves of living wheat grass plants were sprayed three times with 10^{-2} diluted 263K brain homogenate. Plants were left to grow for a period of 0, 3, 7, 14, 21, 28, 35, and 49 days. Thereafter, leaves were collected washed five times with water, dried, and used to detect PrP^{Sc} signal by serial rounds of PMCA. The experiment was done in two independent replicates (Rep 1 and 2) for each time point. F, non-amplified control. 1, 2, and 3, number of PMCA rounds performed. Each round consisted of 96 PMCA cycles (2 days). All samples were digested with PK, except the normal brain homogenate (PrP^{C}) used as a migration control.

species, including humans, our results may have far-reaching implications for animal and human health. Currently, the perception of the risk for animal-to-human prion transmission has been mostly limited to consumption or exposure to contaminated meat; our results indicate that plants might also be an important vector of transmission that needs to be considered in risk assessment.

EXPERIMENTAL PROCEDURES

Biological Samples

This study used brain samples from animals and humans infected with various prion strains. Rodents (Syrian golden hamsters and 129S mice) were experimentally infected by intra-peritoneal route with various prion strains (263K and Hyper for hamster and 301C for mouse). The onset of the disease was monitored by the appearance of the clinical signs, using our previously described procedures (Castilla et al., 2008). Animals were sacrificed when they reached a severe stage of the disease, and the brain was collected and stored at −80°C. For deer material, a piece of brain from a white-tailed deer experimentally infected by CWD was used. For human prions, a piece of brain from a patient affected by variant Creutzfeldt-Jakob disease (vCJD) was used. For all these samples, 10% (w/v) brain homogenates (BHs) were prepared in PBS plus complete protease inhibitor cocktail (Roche). When used in protein misfolding cyclic amplification (PMCA), the BH was clarified by a short, low-speed centrifugation at 800 × *g* for 1 min. The BH was stored at −80°C until use.

For our studies, we also used urine and feces from hamsters infected by 263K prions and deer affected by CWD. Urine and feces from terminally sick hamsters was collected using metabolic cages, as described (Gonzalez-Romero et al., 2008). For cervids, urine and feces were collected as previously described from a CWD-affected white-tailed deer (Haley et al., 2009).

All animal experimentation was performed following NIH guidelines and approved by the Animal Welfare Committees of the University of Texas Medical School at Houston and the Colorado State University.

Exposure of Plant Tissue to Infectious Prions

Leaves and roots, grown from organic wheatgrass seeds (*Triticum aestivum*), were used for inoculation experiments. A 2-cm^2 piece (4 cm^2 total surface considering back and front) of wheat grass leave and a 15-mg piece of a pre-washed root were placed in a 2-ml reaction tube and incubated with 300 µl of prion-infected BH at the indicated dilution in PBS by gently rotating for 16 hr at room temperature. Afterward, the plant tissue was washed carefully five times with 1 ml tap water to remove unbound prion protein. A short spin (3 s) was included to remove remaining liquids. The presence of PrP^{Sc} attached to the plant tissue was measured by serial PMCA.

For contamination of plant tissue with prions present in urine and feces, wheat grass leaves and roots were incubated with 1 ml of whole urine (or 1:2.5 diluted urine for CWD samples) or 1 ml of 20% feces homogenate (5% for CWD samples) for 1 hr gently rotating and processed as described for the BH incubation.

For the experiments aimed to determine the survival of prions attached to living plants, we sprayed the leaves of wheat grass plants three times with a 10^{-2} dilution of 263K BH. Pieces of leaves (3.2 cm^2) from living plants were taken after 0, 3, 7, 14, 21, 28, 32, and 49 days post-treatment, washed five times with 1 ml tap water, and analyzed by PMCA.

Growing of Plants in Prion-Contaminated Soil

Barley grass (*Hordeum vulgare*) plants were grown from seeds placed in 350 g of soil until they reached a height of around 12 cm. Subsequently, the surface of the soil was contaminated with 20 ml of 5% 263K or normal brain homogenate taking especial precaution not to contaminate the plant directly. Plants were grown in this soil for 1 or 3 weeks, and samples of stem and leaves were collected. Figure S5 shows a scheme of the region of stem and leaves used for the experiments. The plant tissue was allowed to dry, and 4 cm of the stem or leaves were grinded and analyzed for PrP^{Sc} by PMCA. To prevent cross-contamination, each sample was minced with separate disposable blades in disposable Petri dishes.

