

A 研究報告（詳細版）

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医薬品
医薬部外品
化粧品
研究報告 調査報告書

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一般的名称	人ハプトグロビン		研究報告の 公表状況	www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/05/WC500166018.pdf/2014/5/2		使用上の注意記載状況・ その他参考事項等
販売名 (企業名)	ハプトグロビン 2000 単位「ベネシス」 (日本血液製剤機構)		公表国 欧州			
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に関する概念書(案) 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、そして先進国において新興の関心事となっている。HEVはノンエンベロープ、一本鎖、RNA(+)ウイルスで、ヘペウイルス科の一つである。発展途上国では、HEV(遺伝子型1と2)は急性肝炎の主な原因で、糞口経路による感染や飲料水の汚染に関連している。先進国では、HEV(遺伝子型3と4)は当初考えられていたよりも、ヒト集団において、より流行していることが分かっている。HEV(遺伝子型3および4はヒトだけでなく、ブタ、イノシシ、シカなどの動物にも感染する。ヒトへのHEV(遺伝子型3と4)の人間共通感染は、汚染された肉や肉製品の消費、或いは感染した動物との接触によって引き起こされる。これらの遺伝子型は遺伝子型1と2よりも一般的に病原性は低いが、いくつかの例外が報告されている。HEV(遺伝子型3)の慢性感染は移植レシピエントの間で新興性の懸念であり、またHIVおよび特定の血液疾患の人で発生する可能性がある。 2. 問題の記述 HEV感染は広まっており、また血液/血漿ドナーはしばしば無症候である。従って、ウイルス血症の献血による危険性がある。HEVは2004年以降輸血感染原因物質として認識されており、輸血に関連した症例は幾つかの国(イギリス、フランス、日本、サウジアラビア、中国人共和国)で報告されている。血液と血漿供血の最近の分析は、ドイツ、スウェーデンおよび英国のHEV感染ドナーを特定している。これらの研究では、ウイルス血症供血の頻度は1:4,000と1:7,000の間であった。ウイルス血症の持続時間は通常4〜6週間の間で、かつウイルス濃度は1mLあたり10^7に達する。結果として、HEV-RNAは医薬品の製造に使われるプール血漿中で検出された。 3. (問題の記述に関する) 議論 ウイルス血症供血の頻度に関する公表された報告書およびプール血漿に関する研究は、医薬品製造の出発原料として使うプール血漿がHEVで汚染されていることを示す。その上、HEVに感染した供血が分画用プール血漿に投入されたことを示す供血後情報の例がある。これは、血漿由来医薬品の安全性について疑問を提起する。プールされウイルス不活化処理されるヒト血漿についての欧州薬局方モノグラフ(1646)は、HEV RNAの検査を含むように改訂中である(実施日付2015年1月1日)。HEV NAT検査法の標準化において用いるためのHEV用WHO国際標準品は確立されている。他の血漿由来製品の製造は、ノンエンベロープウイルスの不活化/除去の工程を含む。しかしながら、HEVに対するこれらの効果は現在不明である。HEVは培養が困難である、そして血漿由来医薬品の製造に使用するウイルス不活化/除去工程へのHEVの感受性についての現在の情報は不足している。 4. 勧告 更なる情報は、HEVに関する血漿由来医薬品の安全性に必要である。従って、専門家のワークショップが、関連する問題に対処するために2014年に組織される。以下の点に対処する必要がある。 ・輸血関連感染とHEV-感染症の臨床経験。						
研究報告の概要						

1

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	・血液および血漿ドナーの焦点を当てた HEV 感染の疫学に関する最新情報。 ・ウイルス血症の持続期間と血液および血漿供血のウイルス量。 ・ドナーのスクリーニングおよびブール血漿の検査 (NAT、参考試料) のための可能な検査方法。 ・HEV 特異的抗体と中和。 ・HEV 用の細胞培養システムの開発についての最新情報およびウイルス不活化/除去の検証のためその実現可能性。 ・HEV の不活性化/除去に関する研究からの最新の経験。 ・HEV のウイルス不活化/除去の評価のためのモデルウイルスの妥当性。 ・有機溶剤-界面活性剤で処理した血漿の安全性。 ・血漿由来医薬品のためのリスク評価および警告文の関与。 ・患者からの報告。 このワークショップは、血漿由来医薬品および/または特に 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に関するリフレクシオンペーパーの開発に関する現在のガイダンスの可能性を含む、更なるアクションが必要とされるものを決定するための基礎を提供する。 (以下、省略)				
	<table border="1"> <thead> <tr> <th data-bbox="874 611 911 913">報告企業の意見</th><th data-bbox="874 913 911 2190">今後の対応</th></tr> </thead> <tbody> <tr> <td data-bbox="911 611 1176 913"> E 型肝炎ウイルス (Hepatitis E virus: HEV) はへペウイルス科 (hepeviridae) へペウイルス属 (hepevirus) に属する直径約 38nm のエンベロープを持たない小球状のウイルスで、内部に約 7.2kb の一本鎖 RNA を内包し、G1 から G4 まで 4 つの遺伝子型が報告されている。万一、原料血漿に HEV が混入したとしても、Murine encephalomyocarditis virus (EMC) 及び Canine parvovirus (CPV) をモデルウイルスとした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。 </td><td data-bbox="911 913 1176 2190"> 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。 </td></tr> </tbody> </table>	報告企業の意見	今後の対応	E 型肝炎ウイルス (Hepatitis E virus: HEV) はへペウイルス科 (hepeviridae) へペウイルス属 (hepevirus) に属する直径約 38nm のエンベロープを持たない小球状のウイルスで、内部に約 7.2kb の一本鎖 RNA を内包し、G1 から G4 まで 4 つの遺伝子型が報告されている。万一、原料血漿に HEV が混入したとしても、Murine encephalomyocarditis virus (EMC) 及び Canine parvovirus (CPV) をモデルウイルスとした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。
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EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

1 25 April 2014
2 EMA/CHMP/BWP/78086/2014
3 Biologics Working Party (BWP)

4 Concept paper on viral safety of plasma-derived medicinal
5 products with respect to hepatitis E virus
6

Agreed by Biologics Working Party	March 2014
Adopted by CHMP for release for consultation	25 April 2014
Start of public consultation	2 May 2014
End of consultation (deadline for comments)	31 July 2014

7
8 Comments should be provided using this [template](#). The completed comments form should be sent
9 to BPWPsecretariat@ema.europa.eu

10
Keywords virus safety, hepatitis E, plasma-derived medicinal products, blood products



1. Introduction

Hepatitis E virus (HEV) is a causative agent of hepatitis in many countries and of emerging concern in industrialized 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 fecal-oral route and associated with contamination of drinking water. In industrialized 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 contaminated meat or meat products or by contact with infected animals. These genotypes are generally less pathogenic than genotypes 1 and 2, although some exceptions have been reported. Chronic infection with HEV genotype 3 is an emerging concern among transplant recipients and may also occur in persons with HIV and certain hematologic disorders.

2. Problem statement

HEV infection is widespread and blood/plasma donors are often asymptomatic. Therefore, there is a risk for viraemic blood donations. HEV has been recognized 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 Germany, Sweden, and United Kingdom. In these studies, frequency of viraemic donations ranged between 1:4000 and 1:7000. The duration of viraemia is usually between 4 to 6 weeks, and the viral concentration can reach 7 log₁₀ RNA per ml. Consequently, HEV-RNA has been detected in plasma pools used for production of medicinal products.

3. Discussion (on the problem statement)

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 the plasma-derived medicinal products. The Ph. Eur. monograph for human plasma pooled and treated for virus inactivation (1646) is under revision to include a test for HEV RNA (implementation date 1 January 2015). A WHO International Standard for HEV for use in the standardisation of HEV NAT assays has been established. Manufacture of other plasma-derived products includes process steps for inactivation/removal of non-enveloped viruses. However, their effectiveness against HEV is currently unclear. HEV is difficult to cultivate and current information about susceptibility of HEV to virus inactivation/removal steps used in the manufacture of plasma-derived medicinal products is scarce.

4. Recommendation

Further information is needed on the safety of plasma-derived medicinal products with respect to HEV. Therefore, an expert workshop will be organised in 2014 to address the relevant issues. The following points should be addressed.

- Transfusion-associated infections and clinical experience with HEV-infections.
- Latest information on the epidemiology of HEV infection with focus on blood and plasma donors.

- 51 • Duration of viraemia and virus loads of blood and plasma donations.
- 52 • Potential testing methods for screening of donors and testing of plasma pools (NAT, reference
- 53 materials).
- 54 • HEV-specific antibodies and neutralisation.
- 55 • Latest information about the development of cell culture systems for HEV and their feasibility for
- 56 validation of virus inactivation/removal.
- 57 • Latest experience from studies on inactivation/removal of HEV.
- 58 • Relevance of model viruses for evaluation of virus inactivation/removal of HEV.
- 59 • Safety of solvent-detergent treated plasma.
- 60 • Risk assessment for plasma-derived medical products and implication for warning statements.
- 61 • Perspective from patients.
- 62 This workshop will provide the basis for deciding what further action may be needed, including the
- 63 possible update of current guidance on plasma-derived medicinal products and/or development of a
- 64 reflection paper specifically on viral safety of plasma-derived medicinal products with respect to
- 65 hepatitis E virus.

66 **5. Proposed timetable**

67 The workshop is intended to take place on 28-29 October 2014.

68 **6. Impact assessment (anticipated)**

69 Viral safety of plasma-derived medicinal products needs to be kept under review as viruses are
 70 identified that can be present in the plasma starting material. Initiating action with a workshop will
 71 provide an effective means of bringing together and discussing the currently available information on
 72 this topic. This will then allow further actions to be identified.

73 **7. Interested parties**

74 Blood products working party (BPWP).

75 Patient organisations (e.g. haemophilia patients (EHC, WFH), patients with primary immunodeficiencies
 76 (EPPIC, IPOPI)).

77 Industry organisations (IPFA, PPTA) and manufacturers of plasma-derived medicinal products.

78 The workshop may also be of interest to ECDC and blood competent authorities.

79 **8. References to literature, guidelines, etc.**

80 Guideline on plasma-derived medicinal products, EMA/CHMP/BWP/706271/2010

81 [http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/07/WC50010962](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/07/WC500109627.pdf)
 82 [7.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/07/WC500109627.pdf)

83

84 Guideline on the warning on transmissible agents in summary of product characteristics (SmPCs) and
85 package leaflets for plasma-derived medicinal products. EMA/CHMP/BWP/360642/2010 rev. 1
86 [http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/12/WC50011900](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/12/WC500119001.pdf)
87 [1.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/12/WC500119001.pdf)

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	—	研究報告の 公表状況	http://www.promedmail.org/direct.php?id=2397091	公表国 仏国	
販売名(企業名)	—				
研究報告の概要	フランスポリネシア領で2013年からジカウイルスの流行があり、輸血によるジカウイルスの伝播を防止するため、核酸増幅検査を行ったところ、2013年11月～2014年2月における供血症時に無症候の供血症者1505中42人(3%)が陽性であった。 アルボウイルスの輸血感染の主な課題は、無症候感染率が高いことで、デングウイルス、西ナイルウイルスでは約75%と推定されるが、ジカウイルスにおける推定値はない。 アルボウイルスの輸血感染は西ナイルウイルス、デングウイルス、チクングニヤウイルスで報告されており、チクングニヤウイルスのリスクが高い。インド洋のリユニオン島、イタリヤなどでチクングニヤウイルス感染が発生したときには、献血を中止し、他の地方から輸入している。 フランス領ポリネシアは地域的に孤立しており、外部血液センターから新鮮な血液製剤を供給することは不可能であることから、献血を継続する必要があるため可能な限り早期にジカウイルスに対する核酸増幅検査(RT-PCR)を実施することを決定し、2013年11月から実施した。 現在までフランスポリネシア領でジカウイルス陽性血の輸血によるジカウイルス感染は報告されていないが、監視は継続している。 2013年4月から実施のデングウイルスの核酸増幅検査において陽性供血症者は検出されていないが、これは無症候性供血症者の血中のウイルスレベルが低いことに関係しているかもしれないが、我々は恐らくデングウイルス-1及びデングウイルス-3の流行のレベルが低いと考えている。 今回の知見はジカウイルスの輸血感染を防止するために核酸増幅検査を行うべきであることを示唆している。ヨーロッパ疾病予防対策センターが推奨するように、血液安全局は警戒し、ジカウイルス感染発生地域から戻った人の供血を延期することを検討する必要がある。ヒトスジシマ蚊の繁生地域における将来のジカウイルス感染の大流行に対応するための準備計画には、血液供給を維持するための非常時計画が含まれるべきである。				
報告企業の意見	今後の対応 今後ともジカウイルスに関連する情報に留意していく。				
血漿分画製剤でのジカウイルス感染伝播の報告はなく、製造工程中にモデルウイルスであるウシ下痢症ウイルス(BVDV)の不活化除去が確認された工程を設けているが、今後とも関連情報に留意していく。					

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Published Date: 2014-04-11 15:14:18
 Subject: PRO/EDR> Zika virus - Pacific (12): French Polynesia, donated blood
 Archive Number: 20140411.2397091

ZIKA VIRUS - PACIFIC (12): FRENCH POLYNESIA, DONATED BLOOD

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 International Society for Infectious Diseases
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Date: Thu 10 Apr 2014

Source: Eurosurveillance Volume 19, Issue 14 [summ., edited]

<http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20761>

Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014.

Summary

Since October 2013, French Polynesia has experienced the largest documented outbreak of Zika virus (ZIKAV) infection. To prevent transmission of ZIKAV by blood transfusion, specific nucleic acid testing of blood donors was implemented. From November 2013 to February 2014: 42 (3 per cent) of 1505 blood donors, although asymptomatic at the time of blood donation, were found positive for ZIKAV by PCR. Our results serve to alert blood safety authorities about the risk of post-transfusion Zika fever.

Zika virus infection in French Polynesia: implications for blood transfusion

French Polynesia, in the South Pacific, has experienced the largest reported outbreak of ZIKAV infection, which began in October 2013, with an estimated 28 000 cases in February 2014 (about 11 per cent of the population), concomitantly with the circulation of dengue virus (DENV) serotypes 1 and 3. To the best of our knowledge, the occurrence of ZIKAV infection resulting from transfusion of infected blood has not been investigated. Since other arboviruses have been reported to be transmitted by blood transfusion, several prevention procedures were implemented in date to prevent transfusion of ZIKAV through transmission in French Polynesia, including nucleic acid testing (NAT) of blood donors. We report here the detection of ZIKAV in 42 of 1505 blood donors, who were asymptomatic at the time of blood donation.

Discussion

The main challenge in the prevention of arbovirus transfusion-derived transmission is the high rate of asymptomatic infections: this has been estimated at over 75 per cent for DENV and West Nile virus (WNV). For ZIKAV, there is no estimate available of the percentage of asymptomatic infections.

Arbovirus transfusion-derived transmission has been reported principally for WNV, DENV and chikungunya virus (CHIKV). For CHIKV, the risk was evaluated as high.

During the outbreaks of CHIKV infection in Italy (2007) and in Reunion Island in the Indian Ocean (2005-2007), blood donation was discontinued and blood products were imported from blood bank centres elsewhere. In French Polynesia, due to its geographically isolated location, it was impossible to be supplied with fresh blood products from blood bank centres outside French Polynesia.

Due to the potential risk of ZIKAV transfusion-derived transmission, the need to continue blood donations and the lack of a licensed test for ZIKAV diagnosis, we decided to implement ZIKAV NAT as soon as possible, using a modified RT-PCR. The protocol was implemented in November 2013, when agreement from the French Polynesian health authorities was obtained. The specificity of this RT-PCR assay has been previously evaluated and was confirmed by sequencing analysis conducted during the outbreak in French Polynesia and its sensitivity was similar to that previously evaluated.

We detected an unexpectedly high number of positive asymptomatic blood donors (42/1505; 3 per cent). To date, no post-transfusion ZIKAV infection has been reported in recipients of ZIKAV-positive blood in French Polynesia; however, haemovigilance studies are still ongoing.

Due to concomitant circulation of DENV serotypes 1 and 3 since early 2013, multiplex NAT testing for DENV has been implemented from April 2013: no DENV-positive donor has yet been detected. While this might be related to a low level of viraemia in asymptomatic donors, we consider it was probably due to the low level of DENV-1 and DENV-3 circulation. Pathogen inactivation of platelet concentrates using a photochemical treatment (amotosalen)

of blood products and ultraviolet A light inactivation was also implemented.

The management of a dual outbreak of ZIKAV and DENV infection was challenging because we had to test all blood donors for both pathogens, which was time consuming and expensive. In addition, in our blood bank centre, the mean delay between blood donation and production of fresh blood product available for transfusion is generally 24 hours. During the outbreaks, the mean delay was 3 days.

This report serves as a reminder of the importance of quickly adapting blood donation safety procedures to the local epidemiological context. Moreover, it should help in anticipating the needs in other parts of the Pacific region, such as in New Caledonia (South Pacific), where an outbreak of ZIKAV infection started in February 2014.

Our findings suggest that ZIKAV NAT should be used to prevent blood transfusion-transmitted ZIKAV. As recommended by the European Centre for Disease Prevention and Control, blood safety authorities need to be vigilant and should consider deferral of blood donors returning from areas with an outbreak of ZIKAV infection. In areas endemic for *Aedes* species, a preparedness plan to respond to future outbreaks of ZIKAV infection should include emergency plans to sustain the blood supply.

[The full report with references may be found at the URL above. - Mod.SH]

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[The response of the health authorities in French Polynesia in dealing with maintenance of a safe blood supply during a significant outbreak of a virus disease new to the area is outstanding. The logistical complications due to the geographic isolation of the islands were overcome with scientifically sound, innovative approaches for blood screening.

Maps showing the location of the island archipelagos in French Polynesia mentioned above can be accessed at <http://www.worldatlas.com/webimage/countrys/oceania/pf.htm> and at <http://healthmap.org/r/4tD1>. - Mod.TY]

See Also

Zika virus - Pacific (11): French Polynesia, perinatal transmission [20140405.2383237](#)
Zika virus - Pacific (09) [20140328.2365267](#)
Zika virus - Pacific (08) [20140316.2335754](#)
Zika virus - Pacific (07): Easter Island, French Polynesia [20140309.2322907](#)
Zika virus - Pacific (06): French Polynesia, New Caledonia [20140303.2309965](#)
Zika virus - Pacific (04): French Polynesia [20140123.2227452](#)
Zika virus - Pacific (02): French Polynesia [20140110.2165365](#)
Zika virus - Pacific: French Polynesia [20140108.2159822](#)
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 処方せん医薬品^{※1)}

PPSB®-HT 静注用500単位「ニチヤク」

献血由来

生物学的製剤基準 乾燥人血液凝固第Ⅸ因子複合体

PPSB®-HT for I. V. injection 200 units & 500 units 「NICHYAKU」

注1) 注意—医師等の処方せんにより使用すること

本剤は、貴重なヒト血液を原料として製剤化したものである。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、ヒト血液を原料としていること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〕

【組成・性状】

1. 組成

本剤は、人血漿中の血液凝固第Ⅸ因子複合体を含む凍結乾燥製剤で、1瓶中に下記の成分を含有する。

成分	200単位製剤	500単位製剤
有効成分	血液凝固第Ⅸ因子 200単位	500単位
添加物	ヘパリンナトリウム 50ヘパリン単位	125ヘパリン単位
添加物	クエン酸ナトリウム水和物 120mg	300mg
添加物	塩化ナトリウム 48mg	120mg

添付溶解液: 日本薬局方注射用水 10mL 25mL

本剤の主成分である血液凝固第Ⅸ因子は、日本において採取された献血血液を原料としている。また、添加物としてブタ腸粘膜由来のヘパリンを使用している。

2. 製剤の性状

本剤は白色の凍結乾燥注射剤である。本剤を添付の溶解液(日本薬局方注射用水)で溶解したとき、無色ないし淡黄色の澄明な液剤となり、そのpHは6.4~7.4、浸透圧比(生理食塩液に対する比)は0.8~1.2である。

【効能・効果】

血液凝固第Ⅸ因子欠乏患者の出血傾向を抑制する。

【用法・用量】

本剤を添付の日本薬局方注射用水10mL(200単位製剤)あるいは25mL(500単位製剤)で溶解し、通常1回血液凝固第Ⅸ因子量200~1,200単位を静脈内に緩徐に注射する。用量は、年齢・症状に応じ適宜増減する。〔溶解方法については末尾を参照してください。〕

<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、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) IgA欠損症の患者〔抗IgA抗体を保有する患者では過敏反応を起こすおそれがある。〕
- (2) 溶血性・失血性貧血の患者〔ヒトバロウイルスB19の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
- (3) 免疫不全患者・免疫抑制状態の患者〔ヒトバロウイルスB19の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕

2. 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。

- ※(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抗体及び抗HTLV-I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、HIV、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。その後の製造

工程である65℃、96時間の加熱処理及びウイルス除去膜による過処理は、HIVをはじめとする各種ウイルスに対し、不活化・除去作用を有することが確認されているが、投与に際しては、次の点に十分注意すること。

- 1) 血漿分画製剤の現在の製造工程では、ヒトバロウイルスB19等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
- 2) 肝炎ウイルス感染のリスクを完全に否定できないので、観察を十分に行い、症状があらわれた場合には適切な処置を行うこと。
- 3) 現在までに本剤の投与により変異型クローンツェルト・ヤコブ病(vCJD)等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全に排除できないので、投与の際には患者への説明を十分に行い、治療上の必要性を十分検討の上投与すること。

※(2) アナフィラキシーを起こすことがあるので、観察を十分に行うこと。

- (3) 患者の血中に血液凝固第Ⅸ因子に対するインヒビターが発生するおそれがある。本剤を投与しても予想した止血効果が得られない場合には、インヒビターの発生を疑い、回収率やインヒビターの検査を行うなど注意深く対応し、適切な処置を行うこと。
- (4) 大量投与によりDICを起こす危険性を完全に否定できないので、観察を十分に行うこと。

3. 副作用

試験時に安全性評価対象となった血友病B44症例に対し延べ406回の投与を行った結果、血管痛1件、発汗1件がみられたが、いずれも一過性の軽度な副作用で無処置にて回復した。^{1,2)}

以下の副作用は、自発報告等で認められたものである。

(1) 重大な副作用

※(1) アナフィラキシー(頻度不明):

アナフィラキシーを起こすことがあるので、観察を十分に行い異常が認められた場合には投与を中止し、適切な処置を行うこと。

2) DIC(頻度不明):

大量投与によりDICを起こすことがあるので、観察を十分に行い異常が認められた場合には投与を中止し、適切な処置を行うこと。

(2) その他の副作用

	頻度不明
過敏症 ^{※2)}	発熱、顔面紅潮、蕁麻疹等
その他	悪寒、腰痛

注2) 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にのみ投与すること。

〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトバロウイルスB19の感染の可能性を否定できない。感染した場合には胎児への障害(流産、胎児水腫、胎児死亡)が起こる可能性がある。〕

6. 適用上の注意

(1) 調製時：

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) 溶解時に沈殿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。また、一度溶解したものは1時間以内に使用すること。

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。

3) 他剤と混注しないこと。

(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) 使用済の医療機器等の処理については、主治医の指示に従うこと。

【薬物動態】

1. 血友病B 19症例に本剤を平均投与量約1.5mL/kg投与したときの第Ⅰ相及び第Ⅱ相の血中半減期は、平均8.2時間及び20.3時間であった。"

2. 血友病B 21症例に本剤を平均投与量約1.5mL/kg投与したところ、血液凝固第Ⅲ因子の回収率は平均64.8%であった。"

【臨床成績】

血友病B 23症例303件の出血（出血部位：足関節95件、肘関節59件、膝関節19件、筋肉64件等）に対し、本剤を投与した結果、著効195件、有効91件で、有効率は94.4%（286件/303件）であった。"

【薬効薬理】

1. 血友病Bに本剤を投与したところ、活性化部分トロンボラスチン時間（APTT）は、投与直後から著明な改善（短縮）がみられ、投与48時間後でも有意な短縮を示した。"

2. 本剤の投与により、血友病Bに欠乏している血液凝固第Ⅲ因子を補充し、出血を抑制することができる。

【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、医薬品の名称（販売名）、製造番号、投与日又は処方日、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】