Serial Replication of Prions In Vitro by PMCA

10% normal brain homogenates (NBHs) from healthy animals, perfused with PBS plus 5 mM EDTA, were prepared as described before and used as a substrate for PMCA (Morales et al., 2012). NBH prepared from Golden Syrian hamster and 129S mice were used as substrates for prions replication of hamster and mouse PrP^{Sc}, respectively. Transgenic mice overexpressing human PrP with MM at position 129 or transgenic mice overexpressing cervid PrP were used to amplify vCJD and CWD, respectively.

For the positive control reaction, 10% BH from prion-infected animals was serially diluted into NBH and loaded onto 0.2-ml PCR tubes. To determine the presence of PrP^{Sc} in urine and feces, 1 ml of whole urine or 1 ml 20% feces homogenate from 263K-infected hamsters was ultracentrifuged for 1 hr at 45,000 rpm, and, after washing in 1 ml PBS and centrifuging again, the pellet was directly added to the PMCA tube containing NBH substrate.

In order to amplify PrP^{Sc} bound to plant tissue, the contaminated tissue was placed in a reaction tube with 120 µl NBH. NBH alone was used as a negative control. Each PMCA tube, supplemented with three Teflon beads (Hoover Precision Products) was placed in a microsonicator (Qsonica Model Q700) and submitted to PMCA cycles consisting of incubation at 37°C and brief sonication. Hamster and mouse prions were amplified using cycles of 29 min 40-s incubation followed by 20-s sonication at ~260 W. For human and cervid prions, the substrate was supplemented with 0.05% Digitonin and 5 mM EDTA, and the sonication time was increased to 40 s at 260–280 W. After a round of 96 cycles, 10 µl of the amplified sample was transferred into 90 µl NBH, and another PMCA round was performed until detection limit was reached.

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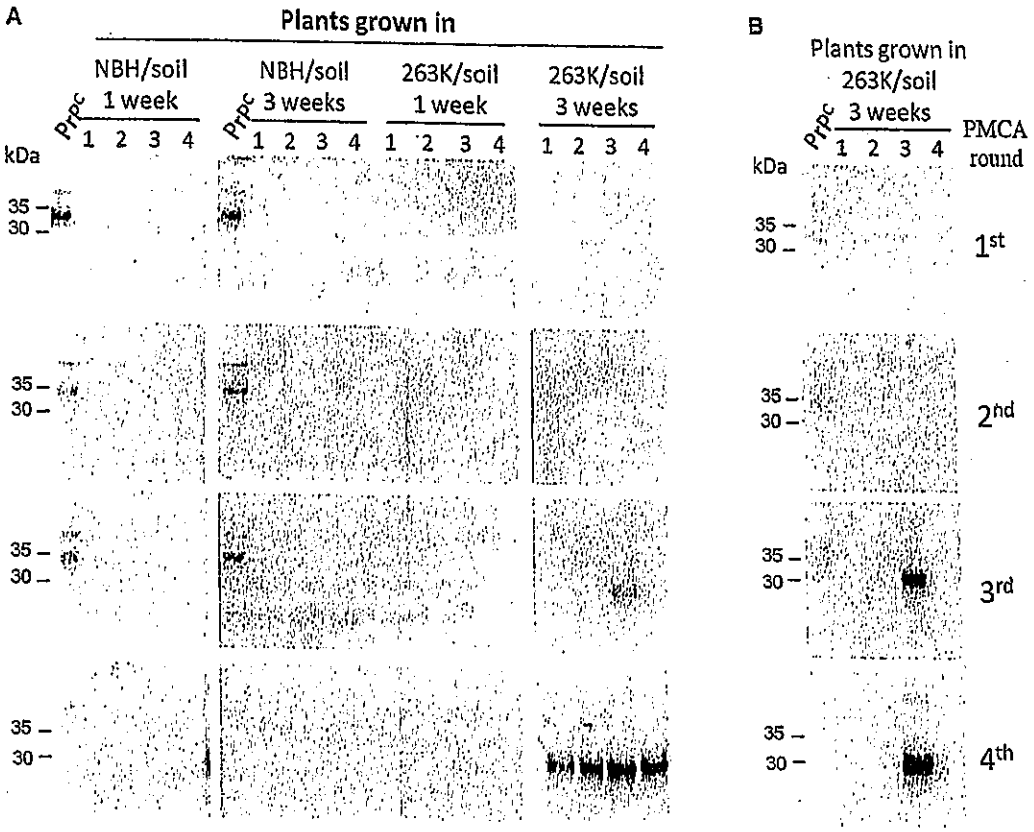