PPSB-HT静注用200単位 血液凝固第Ⅲ因子 200単位含有 1瓶
「ニチャク」 溶解液（日本薬局方注射用水） 10mL 1瓶添付

PPSB-HT静注用500単位 血液凝固第Ⅲ因子 500単位含有 1瓶
「ニチャク」 溶解液（日本薬局方注射用水） 25mL 1瓶添付

※別箱に下記のPPSB-HT静注用200単位「ニチャク」用輸注器セット及びPPSB-HT静注用500単位「ニチャク」用輸注器セットがあります。

溶解移注針、ディスプレイザブル注射筒、
ディスプレイザブル採液針、静脈針、翼状針 各1本
絆創膏、パッド付絆創膏 各1枚
消毒綿 2枚

【主要文献】

1) 安部 英，他：臨牀と研究，64：1327，1987。

2) 安部 英，他：臨牀と研究，66：287，1989。

**【文献請求先・製品情報お問い合わせ先】

◇文献請求・副作用等

日本製薬株式会社 医薬情報グループ

〒101-0031 東京都千代田区東神田一丁目9番8号

TEL 03-3864-8413 FAX 03-3864-8836

【受付時間 9:00～17:30/土日祝日・弊社休業日を除く】

◇その他のお問い合わせ

日本製薬株式会社 医薬学術グループ

〒101-0031 東京都千代田区東神田一丁目9番8号

TEL 03-3864-8414 FAX 03-3864-8410

【受付時間 9:00～17:30/土日祝日・弊社休業日を除く】

この製品は献血血液から製造されています。

製造販売元 **日本製薬株式会社**

東京都千代田区東神田一丁目9番8号

販売 **武田薬品工業株式会社**

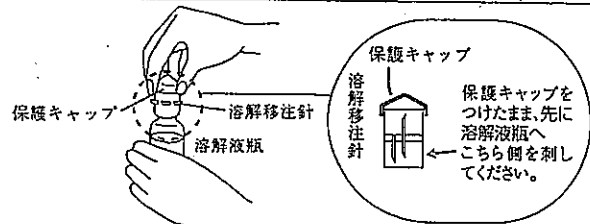
大阪市中央区道修町四丁目1番1号

＜溶解・ろ過の方法＞

製品瓶内は陰圧になっていますので、取り扱いに十分注意し、下記の手順に従って溶解してください。

1. 冷蔵庫より取り出した製品瓶と溶解液瓶をそのままの状態ですべて室温までおいてください。
2. 製品瓶、溶解液瓶のプラスチックキャップをはずし、ゴム栓表面を消毒してください。
3. 溶解移注針（以下「移注針」）の保護キャップのついている側を上にし、針を溶解液瓶のゴム栓にまっすぐ垂直に深く刺し込みます。

必ず溶解液瓶に先に刺し込んでください。製品瓶に先に刺し込むと陰圧が破壊され、溶解液がうまく移行しなくなります。



4. 移注針の保護キャップをはずし、溶解液瓶を移注針ごと逆さにし、製品瓶のゴム栓にまっすぐ垂直に深く刺し込むと溶解液が製品瓶内に移行します。

溶解液の移行中に瓶が倒れると溶解液が製品瓶内に移行しなくなることがありますので、ご注意ください。

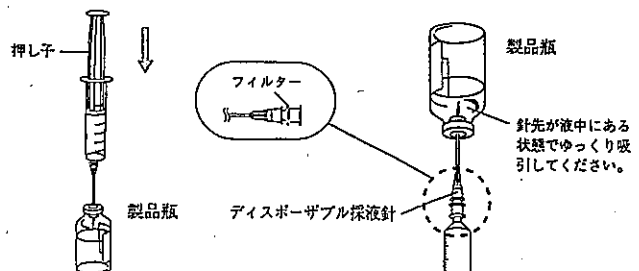


5. 溶解液の移行が終了したら、移注針を持って溶解液瓶と一緒に引き抜きます。

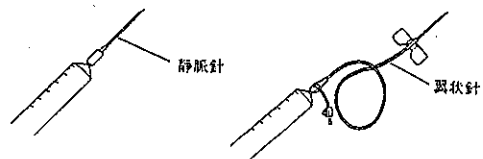


6. 薬液を泡立たせないように、製品瓶をゆるく振り、完全に溶解させてください。通常1～2分で完全に溶解します。
7. 溶解後、ディスプレイザブル採液針をディスプレイザブル注射筒にセットし、溶解液量と同じ容量分の空気（200単位製剤は10mL、500単位製剤は25mL）を吸引した後、ディスプレイザブル採液針を製品瓶に刺し、「押し子」を押して空気を注入します。その後、「押し子」を押したまま製品瓶を逆さにし、針先を液面から上に出さないようにして、薬液をゆっくり吸引してください。

薬液を吸引するとき、針先が液面から上に出て、空気を吸引すると薬液の吸引が困難になりますので、ご注意ください。



8. 薬液の吸引終了後、注射筒からディスプレイザブル採液針を取りはずし、添付の静脈針あるいは翼状針を装着してゆっくりと静脈内に注射してください。



溶解移注針、採液針、静脈針、翼状針、注射筒はディスプレイザブルですので再使用はしないでください。

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		厚生労働省処理欄
乾燥濃縮人アンチトロンビンIII			2014年03月05日	該当なし		
一般的名称	乾燥濃縮人アンチトロンビンIII		研究報告の公表状況	公表国		
販売名(企業名)	①ノイアート静注用 500 単位 (日本血液製剤機構) ②ノイアート静注用 1500 単位 (日本血液製剤機構)		Journal of Clinical Virology 2013; 58(4): 722-725	フランス		
1. 背景 新しいウイルス感染症は、公衆衛生にとって常に脅威である。現時点では、血液の安全性はドナーの選択法の改善、病原体の軽減、また既知のウイルスの核酸検査および抗原/抗体スクリーニング法に依存している。しかし、ウイルス DNA ローディングの低さ、宿主ならびにミトコンドリア DNA の多さ、また全てのウイルス間で保存された遺伝子の欠如といった方法論的な欠点新しい血液感染性ウイルスを検出困難なものとし、頻繁に輸血を受ける症例においては脅威になると考えられる。 サラセミアは、グロビン鎖の合成障害を特徴とする遺伝子疾患群である。サラセミアに罹患した患者は約1カ月に1回の輸血を繰り返して受ける必要があるため、血液感染性ウイルスに感染するリスクが高く、そのような感染性から分離された。 マルセイユウイルスはアメラバに感染する病原体であり、当初は環境試料から分離された。マルセイユウイルスは 250nm の二十面体カプシド内に 368K 塩基対の二本鎖 DNA ゲノムを有している。蓄積しつつあるデータから、ヒトにおけるマルセイユウイルスの存在が強く示されている。マルセイユウイルスに相同の配列は、様々なヒトのメタゲノム試料中に見出されている。さらに、マルセイユウイルス科のメンバーであるセネガウイルスが無症候性の患者の糞便から分離されている。また、我々も先頃、無症候の血液ドナーの血液中 Giant Blood Marcellivirus (GBM) として知られる新しい巨大ウイルスを確認した。GBM は 0.45 μm のフィルターを通した血清のウイルスメタゲノミクス分析によって検出され、in vitro でヒト T 細胞に感染することが明らかにされた。 2. 目的 本研究では、無症候の血液ドナーと輸血を受けた患者で構成された2つのコホートについて、マルセイユウイルスとそれに関連したウイルスの感染率を ELISA および PCR を用いて評価した。 3. 試験デザイン 3.1 試験料 無症候の血液ドナー (n=174; 男性/女性: 91/83 例; 年齢の中央値=46 歳; 範囲=21~70 歳) およびサラセミア患者 (n=22; 男性/女性: 12/10 例; 年齢の中央値=23.5 歳; 範囲=8~49 歳) から採取した血清をそれぞれ血液供給センター (Montpellier, フランス) および Timone Hospital (Marseille, フランス) から入手した。血液ドナーは検査の結果、HIV-1/2、ヒト T 白血病ウイルス、B 型肝炎ウイルス表面抗原および抗 B 型肝炎ウイルスコア抗原ならびに C 型肝炎ウイルス (HCV) 陰性であったが、サイトメガロウイルスに関する血清学的検査では、陰性または過去の感染が示された。サラセミア患者の血清学的検査の結果を表 S1 に示す。対照血清 (n=9) も 6~12 カ月齢の小児から採取した (Timone Hospital)。本試験は非介入的試験であり、通常以上の処置を必要としなかった。生物学的材料および臨床データは臨床ウイルス学における標準的診断のためのみ、医師の処方通りに入手されたものであり、臨床試料の特別な採取、試料採取プロトコルの変更または患者への補足的な質問は必要としなかった。データは匿名化されたデータベースから抽出し、分析した。フランス公衆衛生法 (CSP Art L1121-1.1) により、このタイプの試験は書面でのインフォームドコンセントの取得は免除された。本試験は地域の倫理審査委員会 "Comité d'éthique de l'IFR 48, Service de Médecine Légale" により承認され、その登録番号は N° 13-016 および N° 13-025 であった。						
使用上の注意記載状況・その他参考事項等 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、プールの試験血漿については、HIV-1、HBV 及び HCV について核増幅検査 (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人アネトロンビンIII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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3.2 ウイルス DNA の検出

血清試料由来の DNA は High Pure Viral Nucleic Acid Kit (Roche Applied Science, Mannheim, ドイツ) を用いて抽出した。次のプライマーを用いて PCR を実行した: マルセイユウイルス ORF152 を標的とした ATPase_T19F (5' AGACCCAACTCGCAGCTTA-3') および ATPase_T19R (5' -CCGGAAGATTCCAGTTCA-3')。Phusion DNA Polymerase (Thermo Fisher Scientific, Illkirch, フランス) を用いた増幅は、95°C 30 秒の初期分解ステップで開始し、次いで 98°C で 10 秒、53°C で 30 秒および 72°C で 20 秒のサイクルを 35 回実行した。シーケンシング反応は、BigDye® Terminator vl.1 Cycle Sequencing Kit (Life Technologies, Saint Aubin, フランス) を使用説明書に従って用い、実行した。配列は ChromasPro ソフトウェアを用いて回折し、BioEdit ソフトウェアの ClustalW ツールを用いてアラインした。

3.3 酵素結合免疫吸着法 (ELISA)

ELISA は既報の手順で実施した。IgG レベルの結果の平均値は、2 つの独立した試験から算出した。各プレートに陰性 ($OD_{490nm}=0.182$; $OD_{490nm}=0.139$) および陽性対照 (OD_{490nm} はそれぞれ 0.474 および 0.487) を含めた。血液ドナーおよびサラセミア患者の閾値 (それぞれ $OD_{490nm}=0.260$ および 0.233) は相対陽性率 (RPP%) の式、 $RPP\% = \frac{OD_{\text{検体}} - OD_{\text{陰性対照}}}{OD_{\text{陽性対照}} - OD_{\text{陰性対照}}}$ を用いて算出した。ELISA の結果の相対的特異性を 97.1%、また相対的感度を 97.2% と予測し、RPP=97% を用いた。

3.4 統計解析

統計解析では PASW Statistics ソフトウェアバージョン 17.0 (SPSS Inc., Chicago, IL, 米国) を用い、カイ二乗検定を行った。p 値 0.05 以下を統計的に有意とみなした。

4. 結果

196 例の血清試料を分析したところ、血液ドナー 22/174 例 (12.6%) およびサラセミア患者 5/22 例 (22.7%) から閾値を超える IgG レベルが検出された (表 1、表 S1)。特にサラセミア患者 1 例 (#21) では、最後の輸血後 22 日目の血清反応が陽性であった ($OD_{490nm}=1.6$ 倍)。マルセイユウイルス ORF152 の保存的な 200 塩基対領域を増幅することによって、さらにウイルス DNA を検出した。その結果、血液ドナー 7 例 (4%) およびサラセミア患者 2 例 (9.1%) でマルセイユウイルスが検出された。この 7 例の血液ドナー中、#45 (Acc 番号 #KF223992) および #127 (Acc 番号 #KF223993) の 2 例では、それぞれ第 136 位 (A/G) および 55 位 (T/C) に一塩基多型が認められた (図 1)。PCR 陽性の血液ドナー 4 例およびサラセミア患者 1 例では IgG レベルも高かったことが注目される (表 1、表 S2)。

5. 考察

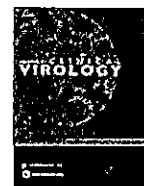
本試験で我々は、血液ドナーの間ではマルセイユウイルスの存在が珍しくないことを示した。実際、分析した血清試料の 12.6% は抗マルセイユウイルス IgG 値がカットオフ値を上回っていたが、4% で循環血中にマルセイユウイルス DNA が認められた。これに対し、サラセミア患者のコホートは IgG および PCR 陽性の患者の比率がそれよりも高かった。しかし、観察された差は統計的に有意 (p 値 > 0.05) ではなかったことから、観察された現象の重要性を推定するためには更に多くの試料が必要かもしれないことが示された。興味深いことに、我々はサラセミア患者の血清が血液ドナーよりも若い時点で抗マルセイユウイルス IgG 陽性となることを認めており (p 値 < 0.0001)、頻回の輸血がマルセイユウイルス伝播の危険因子となりうることを示唆された。しかし、サラセミア患者 (濃縮赤血球の輸血を 1 カ月 1 回程度受けていた) で検出された抗マルセイユウイルス IgG が血清反応陽性の血液ドナーから受動的に移行した可能性も考えられた。したがって、血液ドナーおよび血液レシピエントであるサラセミア患者の輸血前後のモニタリングは、ヒトにおけるマルセイユウイルス伝播の解明に役立つはずである。

Marseilleviridae 科の別のウイルスである Lausannevirus の最近の研究では、無症状のスイス人男性に有意なレベルの血清陽性反応が認められた。注目される点として、このような Lausannevirus の血清反応陽性は、頻繁な屋外スポーツと牛乳の消費の両方に関与しており、マルセイユウイルスが特定の屋外環境にも関連することが示唆される。

マルセイユウイルスが明らかに健康な人から検出されたことは、この感染症が機能的免疫系を有する人においては亜臨床または無症

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	候性である可能性を示唆している。興味深いことに、我々は 174 例のドナー中 4 例でウイルス DNA と IgG を同時に検出しており、マルセイユウイルスが状況によっては、特異的な液性応答があったとしても、急性期を超えて存在し続けることが示唆された。ヒトにおけるマルセイユウイルス感染症の発現率、有病率および臨床的重要性を解明するためには、より長い追跡期間を設定し、様々な解剖的部位由来の試料を用いた包括的研究を実施することが興味深いと思われる。	
	報告企業の意見	
	マルセイユウイルス (Marseillevirus) はアเมอร์バに感染する病原体で、250nm の二十面体カプシド内に 368K 塩基対の二本鎖 DNA ゲノムを有している。万一原料血漿にマルセイユウイルスが混入したとしても、製造工程中の各種除菌ろ過処理、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により、不活化・除去されると考えている。	今後の対応 本報告は本剤の安全性に影響を与えないと考えられるので、特段の措置はとらない。



Short Communication

Marseillevirus prevalence in multitransfused patients suggests blood transmission

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ABSTRACT

Background: Emerging viral infections in humans are appearing at an increasing rate. Recently, we identified a new Marseillevirus, named Giant Blood Marseillevirus (GBM), by performing viral metagenomics on asymptomatic blood donors.