Figure 5. Uptake of Prions by Plants Grown in PrP^{Sc}-Contaminated Soil

The soil of barley grass plants, grown from seeds, was carefully contaminated on day 5 with 20 ml of 5% 263K brain homogenate and as control with the same amount of normal brain homogenate (NBH). One or 3 weeks after infection, plant samples were taken, dried, and minced. The grinded tissue corresponding to either the stem (A) or leaves (B) was analyzed for the presence of PrP^{Sc} by PMCA. Western blots of four different samples (1, 2, 3, or 4) of stems or leaves taken from plants grown for 1 or 3 weeks in 263K BH (or NBH as control) are shown. The results of four consecutive serial rounds of PMCA are depicted. Each round consisted of 96 PMCA cycles (2 days). All samples, except the normal brain homogenate used as a migration control (PrP^{C}), were digested with PK, as indicated in Experimental Procedures.

PK Digestion Assay and Western Blotting

To detect PrP^{Sc}, the samples were incubated in the presence of PK (50 μg/ml) for 1 hr at 37°C with shaking (450 rpm) in a thermomixer. When digesting samples resulting from human and cervid PMCA, 0.2% SDS was added to the PK reaction (100 μg/ml PK). The PK digestion was stopped by adding SDS sample buffer, 33 mM DTT, and boiling the samples for 10 min.

The proteinase resistant PrP was fractionated by SDS-PAGE, electroblotted into Hybond-ECL nitrocellulose membrane (Amersham GE Healthcare), and probed with 6D11 (1:5,000) for hamster, mouse, and cervid PrP^{Sc} or 3F4 (1:10,000) for human samples. The immunoreactive bands were visualized by enhanced chemiluminescence assay ECL Prime Western Blotting Detection system (GE Healthcare) using a Bio-Rad image analysis system.

Bioassay

Groups of five golden Syrian hamsters (females 6–10 weeks old) purchased from Harlan laboratories were orally inoculated with 3 U (3 × 2 cm^2 leaves or 3 × 15 mg roots) of leaves or roots previously exposed to 263K BH as indicated above. Hamsters orally injected with three similar units of leaves or roots treated with 10% NBH were used as control. The onset of clinical disease was measured by scoring the animals twice a week using our previously described scale (Castilla et al., 2008). Stage 1: normal animal; stage 2: mild behavioral abnormalities, including hyperactivity and hypersensitivity to noise; stage 3: moderate behavioral problems, including tremor of the head, ataxia, wobbling gait, head bobbing, irritability, and aggressiveness; stage 4: severe behavioral abnormalities, including all of the above plus jerks of the head and body and spontaneous backrolls. Animals scoring level 4 during 2 consecutive weeks were considered sick and were sacrificed. Brains were extracted and disease was confirmed by biochemical and histological analysis. The right cerebral hemisphere was frozen and stored at −70°C for biochemical studies of PrP^{Sc}, and the left hemisphere was used for histology analysis.

Neuropathology

Brains were harvested and left hemisphere fixed in Carnoy fixative (Giaccone et al., 2000), dehydrated, and embedded in paraplast. 10-μm serial sections were stained with H&E or immunostained with monoclonal antibodies to PrP (6H4, 1:1,000; Prionics) and to reactive astrocytes (GFAP, 1:2,000; Abcam). Before PrP immunostaining, the sections were treated with proteinase K (10 μg/ml, 5 min, room temperature) and guanidine isothiocyanate (3 M, 20 min, room temperature). To prevent unspecific bindings, Animal Research Kit (ARK, Dako) was used. Immunoreactions were visualized using 3-3′-diaminobenzidine (DAB, Dako) as chromogen.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.04.036.