Objectives: To study and compare the prevalence of Marseillevirus between asymptomatic blood donors and thalassemia patients.

Design: Here, we present a combined molecular and serological study on 174 asymptomatic blood donors and 22 patients with thalassemia who receive repeated blood transfusions to estimate the prevalence of Marseillevirus in these two populations.

Results: We identified Marseillevirus genomic DNA in 4% of donors, whereas 9.1% of the thalassemia patients were positive for this virus. Moreover, IgG seropositivity was detected in 22.7% of patients in the thalassemia group, whereas this seropositivity was observed in 12.6% of the blood donor population.

Conclusion: These results suggest that Marseillevirus infection is not rare in healthy persons and may be transmitted by transfusion, thus raising speculation regarding the long-term consequences of this viral infection, particularly in patients requiring repeated blood transfusions.

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1. Background

New viral infections represent constant threats to public health [1–3]. Currently, blood safety depends on improved donor selection procedures, pathogen reduction, and nucleic acid testing and selective antigen/antibody screening for known viruses [4]. However, methodological limitations, such as a low viral DNA load, the abundance of host and mitochondrial DNA, and the absence of genes that are conserved among all viruses, make detecting emerging blood-borne viruses elusive and may represent a threat in the case of frequent blood transfusions [5].

The thalassemia group of genetic diseases are characterized by globin chain production disorders [6]. Patients with thalassemia require iterative blood transfusions, approximately once

per month, leading to higher risks of blood-borne viral infections, representing a secondary cause of death [7–10].

Marseilleviruses are amoeba-infecting pathogens that were initially isolated from environmental samples [11–13]. Marseillevirus possesses a 368 kilobase-pair-long double-stranded DNA genome, enclosed in a 250 nm icosahedral capsid. Accumulating data emphasize the presence of Marseilleviruses in humans. Sequences homologous to Marseilleviruses were found in various human metagenomics samples [14]. Moreover, Senegalvirus, a member of the *Marseilleviridae* family, was isolated from a human stool sample in an asymptomatic patient [14,15]. Finally, we recently identified a new giant virus known as Giant Blood Marseillevirus (GBM) [16] in the blood of asymptomatic blood donors. GBM was detected by performing viral metagenomics on 0.45 µm filtered sera and was found to infect human T-cells *in vitro*.

2. Objectives

In this study, we investigated the prevalence of Marseillevirus and related viruses using ELISA and PCR to test the sera of two

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cohorts composed of asymptomatic blood donors and thalassemia patients.

3. Study design

3.1. Samples

Sera collected from asymptomatic blood donors ($n=174$; male/female: 91/83; median age = 46 years; range = 21–70 years) and thalassemia patients ($n=22$; male/female: 12/10; median age = 23.5 years; range = 8–49 years) were obtained from Blood supply center (Montpellier, France) and the Timone Hospital (Marseille, France) respectively. The blood donors tested negative for HIV-1/2, human T leukemia virus, hepatitis B virus surface antigen and anti-hepatitis B core antibody and hepatitis C virus (HCV), whereas serology to cytomegalovirus indicated negativity or past infection. Serological data for the thalassemia patients is summarized in Table S1. Control sera ($n=9$) were also collected from 6- to 12-month-old children (Timone Hospital). This study was a non-interventional and did not require more than the routine procedures. Biological material and clinical data were only obtained for standard diagnosis in clinical virology, as prescribed by physicians, and did not require any specific collection of clinical samples, change in sampling protocol or supplementary questioning of the patient. Data were collected and then analyzed from an anonymized database. According to the French Law of Public Health (CSP Art L 1121-1.1), this type of study is exempt from written informed consent. This study was approved by the local ethics committee "Comité d'éthique de l'IFR 48, Service de Médecine Légale," under the accession numbers N°13-016 and N°13-025.

3.2. Viral DNA detection

DNA from serum samples was extracted using the High Pure Viral Nucleic Acid Kit (Roche Applied Science, Mannheim, Germany). PCR was performed using the following primers: ATPase_T19F (5'-AGACCCAACTCGCAGCTTA-3') and ATPase_T19R (5'-CCGGAAGATTCCAAGTTCA-3') to target the *Marseillevirus* *orf* 152. Amplification using *Phusion* DNA Polymerase (Thermo Fisher Scientific, Illkirch, France) started with an initial denaturation step at 95 °C for 30 s, followed by 35 cycles at 98 °C for 10 s, 53 °C for 30 s and 72 °C for 20 s. Sequencing reactions were carried out with the reagents of the BigDye® Terminator v1.1 Cycle Sequencing Kit (Life Technologies, Saint Aubin, France) according to the manufacturer's instructions. Sequences were analyzed using ChromasPro software and aligned using the ClustalW tool of BioEdit software.

3.3. Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed as previously described [16]. Average results for the IgG levels were obtained from two independent experiments. Negative ($OD_{490\text{ nm}} = 0.182$; $OD_{490\text{ nm}} = 0.139$) and positive controls ($OD_{490\text{ nm}} = 0.474$ and 0.487 , respectively) were included in each plate. Thresholds for the blood donors and thalassemic patients ($OD_{490\text{ nm}} = 0.260$ and 0.233 , respectively) were calculated using the relative percentage of positivity (RPP%) formula $RPP\% = \frac{OD_{\text{threshold}} - OD_{\text{neg. control}}}{OD_{\text{pos. control}} - OD_{\text{neg. control}}}$. RPP = 27% was used, for which a 97.1% relative specificity and 97.2% relative sensibility of the ELISA results were predicted.

3.4. Statistical analysis

Statistical analysis was performed using PASW Statistics software version 17.0 (SPSS Inc., Chicago, IL, USA) to carry out Chi²

Table 1

Serological and molecular survey of *Marseillevirus* infection in 174 blood donors and 22 thalassemia patients.

	Blood donors	Thalassemia patients	p-Value ^a
Sample number	174	22	
Male/female (male %)	91/83 (52.3%)	12/10 (54.5%)	
Median age (range)	46 (21–70)	23.5 (8–49)	
IgG positive			
Number	22/174 (12.6%)	5/22 (22.7%)	0.165
Male/female (male %)	10/12 (45.5%)	3/2 (60%)	
Median age (range)	47.5 (22–70)	30 (17–49)	<0.0001
PCR positive			
Number	7/174 (4%)	2/22 (9.1%)	0.267
Male/female (male %)	3/4 (42.9%)	1/1 (50%)	
Median age (range)	37 (21–61)	31 (21–41)	
IgG positive/PCR positive			
Number	4/174 (2.3%)	1/22 (4.5%)	0.452
Male/female (male %)	2/2 (50%)	1 (0%)	
Median age (range)	31 (22–53)	41	

Summary results from serological and molecular testing for the presence of *Marseillevirus* IgGs and DNA in serum, performed on 174 blood donors and 22 thalassemic patients. PCR-positive sera were systematically verified using DNA sequencing. See Study design section for further information.

^a Chi² test.

tests. P-values less than or equal to 0.05 were considered to be significant.

4. Results

By analyzing 196 serum samples, we detected IgG levels above the threshold values in 22/174 (12.6%) of the blood donors and 5/22 (22.7%) of the thalassemic patients (Table 1, Table S1). Notably, in one patient with thalassemia (#21), we detected seropositivity 22 days after the last transfusion ($OD_{490\text{ nm}}$ increase = 1.6-fold).

We further performed viral DNA detection by amplifying a conserved 200 base pair region in the *Marseillevirus* ORF152. We found *Marseillevirus* DNA in the sera from 7 (4%) blood donors and 2 (9.1%) thalassemia patients. Two of the seven blood donors, #45 (Acc. Number # KF223992) and #127 (Acc. Number # KF223993) presented single nucleotide polymorphisms at positions 136 (A/G) and 55 (T/C), respectively (Fig. 1). It is worth noting that 4 of the blood donors and 1 of the thalassemia patients who were PCR positive also presented elevated IgG levels (Table 1, Table S2).

5. Discussion

In the present study, we showed that the presence of *Marseillevirus* among blood donors is not uncommon. In fact, we found that 12.6% of the analyzed serum samples had anti-*Marseillevirus* IgG levels above the cut-off values, whereas 4% presented circulating *Marseillevirus* DNA. In comparison, the thalassemia patient cohort presented a higher prevalence of IgG- and PCR-positive subjects. However, the observed difference was not statistically significant ($p\text{-value} > 0.05$), indicating that additional samples may be required to estimate the significance of the observed phenomenon. Interestingly, we observed that thalassemia patients acquired IgG seropositivity toward *Marseillevirus* at a younger age compared to those in the blood donors cohort ($p\text{-value} < 0.0001$), suggesting that frequent blood transfusions may represent a risk factor for *Marseillevirus* transmission. However, the IgG to *Marseillevirus* detected in thalassemia patients (who received transfusions of concentrated erythrocytes approximately once per month) may have been transferred passively from the seropositive blood donors. Therefore, additional pre and post transfusion monitoring of blood donors and thalassemic blood recipients should shed light on *Marseillevirus* transmission in humans.

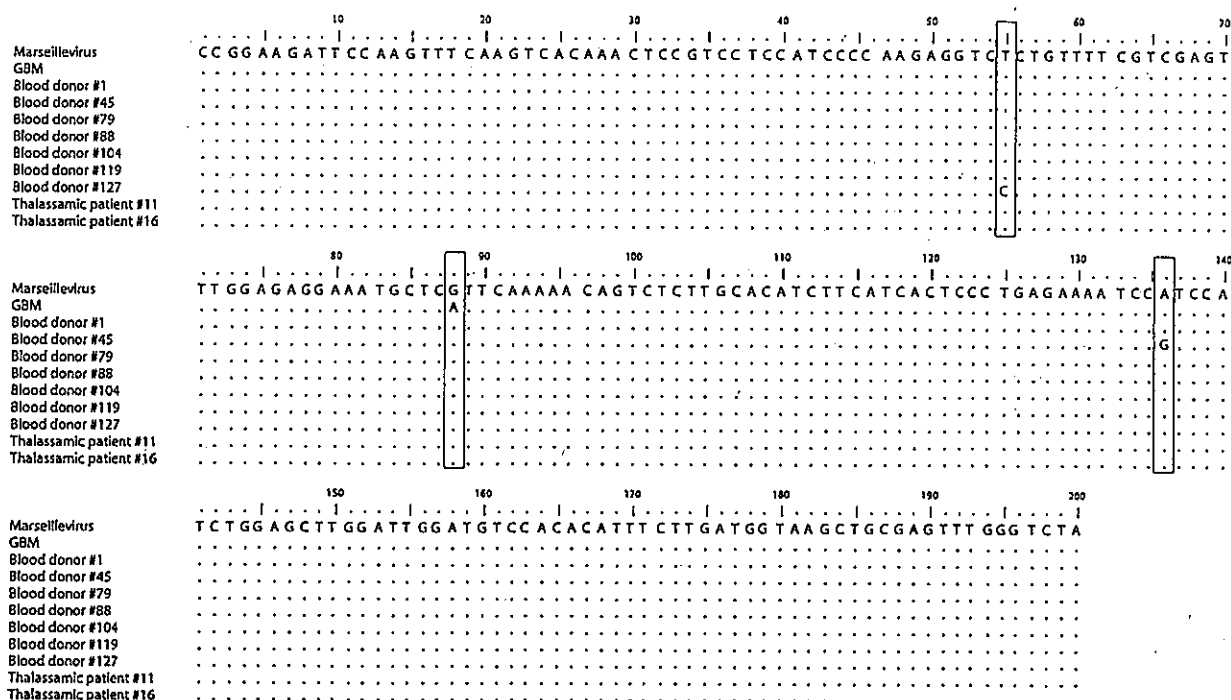


Fig. 1. Multiple alignment of partial D5 helicase gene sequence. Sequences corresponding to the 200 bp amplicons obtained from the blood donors and thalassemia patients were aligned to a Marseillevirus reference sequence using the ClustalW alignment tool. Only differences to the reference sequence, corresponding to single nucleotide polymorphisms (in black rectangles), are provided.

A recent study of another virus from the *Marseilleviridae* family, Lausannevirus, identified a significant level of seroprevalence in asymptomatic Swiss men [17]. It is worth noting that the Lausannevirus seroprevalence correlated with both frequent outdoor sport practice and milk consumption, suggesting that Marseilleviruses may also be associated with specific outdoor environments [17].

The fact that Marseillevirus was detected in apparently healthy individuals suggests that this infection may be subclinical or asymptomatic in persons with a functional immune system. Interestingly, we concurrently detected viral DNA and IgG in 4 out of 174 donors, which suggests that in some situations, Marseillevirus may persist beyond the acute phase despite the presence of a specific humoral response. It would be interesting to perform more comprehensive studies with longer periods of follow-up and on samples from various anatomical sites to shed light on the incidence, prevalence and clinical significance of Marseillevirus infections in humans.

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Competing interests

None for all authors.

Ethical approval

This study was approved by the local ethics committee "Comité d'éthique de l'IFR 48, Service de Médecine Légale," under the numbers N°13-016 and N°13-025.

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We are grateful to Jean-François Delfraissy for the fruitful discussion, Hervé Richet for his help with the statistical analysis and Lina Barassi for Marseillevirus production.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jcv.2013.10.001>.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2014. 4. 5	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人赤血球液	研究報告の公表状況		公表国 米国	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)	○バベシア:低温保存が血液バッグ中の原虫の生存・増殖に与える影響 背景:バベシア属は輸血用血液における重大な感染脅威である。血液の保存状態は輸血感染バベシア症の発生率及び重症度を左右するため、保存状態がバベシア属の生存能に与える影響を理解することは重要である。 研究デザインと方法:白血球除去赤血球(RBC)の血液バッグに <i>Babesia divergens</i> を添加後4℃で0～31日間保存し、原虫の存在、形態、生存能を調べた。血液バッグから経時的にRBCを採取後、血液スメア標本を作製し、顕微鏡で観察した。また、バッグから採取したRBCを原虫の増殖に最適な条件下で8日間培養し、原虫の生存能を評価した。 結果:4℃で24時間保存した後、血液バッグ内の原虫は大幅に減少し、保存期間を通じて減少し続けた。減少に伴い形態変化した原虫が増加した。しかし、より長期間冷蔵保存された原虫が指数増殖期に到達するのには時間の遅れが見られたものの、生存能は31日間の冷蔵保存中を通じて維持された。 結論: <i>B. divergens</i> の冷蔵保存において原虫の形態は変化し、原虫数は減少するが、4℃で31日間の保存後にも生存し、感染するためには十分な数の原虫が存在する。			使用上の注意記載状況・その他参考事項等 赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 赤血球液-LR「日赤」 照射赤血球液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク
研究報告の概要	報告企業の意見 <i>B. divergens</i> は冷蔵保存下で形態が変化し、数は減少するが、4℃で31日間の保存後にも生存し、感染するために十分な数の原虫が存在することが明らかとなったとの報告である。 今後の対応 日本赤十字社では問診時にバベシア症の既往歴を確認し、該当する場合は献血不適として扱う。今後引き続き、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。				

4

BLOOD COMPONENTS

Babesia: impact of cold storage on the survival and the viability of parasites in blood bags

Jery R. Cursino-Santos, Andy Alhassan, Manpreet Singh, and Cheryl A. Lobo

BACKGROUND: *Babesia* represents one of the major infectious threats to the blood supply since clinically silent infections in humans are common and these can be life-threatening in certain recipients. It is important to understand the effect of blood storage conditions on the viability of *Babesia* as this will impact the occurrence and severity of transfusion-transmitted babesiosis.

STUDY DESIGN AND METHODS: *Babesia divergens* was introduced into blood bags containing leukoreduced red blood cells (RBCs) and stored at 4°C for 0 to 31 days. Samples were withdrawn for assessment of the presence, morphology, and viability of parasites. Blood smears were made immediately on removal from blood bags at different time intervals and evaluated by blood film microscopy. RBCs withdrawn from the bags were also cultured for 8 days using conditions optimal for parasite reproduction and growth to allow assessment of parasite viability.

RESULTS: After 24 hours of storage at 4°C, there was a substantial reduction of parasitemia in the blood bags, which was maintained throughout storage. This decrease was accompanied by a change in morphology of parasites, with the number of altered parasites increasing through the period of storage. However, viability was maintained through 31 days of cold storage with a lag in achieving exponential growth seen in the parasites subjected to longer periods of refrigeration.

CONCLUSION: Refrigeration of *B. divergens* leads to an alteration of parasite morphology and a decrease in parasite numbers. However, there are sufficient parasites that are robust enough to survive 31 days of storage at 4°C and yield high end-point parasitemia.

Babesiosis is a tick-borne zoonotic disease endemic in the Northeast and Midwest United States.¹ There are multiple species of *Babesia* that invade and infect the red blood cells (RBCs) of various animal species; however, only a few of them have been shown to be zoonotic. Primarily among these are *B. microti*, a rodent parasite that is responsible for most of the infection in the United States; *B. divergens*, a cattle parasite seen primarily in Europe,² although *B. divergens*-like variants have been found in the United States;³ and *B. duncani*, a few cases of which have been identified in California.^{4,5}

Besides their natural route of transmission through tick bites, the spread of *Babesia* is becoming increasingly common and problematic through blood transfusions. In fact, babesiosis has become the most frequent transfusion-transmitted infection with approximately 162 cases reported since 1980 and 12 associated fatalities in the period 2005 to 2008.⁶⁻⁹ These figures probably underestimate the actual number of transfusion-associated cases.⁵ This is because the disease is clinically silent in most healthy adults who are the bulk of blood donors. In the absence of a licensed test, current safeguards against babesia remain a questionnaire relating to past history of the infection and have not proven to be effective in protecting against the parasite.¹⁰

ABBREVIATIONS: TTB = transfusion-transmitted babesiosis; ZT = zero time.

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For the parasite to be successfully transmitted by transfusion, it must survive at 4°C, under the same conditions used for storing donor blood.¹¹ A recent article on *Plasmodium falciparum*, the malaria parasite showed that the parasite remains detectable in blood smears up to 28 days, although after 14 days it is no longer viable.¹² As this question has not been reliably investigated for *Babesia*, we performed a similar study, using *B. divergens*, which is currently the only babesial pathogen capable of causing disease in man that can be cultured in human RBCs in vitro. *B. divergens*, however, has been shown to be a transfusion threat only in Europe and not in the United States.

MATERIALS AND METHODS

Parasite propagation

Blood stage cultures of *B. divergens* (Bd Rouen 1986 strain) were maintained in vitro in human A+ RBCs using RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% human serum and 0.24% (wt/vol) sodium bicarbonate solution (Invitrogen). Cells were cultured at 37°C in 90% CO₂, 5% nitrogen, and 5% oxygen, as previously described.¹³ Blood smears were made from cultures and stained with Giemsa to calculate parasitemia (number of infected RBCs in every 100 RBCs).

Preparation of *B. divergens*-infected blood bags

Leukoreduced RBC units were obtained from the New York Blood Center. Units were used on the same day that they were drawn. Parasite cultures were centrifuged, parasitemia was determined by Giemsa staining, and required aliquots of infected RBCs were added to each bag to obtain the desired parasitemia. The zero-time (ZT) sample, which served as the control for the refrigerated stored samples, was withdrawn immediately after thorough mixing and then the bags were stored at 4°C for various time periods.

Determination of ZT parasitemia from various storage-time samples

After removal from 4°C, the bags were thoroughly mixed on a shaker for 30 minutes. Five milliliters of ZT aliquots from the seven storage time points (Days 0, 1, 3, 10, 17, 24, and 31) was withdrawn. The bags were then returned to 4°C after withdrawal of each aliquot. Smears were immediately made from each ZT aliquot and stained with Giemsa, and parasitemia was enumerated.

Culturing and testing of viability of aliquots from blood bags (assessing parasitemia for Days 1-8 after culture)

To determine the viability of parasites in the 5-mL aliquots drawn from the blood bags at the seven storage time points, the blood was centrifuged, washed thrice with

RPMI 1640, and put in in vitro culture using standard conditions (see parasite propagation). Cultures were maintained for 9 days or until a parasitemia of 50% was reached, whichever was earlier. Smears were made and Giemsa stained to analyze growth of the parasites on a daily basis (up to Day 9) until parasite cultures crashed (>50% parasitemia).

RESULTS

Morphology of parasites changes upon storage at 4°C

One of the striking effects of refrigeration on *Babesia* parasites was a distinct change in parasite morphology. *Babesia* parasites maintained at 37°C appear normal (Fig. 1A). Parasites replicate by binary fission and in normal cultures, a variety of these forms can be seen, in one- (ring), two- ("figure 8" stage), or four-celled (Maltese cross) stages (Fig. 1A). Upon storage at 4°C, parasites appear to undergo a condensation or shrinking effect, forming crisis forms, and can be seen to occupy smaller volumes of the RBC compared to nonstressed parasites (Fig. 1B). The number of these "condensed" parasites as well as the degree of condensation of these parasites increases steadily with days of cold stress with the maximum numbers of these abnormal parasites seen when parasites are withdrawn from the blood bags on Days 24 to 31. However, very small numbers of morphologically normal parasites are also present at this time (Day 31 has almost none) and thus it is the ratio of normal to abnormal parasites that decreases through the period of refrigeration. It is not clear what the fate of these abnormal parasites would be when they are put into culture at 37°C, with respect to viability and time needed to propagate.

Parasitemia decreases upon storage at 4°C

For the purpose of parasite enumeration, all stained organisms (normal and abnormal morphology) within the RBCs were counted to obtain parasitemia at different time periods. This was done as it is not possible, by mere examination of the Giemsa-stained smear, to predict the viability and potential reproductive abilities of the altered parasites. Parasitemias on Day 0 (ZT) of all storage time points were assessed to determine the effect of cold temperature on the survival of *B. divergens* and the impact of length of storage at 4°C on the survival of the parasites.