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AUTHOR CONTRIBUTIONS

S.P. designed the studies, carried out the majority of the experiments, analyzed the results, and prepared the final version of the figures. R.M. participated in the in vivo infectivity studies and collaborated with the histological analysis. F.M. performed most of the histological studies. U.K. performed the studies of quantitative PMCA. G.C.T. provided colonies of transgenic mice expressing human and cervid PrP. E.H. provided CWD-infected urine, feces, and brains from white-tailed deer. C.S. is the principal investigator on the project and was responsible for coordinating research activity, analyzing the data, funding, writing the manuscript, and producing the final version of the article.

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No. 10

医薬品 研究報告 調査報告書

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一般的名称	新鮮凍結人血漿	研究報告の公表状況	Christopher Johnson, hristina Carlson, Matthew Keating, Nicole Gibbs, Haeyoon Chang, Jamie Wiepz, and Joel Pedersen Prion 2015 May 26-29, 2015. the United States.	公表国 米国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			

研究報告の概要

○プリオンの植物への取り込み
慢性消耗性疾患(CWD)やスクレイピーの感染性は、環境中では土壌で保存され得るため、土の粒子の摂取や吸入によって動物がプリオンに曝露する機序が考えられる。植物はタンパク質を含めて様々な物質を土から吸収するが、異常プリオン(PrP^{TSE})を吸収し、プリオンの感染性を保存するか否かは判明していない。本研究で我々は、蛍光タグを付けたPrP^{TSE}を用いたレーザー走査共焦点顕微鏡法によって植物の根へのPrP^{TSE}取り込みを調べ、連続蛋白質異常折りたたみ循環増幅(sPMCA)を用いて植物の地上部分のPrP^{TSE}の検出と定量化を試みた。タグを付けたPrP^{TSE}への曝露後、モデル植物であるシロイヌナズナと、農作物であるアルファルファ、オオムギ、トマトの根毛には蛍光が認められたが、タグを付けたコントロールには蛍光は確認されなかった。sPMCAを用い、根のみをPrP^{TSE}に曝露させた水耕栽培のシロイヌナズナ、アルファルファ、トウモロコシにおいて地上部分にPrP^{TSE}の存在が確認された。同部分のPrP^{TSE}濃度は、約4×10^{-10}～1×10^{-9} g PrP^{TSE} / g 植物乾燥重量、あるいは2×10^{5}～7×10^{6}脳内ID_{50}単位 / g 植物乾燥重量であった。プリオン含有培地で栽培したシロイヌナズナの茎および葉はいずれも、マウス脳内に注入した場合、感染性を有していた。我々の得た結果から、プリオンは植物への取り込みが可能であり、汚染された植物は、ヒト、家畜、野生動物においてこれまで認識されなかったプリオン曝露リスクとなることが示唆される。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
感染性プリオン蛋白(PrP^{TSE})含有培地で栽培したモデル植物であるシロイヌナズナの茎および葉はいずれも、マウス脳内に注入した場合、感染性を有していたという報告である。	今後もプリオン検出法等の技術を含め、CJD等プリオン病に関する新たな知見及び情報の収集に努める。

of total PrP^C which was the lowest of all samples analyzed, whereas sheep of the ARR/ARR genotype exhibited 5 times more C1, on average 53% of total PrP^C. Increased total PrP^C expression is associated with the relative level of truncated forms. It is likely that these differences in PrP^C processing contribute to the susceptibility and pathogenesis of prion diseases and they may reflect on diverse biological roles in different species.