Parasitemia at ZT in all 4°C storage time points

Parasites were introduced into bags to obtain a final parasitemia of approximately 0.25% to 0.27% (Table 1, 0 storage time point at ZT), as this level represents an average typical parasitemia in blood donors. Parasitemia represents the number of infected RBCs in every 100 RBCs. We measured ZT parasitemia for the various time periods of storage by taking an aliquot from the bag at the

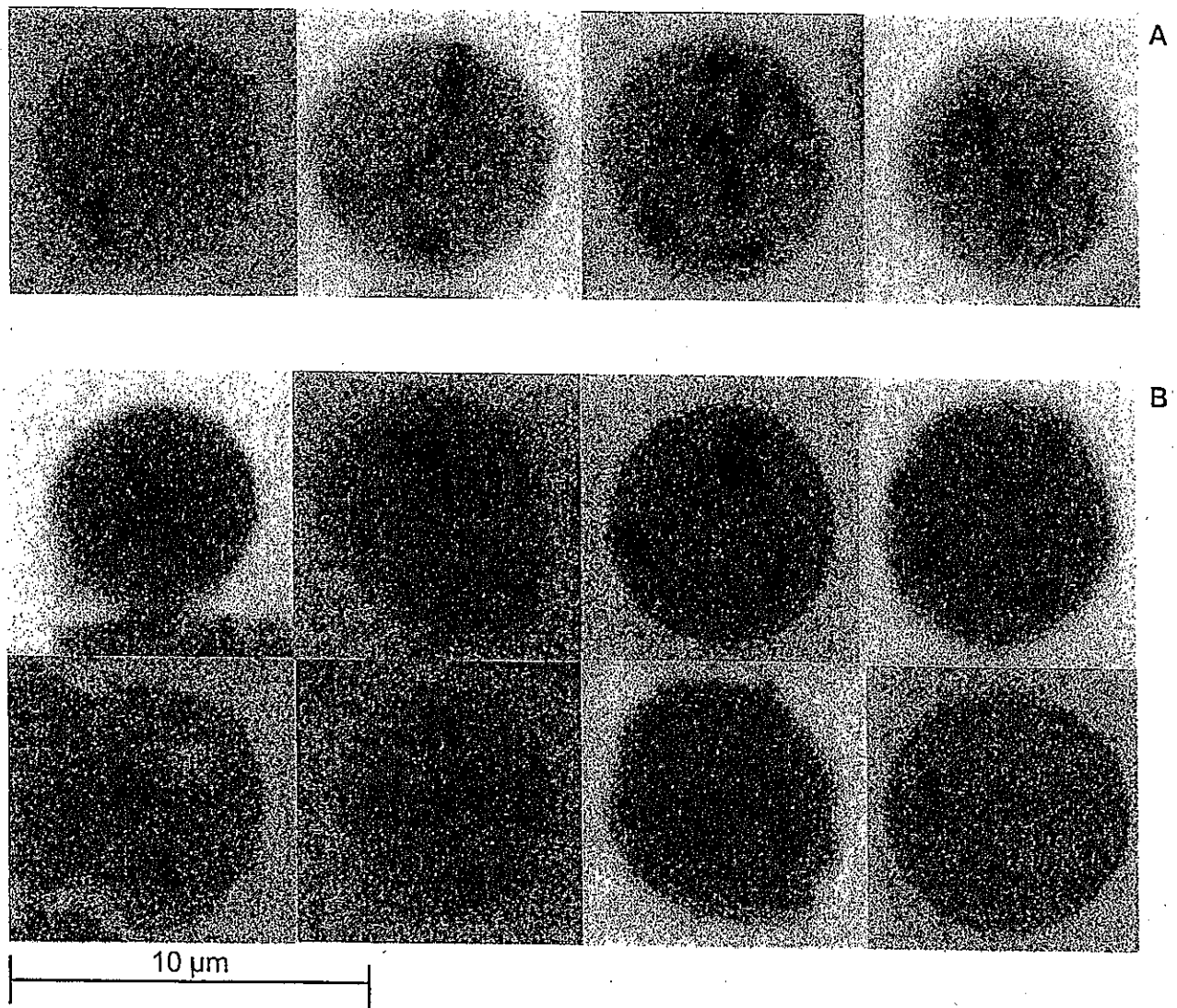


Fig. 1. Morphology of *B. divergens* parasites as seen by Giemsa staining of smears. (A) Grown without cold stress under normal conditions at 37°C. From left to right, ring, two-celled figure 8, four-celled Maltese cross, and multiply invaded RBCs. (B) Smeared from bag after storage at 4°C for 1 to 31 days.

specified time (Days 1, 3, 10, 17, 24, and 31) as soon as the bag is taken out from the refrigerator and mixed thoroughly (ZT for all bags). After just 1 day of storage at 4°C, ZT parasitemia dropped by approximately 30% to 40% and this decrease was maintained through the prolonged period of cold storage (0.25% vs. 0.15% in Bag 1 and 0.27% vs. 0.19% in Bag 2; see Table 1). However, the decrease in parasitemia was not severely impacted by the length of storage at 4°C, up to Day 24 of blood bag storage (0.15% vs. 0.15% in Bag 1 and 0.19% vs. 0.21% in Bag 2, Table 1). Thus, there was no significant difference between the decrease in parasitemia as measured by ZT parasitemia on Day 1 or Day 24 of storage at 4°C. This was unexpected and indicated that the impact of suboptimal temperatures on *Babesia* is immediate and does not increase substantially

over time. However, the readout of this effect is based on Giemsa-stained smears and while differences resulting in severely deformed and disintegrating parasites (Fig. 1) can be seen, we cannot assess the effect of these changes on the survival or viability of the parasite, so all stained parasites were included in the tally, irrespective of morphology. On Day 31, however, there was a further decrease in parasitemia in both bags averaging a 50% to 60% decrease from control smears (Table 1, parasitemia 0.12% in Bag 1 on Day 31 and 0.11% in Bag 2 on Day 31).

Viability of parasites is affected by storage at 4°C

While total numbers of parasites remained relatively constant from Day 1 through Day 24 of cold storage, we

TABLE 1. Monitoring of parasite presence (ZT) and growth (time in culture) at seven storage time points when aliquots of blood were collected directly from the bags and left in culture for noted times, through Day 8

Storage time point at 4°C (days)	Parasitemia (%)															
	Time in culture at 37 °C (days)															
	Bag 1								Bag 2							
	ZT*	1	2	3	4	5	6-7	8	ZT*	1	2	3	4	5	6-7	8
0†	0.25	0.45	0.78	3	8	39	61	Crash‡	0.27	0.70	1.39	2	5	25	57	Crash
1	0.15	0.28	0.64	2	5	27	67	Crash	0.19	0.43	0.91	1	5	18	55	Crash
3	0.18	0.45	1.43	3	7	28	63	Crash	0.22	0.35	0.76	1	4	18	58	Crash
10	0.16	0.47	0.50	2	5	27	67	Crash	0.21	0.38	0.68	1	2	10	59	Crash
17	0.16	0.40	0.48	2	3	13	55	Crash	0.21	0.24	0.37	0.71	2	8	42	Crash
24	0.15	0.13	0.11	0.58	0.91	5	35	Crash	0.21	0.18	0.09	0.28	1.05	1.25	20	45
31	0.12	0.08	0.09	0.52	0.52	0.63	5	11	0.11	0.08	0.12	0.28	0.38	1.34	3	7

* ZT of all storage time points before culturing.

† Control: sample collected immediately after *B. divergens* was inoculated into blood bag and not subjected to cold stress.

‡ Crash: further growth of parasites cannot be supported without addition of fresh RBCs.

probed for effects of the stress on the parasites that would impact their viability by comparing the ability of parasites, stored at 4°C for the different time periods (1, 3, 10, 17, 24, and 31 days) and parasites not exposed to 4°C (0 storage time point) to reproduce when put into in vitro culture.

Control cultures

The time between one-cell division and the next in the asexual erythrocytic *B. divergens* is approximately 8 hours. Thus, control parasites in the blood bag, which were not subjected to low-temperature stress, reproduced normally through successive intraerythrocytic cycles and by Day 6, showed a typical parasitemia of more than 50%.

Parasitemia on Days 1 and 2 of culture in all 4°C storage time points

Parasitemia in RBCs stored for Days 1, 3, 10, and 17 showed typical progression of asexual multiplication and growth after being put into culture. Thus, parasitemia increased steadily, although final levels were a little lower than those found in the control flasks on all days of culture. However, we found that in the case of Day 24 and 31 storage time points, parasitemia on Days 1 and 2 after introduction into culture resulted in a decrease in parasitemia compared to that sample's ZT parasitemia. For example, Day 24 storage time point samples showed a parasitemia of approximately 0.15% at ZT, which dropped to 0.13% on Day 1 and 0.11% on Day 2. Again, for the Day 31 storage time point samples, we record ZT parasitemia of approximately 0.12%, which decreases to 0.08% on Day 1. We hypothesize that this is due to the disintegration and removal of the severely cold-damaged parasites that were enumerated at Day 0, but are no longer around to contribute to parasitemia on Days 1 and 2 of culture. Thus, Day 1 and 2 parasitemias after being put into culture may represent a better variable to measure the initial effect of refrigeration on these parasites. Figure 2 shows this difference in early culture parasitemia among aliquots withdrawn after different periods of storage at 4°C.

Parasitemia on Days 3 to 8 of culture in all 4°C storage time points

The assessment of parasitemia from RBCs stored for various lengths of time at 4°C allowed us to separate them into two groups. The first is parasitized RBCs stored up to Day 10 storage time point at 4°C, where we see that while the parasitemia was lower at ZT, the parasites recovered efficiently on subsequent days in culture to produce parasitemias similar to the control flasks by Day 3 in culture (parasitemia averaging 2.5% in Bag 1 and 1.4% in Bag 2). These parasites, as did the control, reached more than 50% parasitemia by Day 6 in culture. The second group encompasses those parasites recovered from the blood bags after 17 or more days of cold storage. These parasites experienced a lag in multiplication rates and this

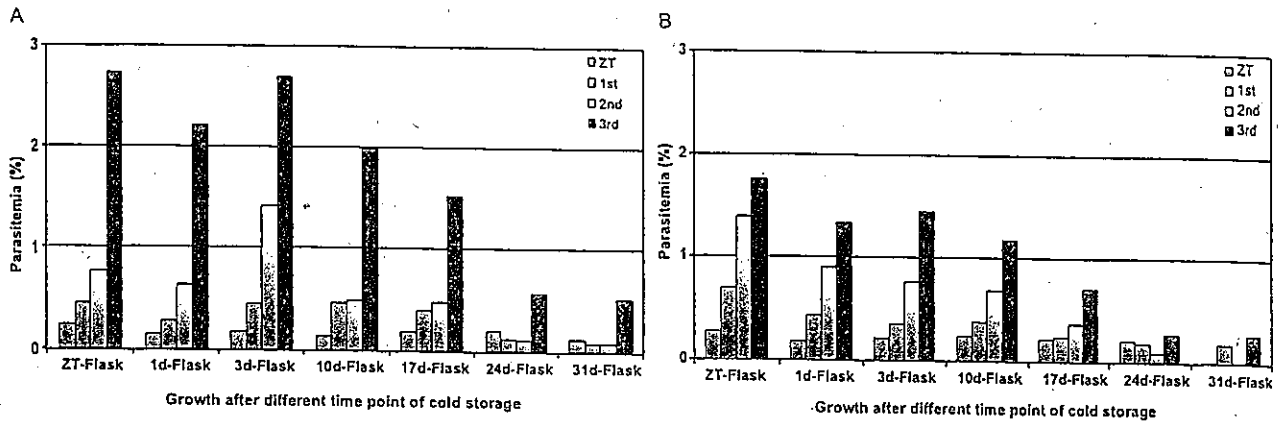


Fig. 2. Growth curves of parasites in the early period of culture showing a distinct lag in growth as time of storage at 4°C increases. ZT Flask = no cold storage (ZT); culture started from bag stored for 1 day (1d), 3 days (3d), 10 days (10d), 17 days (17d), 24 days (24d), and 31 days (31d). (A) Bag 1; (B) Bag 2.

was seen in the time taken by these parasites to reach 50% parasitemia once put into culture. Thus, aliquots withdrawn on Day 17 of storage time took 7 days to reach approximately 50% parasitemia while parasites subjected to 24 days of cold storage required 8 days to achieve 50% parasitemia. Parasites recovered after 31 days of storage had not reached 50% parasitemia levels on Day 8, the end point of our study. Thus, although ZT parasitemias were similar from Day 1 to Day 24 of cold storage, the effect of the low temperatures did impact the viability of *B. divergens*. This difference in viability may be a reflection of the increased amounts of condensed parasites seen in the aliquots withdrawn from the blood bags at later time points. Thus, in vitro culture is a needed analytical tool to probe these differences in the parasites.

DISCUSSION

From a blood safety perspective, transfusion-transmitted infections involving *Babesia* have become increasingly problematic, with progressively more reported each year.¹⁴ In the period from 2005 to 2010, 3.6% (11/307) of transfusion-related fatalities reported to the Food and Drug Administration (FDA) were due to transfusion-transmitted babesiosis (TTB).¹⁵ This risk of transmission of *Babesia* via transfusion is intimately linked to its capacity to survive blood collection and blood storage procedures. In this study, the focus was on determining the impact of cold storage (4°C) on the viability of the parasite that was introduced into bags at donor-typical levels of parasitemia (Lobo et al., unpublished observations, 2012). As blood bags are stored for more than 31 days, we followed parasite viability by assessing blood samples at weekly intervals during the period of storage.

Systematic laboratory screening of donor blood in the form of FDA-licensed serological and nucleic acid testing assays is available for many pathogens like blood-borne

viruses to prevent their spread by transfusion.¹⁶ Unfortunately, the lack of comparable, sensitive screens available for protozoan parasites like *Babesia* has resulted in the current complete dependence on a donor response questionnaire to safeguard the nation's blood supply.¹⁰ This safeguard, as we can see from the increasing numbers of TTB cases, is not efficient in ensuring the safety of the 5 million or so recipients that receive blood every year. In the absence of a suitable test, small changes to blood storage conditions may have an impact on TTB incidence. Storage of the infected blood for just 24 hours resulted in a precipitous decrease in the number of parasites in the unit, which augurs well for transfusions involving recipients with functioning immune systems as we hypothesize that the reduced parasite numbers will result in a subclinical episode, if any, of babesiosis. Unfortunately, our finding that *B. divergens* can survive and replicate after 31 days of storage at 4°C raises other concerns for transfusion safety. Although parasite levels decreased from Day 0 through Day 31 of storage, the parasite needed very little time (2-3 days) to reach exponential growth. This rapid burst of multiplication resulting in parasitemia over 50% is what makes *Babesia* a particular concern for transfusion recipients that suffer from immunodeficiencies or asplenic recipients. The latter are a special concern because the majority of this group are sickle cell and thalassemic patients who require repeated transfusions, which heightens the overall risk of acquiring the parasite.⁹ The high parasitemia found in in vitro culture is not an artifact but reflects what happens in vivo, in the absence of effective immune responses.

A detailed examination of the reduction in numbers of *Babesia* parasites that survived refrigeration reveals that while the numbers decreased through cold storage, there still remain parasites capable of transmitting infection probably in part due to the vast numbers of RBCs involved in a single transfusion event. We chose a modest starting

parasitemia as this would represent a typical parasitemia in donors who would have to be devoid of any clinical symptoms if they felt well enough to donate blood (Lobo et al., unpublished observations, 2012). This 0.25% parasitemia represents a number of 1.25×10^8 parasites/mL of blood or 3.75×10^{10} parasites totally in the blood unit, assuming an average of 5×10^{10} RBCs/mL of packed blood and 300 mL of packed RBCs/unit. Even when the parasitemia decreases by large percentages, for example, ZT parasitemia on Day 31 of storage showed a 50% decrease in parasitemia, there still remains approximately 10^{10} parasites in the blood unit, which would be capable of causing illness and/or death in certain recipients. The change in parasite morphology through the extended period of storage at 4°C, while significant, does not appear to result in a total loss of infectivity, although parasitemias found in the latter half of the storage period could be resulting from the few morphologically normal parasites that are still present. It would take additional studies to clearly determine the fate of these condensed parasites in contributing to future rounds of asexual multiplication.

This study is the first systematic analysis of the effect of refrigeration temperatures on the presence and persistence of *B. divergens*. An earlier study focused on *B. microti* with significant differences in method.¹⁷ The parasite source in that study was hamster RBCs infected with *B. microti* and as *B. microti* cannot be cultured successfully long term in vitro, the end point assay for viability was a xenodiagnostic assay where aliquots of infected blood were inoculated into hamsters to examine the ability to multiply and infect hamster RBCs. The sensitivity afforded by in vitro culture of *Babesia* should be superior to that of hamster inoculation. Stability of hamster RBCs versus human RBCs over the 30-day period is an additional confounder to results obtained with that study. Another difference related to conditions of storage of the infected blood as in the earlier study, which was in tubes and not blood bags, thus gaseous exchange which is optimal in blood bags was not facilitated in the tubes, which could have impacted the results. However, the conclusions from that study are on the whole in agreement with ours, that infectivity of *Babesia* remained through 21 days of storage at 4°C, albeit decreasing to 25% on Day 21.¹⁷ This is also corroborated by a case of TTB involving a blood unit stored for 35 days.¹⁸ A similar study with *P. falciparum*, the malaria parasite revealed that parasites can be detected through Day 28 of cold storage; however, after Day 14, the viability of the parasite is in question as no asexual multiplication was observed when the parasites were put into in vitro culture.¹² Unlike *Plasmodium*, *B. divergens* robustly maintained its ability to divide and this resistance to cold shock elevates its threat to the blood supply.

From our study, it becomes clear that while the infectivity of *B. divergens* persists through the shelf-life of stored blood, adding to the problem of preventing TTB,

there is a significant decrease in parasite levels resulting from just 1 day of storage. This may have implications for the use of stored blood in transfusions in areas endemic for *Babesia*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2014. 4. 5	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人赤血球液			公表国 米国	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)	研究報告の公表状況	Paintsil E, Binka M, Patel A, Lindenbach BD, Heimer R. J Infect Dis. 2014 Apr 15;209(8):1205-11. doi: 10.1093/infdis/jit648. Epub 2013 Nov 23.		
研究報告の概要	○室温、無生物環境下での乾燥後に数週間感染性を維持するHCV:伝播リスクに関する影響 背景: 先進諸国において、輸血及び外科手術によるHCV感染はこの10年でほとんど見られなくなった一方、院内感染はHCV感染の新たな原因(20-50%)となっている。医療従事者は医療行為を含む汚染源に接する可能性がある。 汚染源におけるHCVの長期生存能力が罹患率に顕著な影響を及ぼすと仮定し、ウイルス感染性の持続期間及び消毒薬の効果について調査した。 方法: 運搬中に誤って溢った血清や血漿の量を測定し、最大量に相当する血漿33 μ Lに2a HCVレポーターウイルスをスパイクし、24ウェルプレートに誤って溢った血清や血漿の量を測定し、最大量に相当する血漿33 μ Lに2a HCVレポーターウイルスをスパイクし、24ウェルプレートに滴下した。プレートは蓋をせずにそれぞれ4℃、22℃及び37℃で6週間保存後、ウイルスの感染性を測定した。 結果: 4℃及び22℃で6週間保存した後に、低力価のHCVにおいても感染性が確認された。また、一般的に用いられる消毒剤のHCVに対する不活化効果は、6%次亜塩素酸ナトリウム(10倍希釈) > Cavicide (10倍希釈) > エタノール(70%)の順であった。 結論: この調査結果は、汚染源や医療器具を介した職業性感染の可能性についての仮説を支持する。市販の消毒薬における抗HCV効果は、種類や希釈により幅がある。				
報告企業の意見	今後の対応 日本赤十字社では、HCV抗体検査を実施することに加えて、精度を向上させたNATシステムを導入し、20プールでスクリーニングNATを行い、陽性血液を排除している。また、個別スクリーニングNATの導入についても検討を行っている。HCV感染に関する新たな知見等について、今後も情報の収集に努める。				
	使用上の注意記載状況・その他参考事項等 赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 赤血球液-LR「日赤」 照射赤血球液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク				

5

Hepatitis C Virus Maintains Infectivity for Weeks After Drying on Inanimate Surfaces at Room Temperature: Implications for Risks of Transmission

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Background. Healthcare workers may come into contact with fomites that contain infectious hepatitis C virus (HCV) during preparation of plasma or following placement or removal of venous lines. Similarly, injection drugs users may come into contact with fomites. Hypothesizing that prolonged viability of HCV in fomites may contribute significantly to incidence, we determined the longevity of virus infectivity and the effectiveness of antiseptics.

Methods. We determined the volume of drops misplaced during transfer of serum or plasma. Aliquots equivalent to the maximum drop volume of plasma spiked with the 2a HCV reporter virus were loaded into 24-well plates. Plates were stored uncovered at 3 temperatures: 4°C, 22°C, and 37°C for up to 6 weeks before viral infectivity was determined in a microculture assay.

Results. The mean volume of an accidental drop was 29 μ L (min–max of 20–33 μ L). At storage temperatures 4°C and 22°C, we recovered viable HCV from the low-titer spots for up to 6 weeks of storage. The rank order of HCV virucidal activity of commonly used antiseptics was bleach (1:10) > cavicide (1:10) > ethanol (70%).

Conclusions. The hypothesis of potential transmission from fomites was supported by the experimental results. The anti-HCV activity of commercial antiseptics varied.

Keywords: hepatitis C virus; fomites; infectivity; transmission; nosocomial; virucidal agents.

The global burden of morbidity and mortality from hepatitis C virus (HCV) infection is truly pandemic, with more than 170 million people currently infected [1]. Because there is currently no vaccine for HCV and available treatment regimens are limited by efficacy, cost, and side effects, prevention of HCV transmission remains the primary strategy for curbing the HCV epidemic. HCV is transmitted primarily through parenteral exposure to blood or body fluids contaminated with HCV. Injecting drug use (IDU), mother-to-child

transmission, multiple heterosexual partners, accidental needle injuries, and transfusion of blood or blood products are among the most relevant risk factors for HCV acquisition [2–4].

The epidemiology of HCV has changed in the last decade. Transmission from blood transfusions and surgical procedures have all but disappeared in developed countries [5]. There have been modest but insufficient declines in incidence among IDUs in locations with broad implementation of syringe-exchange programs [6–12]. Nosocomial transmissions of HCV increasingly account for a large proportion of new HCV infections (ie, 20%–50%) in developed countries [13–17]. Thus, the relative impact and burden of nosocomial HCV transmission might be greater now than a decade ago. In an Italian study of 214 patients with acute HCV infections [18], the most relevant risk factors were history of medical procedures (32%; eg, hospitalization, surgery, endoscopy, dialysis, blood transfusion, dental

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treatment, and other invasive procedures) and intravenous drug use (30%). Interestingly, among the patients classified under medical procedures, almost half of them did not have surgery or any invasive procedures while on admission. This has been corroborated by a study from Spain where the investigators found that the only documented risk factor among patients with acute HCV infection was hospital admission [19]. One can speculate that these patients might have been exposed to HCV-contaminated surfaces during hospitalization. We hypothesized that occupational and iatrogenic HCV infections may be due, in part, to the virus's ability to remain viable on fomites and other hospital equipment for prolonged periods.

We recently established a microculture assay for propagation of cell culture-derived HCV (HCVcc) in small volumes by using a genetically engineered reporter virus derived from the HCVcc clone [20, 21]. Using our microculture assay system, we performed a set of experiments to replicate the circumstances in which healthcare workers or patients may come into contact with HCV that has dried on surfaces. These circumstances include preparing plasma, handling hemodialysis equipment, and placing and removing venous lines. To our knowledge, this is the first study to closely simulate conditions that lead to nosocomial transmission of HCV.

MATERIALS AND METHODS

Plasmids and Viruses

The construction of the Jc1/GLuc2A reporter virus, a derivative of the chimeric genotype 2a FL-J6/JFH with a luciferase gene from *Gaussia princeps* inserted between the p7 and NS2 genes, has been described previously [21, 22]. Viral stocks of Jc1/GLuc2A reporter virus were prepared by RNA transfection of Huh-7.5 cells. The titer of HCVcc was quantified by infecting cells with serial dilutions of the stock virus and determining the dilution that will infect 50% of the wells using the method of Reed and Muench [23].

Cell Culture

Human hepatoma cells that are highly permissive for HCVcc (Huh-7.5 subline) [24] were maintained as subconfluent, adherent monolayers in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum and 1 mM nonessential amino acids (Invitrogen, Carlsbad, CA) at 37°C and 5% CO₂.

Determination of the Volume of Accidentally Misplaced HCVcc-Contaminated Plasma on Surfaces

The most likely circumstances in which healthcare workers or patients will come into contact with HCV dried on surfaces are following spillage of HCV-contaminated blood, serum, or plasma during the course of preparing a blood sample for

analysis or removing a venous line. To simulate such accidents, we obtained EDTA-anticoagulated blood from human immunodeficiency virus (HIV) and HCV seronegative donors. The tube was centrifuged at 2000 rpm for 15 minutes, and a rubber dropper was used to aspirate and transfer the plasma into several vials for storage, as per practice and recommendation of the clinical microbiology laboratory at Yale-New Haven Hospital. The procedure was done in a biosafety cabinet with a foil mat to collect accidental drops of plasma. The experiment was performed on 2 occasions; at each occasion, 10 accidental drops were weighed. The volume of the drop was calculated based on the formula: 1 mL weighs 1 g. The mean, with standard deviation of the mean, and maximum volumes were calculated.

Desiccation of Displaced HCVcc-Contaminated Plasma Drops on Work Surfaces

To determine how quickly plasma dries on surfaces, we seeded the wells in uncovered 24-well tissue culture plates with the maximum accidentally dropped volume (33 μ L). The 24-well plates were stored in a refrigerator at 4°C, on a benchtop at 22°C, and in an incubator at 37°C and observed every 60 minutes until all replicates (20 drops) had dried. The time to dryness in these storage conditions was recorded. In a separate experiment, we recorded the temperature and humidity using an analog thermohygrometer (General Tools, New York, NY) 3 times a day (7–9 AM, 12 noon–1 PM, and 3–5 PM) for a week in order to determine the effect of humidity on time to dryness. The mean humidity, with standard deviation of the mean, was calculated.