P.157: Uptake of prions into plants

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Soil may preserve chronic wasting disease (CWD) and scrapie infectivity in the environment, making consumption or inhalation of soil particles a plausible mechanism whereby naïve animals can be exposed to prions. Plants are known to absorb a variety of substances from soil, including whole proteins, yet the potential for plants to take up abnormal prion protein (PrP^{TSE}) and preserve prion infectivity is not known. In this study, we assessed PrP^{TSE} uptake into roots using laser scanning confocal microscopy with fluorescently tagged PrP^{TSE} and we used serial protein misfolding cyclic amplification (sPMCA) and detect and quantify PrP^{TSE} levels in plant aerial tissues. Fluorescence was identified in the root hairs of the model plant *Arabidopsis thaliana*, as well as the crop plants alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*) and tomato (*Solanum lycopersicum*) upon exposure to tagged PrP^{TSE} but not a tagged control preparation. Using sPMCA, we found evidence of PrP^{TSE} in aerial tissues of *A. thaliana*, alfalfa and maize (*Zea mays*) grown in hydroponic cultures in which only roots were exposed to PrP^{TSE}. Levels of PrP^{TSE} in plant aerial tissues ranged from approximately 4×10^{-10} to 1×10^{-9} g $PrP^{TSE} \bullet g^{-1}$ plant dry weight or 2×10^5 to 7×10^6 intracerebral ID_{50} units$\bullet g^{-1}$ plant dry weight. Both stems and leaves of *A. thaliana* grown in culture media containing prions are infectious when intracerebrally-injected into mice. Our results suggest that prions can be taken up by plants and that contaminated plants may represent a previously unrecognized risk of human, domestic species and wildlife exposure to prions.

P.158: Evaluation of prion vaccine administered with vaccine enhancing agent

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Transmissible spongiform encephalopathy (TSE) is a neurodegenerative disorder characterized by pathologic accumulation of a misfolded form of a normal cellular protein in neurons. Emergence of TSEs in wildlife populations and the ability of some TSEs to cross species barriers have prompted concern regarding the lack of treatment options or prevention strategies. Efforts at vaccine development have been hampered by the difficulty of overcoming self-tolerance. Studies in our lab have demonstrated that vaccine induced immunity is often diminished due to the recruitment of anti-inflammatory myeloid cells. We hypothesized that utilizing an effective antigen while inhibiting monocyte migration could elicit a more effective anti-prion response.

The vaccine was formulated using a peptide fragment of the human prion protein (PrP106-126). This peptide spontaneously forms fibrillar aggregates and is thought to mediate the conversion from the normal cellular prion protein (PrPC) to the pathogenic form (PrPSC). To enhance vaccine efficacy, a monocyte migration inhibitor was administered (RS102895). To further target the pathogenic PrPSC, the peptide was reconstituted in an acidic solution and incubated at 37°C to increase fibrillization. Antibody responses were assessed using

別紙様式第2-1

No. 11

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
				2015. 5. 26	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		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		Paul Brown, Diane Ritchie, James Ironside, Christian Abee, Thomas Kreil, and Susan Gibson Prion 2015 May 26-29, 2015. the United States.	米国

研究報告の概要

○リスザルにおけるプリオン血液伝播: Baxter研究
英国における、vCJD伝播の5症例と、2,000人当たりに1人とされる無症候性感染の報告は、ヒトの同疾患リスクの情報が引き続き必要であることを強調している。非ヒト霊長類モデルにおける血液製剤の感染性に関する大規模研究が完了し、分析が行われた。ゲルストマン・ストロイスラー・シャインカー病(Gerstmann- Sträussler-Scheinker:GSS)の1症例、sCJDの4症例、vCJDの3症例のうち、GSSの白血球によってのみ5～6年間のサーベイランス期間中に疾患伝播が確認された。sCJDとvCJDに脳内感染させた供血動物の潜伏期および臨床期に行った複数の全血輸血の受血動物は、一律に陰性となった。これらの研究結果、および他のげっ歯類や非ヒト霊長類の研究、ヒトにおける疫学観察研究の結果を併せ、GSS(と恐らく他の家族性TSE)患者からの供血は、vCJD患者からの供血よりリスクが高く、一方、sCJD患者の供血にはリスクはほぼないことが示唆される。“無症候性”vCJDキャリアの数十年に及ぶ潜伏期の問題は、未解決のままである。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
非ヒト霊長類モデルのリスザルによる血液製剤の感染性に関する大規模研究の結果、GSSの白血球によって5～6年間のサーベイランス期間中に疾患伝播が確認されたという報告である。	日本赤十字社は、受付時の問診にてGSS罹患の既往がある場合は献血不可としている。今後もプリオン検出法等の技術を含め、CJD等プリオン病に関する新たな知見及び情報の収集に努める。

target these cells. The normal function of the prion protein (PrP) has remained elusive. PrP undergoes at least 2 internal cleavage events to produce N1/C1 and N2/C2 fragments. We have proposed that these distinct fragments possess differing properties and physiological function. Our previous studies have shown that the N-terminal cleavage fragment designated N2 reduces the production of intracellular reactive oxygen species (ROS) in response to mild stress. Other research groups have shown protective effects of N1. NSC growth is modulated by intracellular ROS levels and NSCs harvested from mice expressing different levels of PrP show a positive correlation between PrP expression and growth. We hypothesized that the N2 fragment and also the longer N1 fragment might be able to modulate NSC growth through their effects on modulating intracellular ROS. We find that both the N1 and N2 fragments halt cellular growth, migration and maturation. NSCs show reduced intracellular ROS detection following N1 or N2 exposure and appear to have entered into a quiescent state. Inhibition of NADPH oxidase produces a similar phenotype in these cells. Our investigations now focus on the role of N1 and N2 modulation of NADPH oxidase signaling pathways in maintaining stem cell quiescence.

P.163: A practical approach to avoiding iatrogenic CJD from invasive instruments

Paul Brown[1] and Michael Farrell[2]

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Potential Creutzfeldt-Jakob disease instrument-contamination events continue to occur that involve widespread hospital and patient concern. This paper proposes a combination of diagnostic tests and instrument handling procedures that, if routinely applied to patients admitted with symptoms of either dementia or cerebellar disease, should eliminate the risk of iatrogenic instrument infection.

P.164: Blood transmission of prion infectivity in the squirrel monkey: The Baxter study

Paul Brown[1], Diane Ritchie[2], James Ironside[2], Christian Abee[3], Thomas Kreil[4], and Susan Gibson[5]

[1]NIH (retired); Bethesda, MD USA; [2]University of Edinburgh; Edinburgh, UK; [3]University of Texas; Bastrop, TX USA; [4]Baxter Bioscience; Vienna, Austria; [5]University of South Alabama; Mobile, AL USA

Five vCJD disease transmissions and an estimated 1 in 2000 'silent' infections in UK residents emphasize the continued need for information about disease risk in humans. A large study of blood component infectivity in a non-human primate model has now been completed and analyzed. Among 1 GSS, 4 sCJD, and 3 vCJD cases, only GSS leukocytes transmitted disease within a 5–6 year surveillance period. A transmission study in recipients of multiple whole blood transfusions during the incubation and clinical stages of sCJD and vCJD in ic-infected donor animals was uniformly negative. These results, together with other laboratory studies in rodents and non-human primates and epidemiological observations in humans, suggest that blood donations from cases of GSS (and perhaps other familial forms of TSE) carry more risk than from vCJD cases, and that little or no risk is associated with sCJD. The issue of decades-long incubation periods in 'silent' vCJD carriers remains open.

感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

１ 基本的な方針

運営委員会に報告する資料においては、

(1) 文献報告は、同一報告に由来するものの重複を廃した一覧表を作成すること。

(2) 8月の運営委員会において、国内の輸血及び血漿分画製剤の使用した個別症例の感染症発生報告は、定期的にまとめた「感染症報告事例のまとめ」を運営委員会に提出する取り扱いとされた。これにより、感染症定期報告に添付される過去の感染症発生症例報告よりも、直近の「感染症報告事例のまとめ」を主として利用することとすること。

２ 具体的な方法

(1) 感染症定期報告の内容は、原則、すべて運営委員会委員に送付することとするが、次の資料概要を作成し、委員の資料の確認を効率的かつ効果的に行うことができるようにする。

① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第2）及び該当文献を添付した**「資料概要A」**を事務局が作成し、送付する。

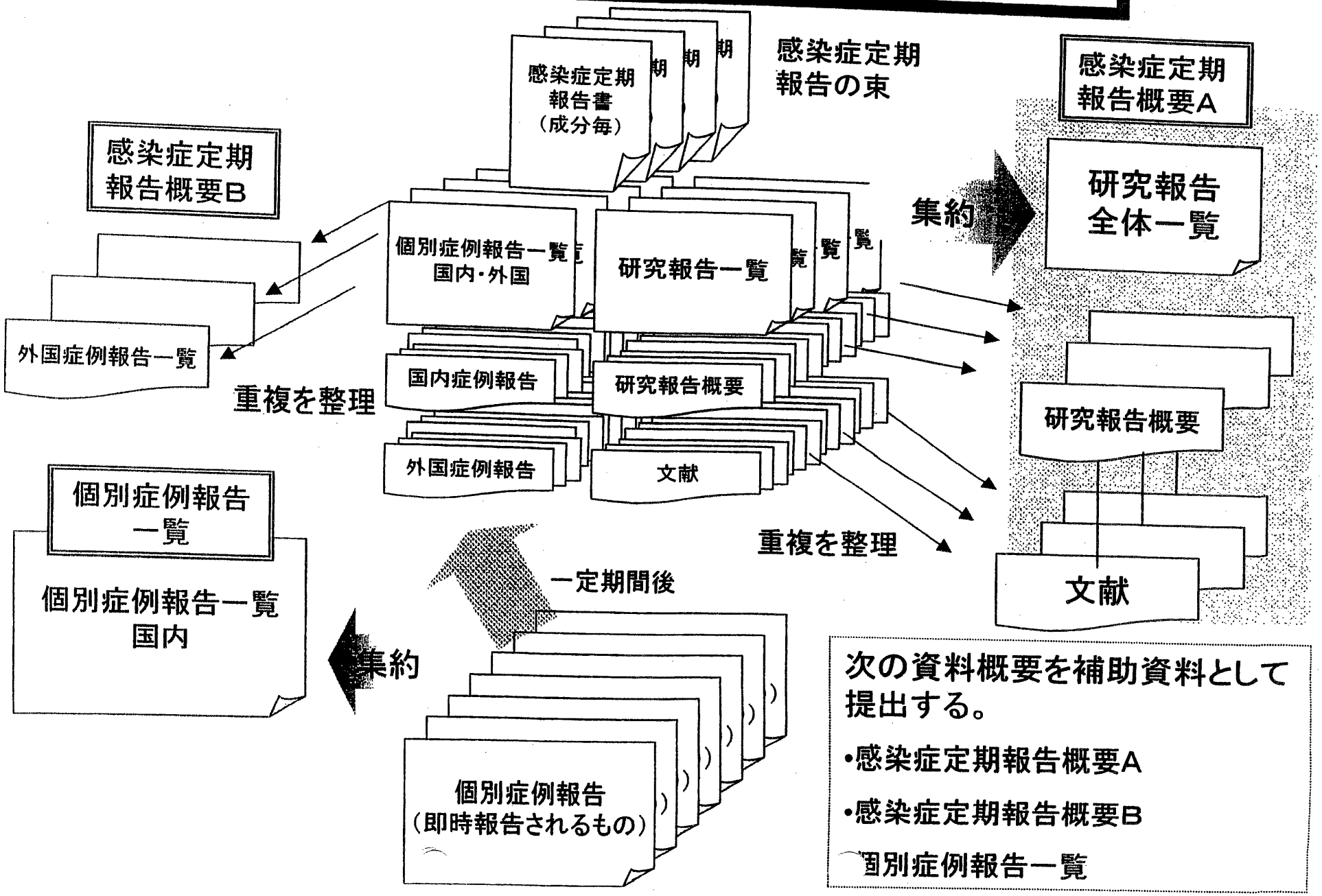
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

③ 感染症発生症例報告のうち、発現国が「国内」の輸血による症例及び血漿分画製剤の使用による感染症症例については、「感染症報告事例のまとめ」を提出することから、当該症例にかかる「資料概要」は作成しないこととする。ただし、運営委員会委員から特段の議論が必要との指摘がなされたものについては、別途事務局が資料を作成する。

(2) 発現国が「外国」の感染症発生症例報告については、国内で使用しているロットと関係がないもの、使用時期が相当程度古いもの、因果関係についての詳細情報の入手が困難であるものが多く、必ずしも緊急性が高くないと考えられるものも少なくない。また、国内症例に比べて個別症例を分析・評価することが難しいものが多いため、緊急性があると考えられるものを除き、その安全対策への利用については、引き続き、検討を行う。

(3) 資料概要A及びBについては、平成16年9月の運営委員会から試験的に作成し、以後「感染症的報告について（目次）」資料は廃止することとする。

感染症定期報告・感染症個別症例報告の取り扱い



次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