Viability of Dried HCVcc on Surfaces

We spotted 33 μ L of plasma spiked with HCVcc on the 24-well plates. They were either immediately tested for viable virus or stored at 4°C, 22°C, and 37°C for up to 6 weeks before testing. Twenty replicates were tested per condition, and the experiment was repeated on 2 occasions. Negative controls comprised of plasma without virus. The day before each time point, 96-well plates were seeded with 6.4×10^3 Huh-7.5 cells/well in 100 μ L of medium and incubated at 37°C in 5% CO₂. To test for infectivity, the dried spots were rehydrated and reconstituted with 100 μ L of culture medium. The medium from the wells was gently aspirated from the cells and replaced with 100 μ L of the reconstituted virus mixture. After 5 hours of incubation, the cells were washed with sterile phosphate-buffered saline (PBS) to remove the input virus; fresh medium was added and incubated for 3 days. After 3 days, culture supernatant was harvested and mixed with 20 μ L of lysis buffer before luciferase activity was measured by using a luciferase assay reagent kit (Promega, Madison, WI) and a luminometer (Synergy HT, BioTek, Winooski, VT). The relative luciferase activity (RLA) was determined to be linearly related to HCV infectivity [16].

Virucidal Effect of Antiseptics on Viability of Contaminated HCVcc on Surfaces

We used 3 antiseptics, bleach (Clorox), ethanol, and cavicide (Metrex), to determine the effect of antiseptics on infectivity of HCVcc-contaminated spots by using a culture media without virus as a negative control. Positive controls consisted of cell culture media with virus. These antiseptics are readily available in hospitals and research laboratories. Bleach is available as 6% sodium hypochlorite and diluted 1:10 in tap water before use, while ethanol is available for use as 70% ethanol [25–27]. Cavicide is ready to use without dilution as per product insert. Prior to testing virucidal activity, it was necessary to determine the cytotoxic effects of the antiseptics on the Huh-7.5 cells. Briefly, 33 μ L of test antiseptic was pipetted onto a 24-well plate. The antiseptic was combined with 297 μ L of culture media (ie, 1:10 dilution), and the mixture was passed through MicroSpin S-400 HR columns (GE Healthcare, Freiburg, Germany) according to the manufacturer's instructions. Next, 300 μ L of column eluate or mixture not passed through the columns was added to Huh-7.5 cells seeded the previous day in a 48-well plate at 3.0×10^4 cells/well in 300 μ L of medium to make a final volume of 600 μ L and then incubated overnight at 37°C. After an additional day of incubation, cell growth was determined with the alamarBlue assay (Invitrogen) per the manufacturer's instructions. Cell growth was determined as a function of relative fluorescence measured at 530 nm excitation and 590 nm emission (Synergy HT plate reader; BioTek). Five replicates were tested per condition, and the experiment was repeated twice.

We modified a previously described protocol to test for the infectivity of HCVcc after exposure to test antiseptic [28]. In brief, an equal volume of test antiseptic was pipetted onto 33 μ L HCVcc-contaminated spots for an exposure period of 1 minute; 264 μ L of culture media was then added to the virus-antiseptic mixture (ie, 1:10 dilution) and reconstituted. To reduce the cytotoxicity of antiseptics, each mixture was passed through a MicroSpin S-400 HR column according to the manufacturer's instructions. Then 300 μ L of eluate that had passed through the column or mixture without column purification was added to Huh-7.5 cells in a 48-well plate at 3×10^4 cells/well in 300 μ L of medium to make a final volume of 600 μ L. The cells were washed with sterile PBS after 4 hours to remove input virus and then incubated in 200 μ L fresh media for 3 days. The infectivity of HCVcc was determined by luciferase assay as described above. Ten replicates were tested per condition, and the experiment was conducted on 3 occasions.

RESULTS

Volume of Accidentally Misplaced HCVcc-Contaminated Plasma

Three experiments were performed, and 10 drops were weighed during each experiment. The mean volume of the drops, based

on the formula: 1 mL weighs 1 g, was 29 ± 5 μ L and the range was 18–33 μ L. Because the maximum drop volume of 33 μ L presents the most risk of transmission, we used 33 μ L throughout our study.

Time to Drying of HCVcc-Contaminated Drops at Different Temperatures

Dried droplets of serum contaminated with HCV may be inconspicuous and, therefore, more likely than a liquid droplet to cause accidental exposure to HCV. We determined how long it took a drop of HCVcc-contaminated plasma to dry at 4°C, 22°C, and 37°C. We determined the mean temperature and relative humidity in the refrigerator, the benchtop, and the incubator over a week. The temperature was $4 \pm 1^\circ\text{C}$, $22 \pm 0^\circ\text{C}$, and $37 \pm 0^\circ\text{C}$ in the refrigerator, the benchtop, and the incubator, respectively. The humidity was $53\% \pm 10\%$, $44\% \pm 5\%$, and $82\% \pm 1\%$ at 4°C, 22°C, and 37°C, respectively. The order of time to dryness was 4, 24, and 28 hours at 22°C (benchtop), 4°C (refrigerator), and 37°C (incubator), respectively. Thus, time to dryness correlated positively with the humidity of the storage condition.

Infectivity of Dried HCVcc on Surfaces at Different Temperatures

We investigated the infectivity of HCVcc after drying on surfaces at different temperatures. Aliquots of 33 μ L of HCVcc-contaminated serum were pipetted into 24-well plates and stored for up to 6 weeks. Twenty spots of dried HCVcc for each combination of storage time and temperature were reconstituted with culture media after storage and introduced into our assay system [20]. The proportion of HCVcc-positive dried spots and the infectivity per HCVcc dried spot were determined. The results presented here came from at least 3 independent experiments.

First, we used a low-titer stock of HCVcc (ie, equivalent to 10^4 infectious units/mL) to determine the infectivity of HCVcc after drying and storage for up to 6 weeks. We observed a negative correlation between storage temperature and HCVcc infectivity (Figure 1A). With an assay detection limit of 1000 RLA (2–3 times above the background luciferase activity), we recovered viable HCVcc from dried spots stored at 37°C until day 7 of storage. In contrast, at storage temperatures of 4°C and 22°C, we recovered replicating HCVcc from all spots for up to 6 weeks of storage. The infectivity, measured by RLA of the reconstituted spots, declined rapidly over time inversely to the storage temperature (Figure 1B). At storage temperatures of 4°C and 22°C, we observed a sharp decline in infectivity over the first 2 weeks followed by persistent but lower infectivity through week 6 (Figure 1B). This is consistent with our previous report of the biphasic decay rate of HCVcc [20].

By using a high-titer stock of HCVcc (equivalent to 10^6 infectious units/mL), we observed a prolonged infectivity of HCVcc

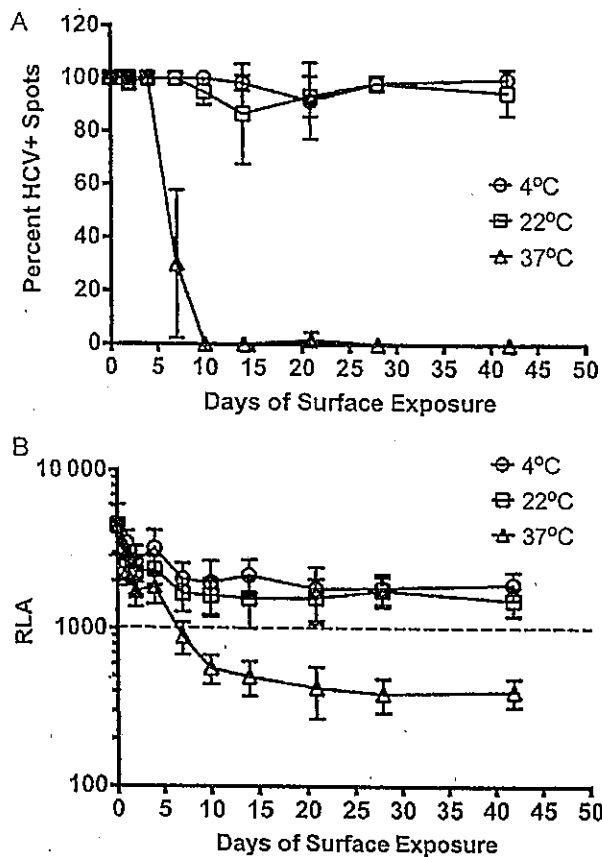


Figure 1. Survival of low-titer hepatitis C virus (HCV) after drying on surfaces. The 33 μ L of HCV-spiked blood was spotted on 24-well plates at 4°C, 22°C, and 37°C for up to 4 weeks before content was flushed to infect Huh-7.5 cells. HCV survival, a function of infectivity, was determined by the relative luciferase units after 3 days of culture. There were 20 drops at each time point. *A*, The percentage of HCV-positive dried spots after storage. *B*, HCV infectivity per positive dried spot. Each value is mean $\pm$ standard deviation from at least 3 independent experiments.

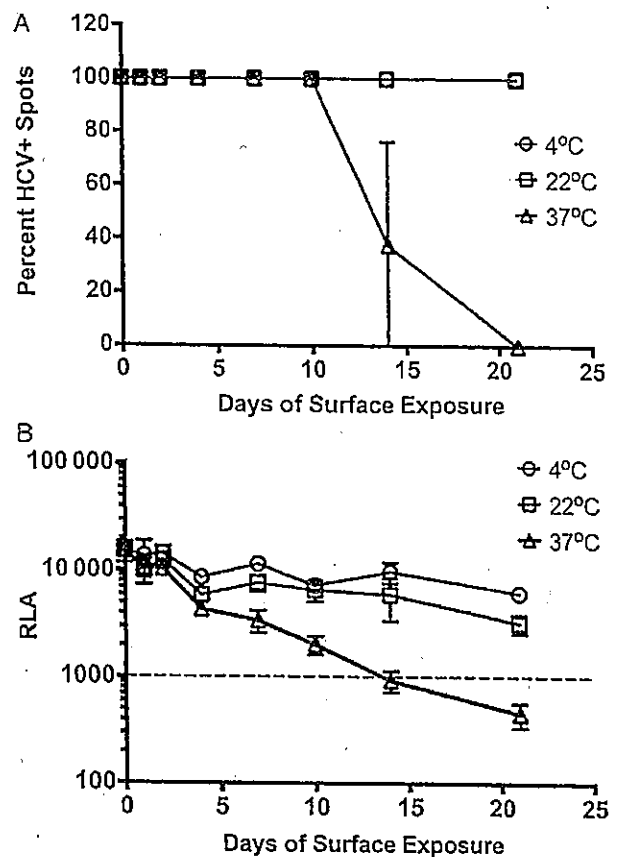


Figure 2. Survival of high-titer hepatitis C virus (HCV) after drying on surfaces. The 33 μ L of HCV-spiked blood was spotted on 24-well plates at 4°C, 22°C, and 37°C for up to 3 weeks before content was flushed to infect Huh-7.5 cells. HCV survival, a function of infectivity, was determined by the relative luciferase units after 3 days of culture. There were 20 drops at each time point. *A*, The percentage of HCV-positive dried spots after storage. *B*, HCV infectivity per positive dried spot. Each value is mean $\pm$ standard deviation from at least 2 independent experiments.

at all storage temperatures. Almost 100% of the contaminated spots stored at 4°C and 22°C remained positive for HCVcc through 3 weeks of storage (Figure 2A). At 37°C, 100% of the spots were positive until 10 days of storage and then declined to 40% at day 14 and to 0% at day 21 (Figure 2A). The infectivity of the HCVcc recovered from the high-titer HCVcc-contaminated spots was in general 2- to 3-fold higher than the RLU of the low-titer HCVcc at each time point. Infectivity was inversely proportional to the storage temperature. We observed a 50% reduction in infectivity at day 3, day 14, and day 21 for storage temperatures 37°C, 22°C, and 4°C, respectively (Figure 2B).

Effect of Antiseptics on Infectivity of HCVcc on Surfaces

To investigate the virucidal effect of bleach, ethanol, and cavi- cide, we first determined the effects of these antiseptics on the growth of Huh-7.5 cells by using the alamarBlue assay. When

we tried undiluted bleach and cavi- cide, which were diluted 1:10 before being added to the tissue culture system, we found them to be uniformly cytotoxic to Huh-7.5 cells, whereas 70% ethanol had no significant effect on cell growth (Figure 3A). Cell growth was almost restored to control levels with a 1:10 dilution of bleach and a 1:20 dilution of cavi- cide following passage of the solution through MicroSpin S-400 HR columns (Figure 3A). Cavi- cide at a 1:10 dilution reduced growth by 70% relative to the control.

Based on the cytotoxicity results, experiments using bleach diluted 1:10 and 1:100, cavi- cide diluted 1:10 and 1:20, and ethanol at 70% and 7% were conducted by using MicroSpin S-400 HR columns [29] prior to adding eluate to the microculture system. After 1 minute of exposure to bleach (1:10 dilution), cavi- cide (1:10), and ethanol (70%), the percentages of positive contaminated HCVcc spots were 0%, 3% $\pm$ 6%, and 13% $\pm$ 6%,

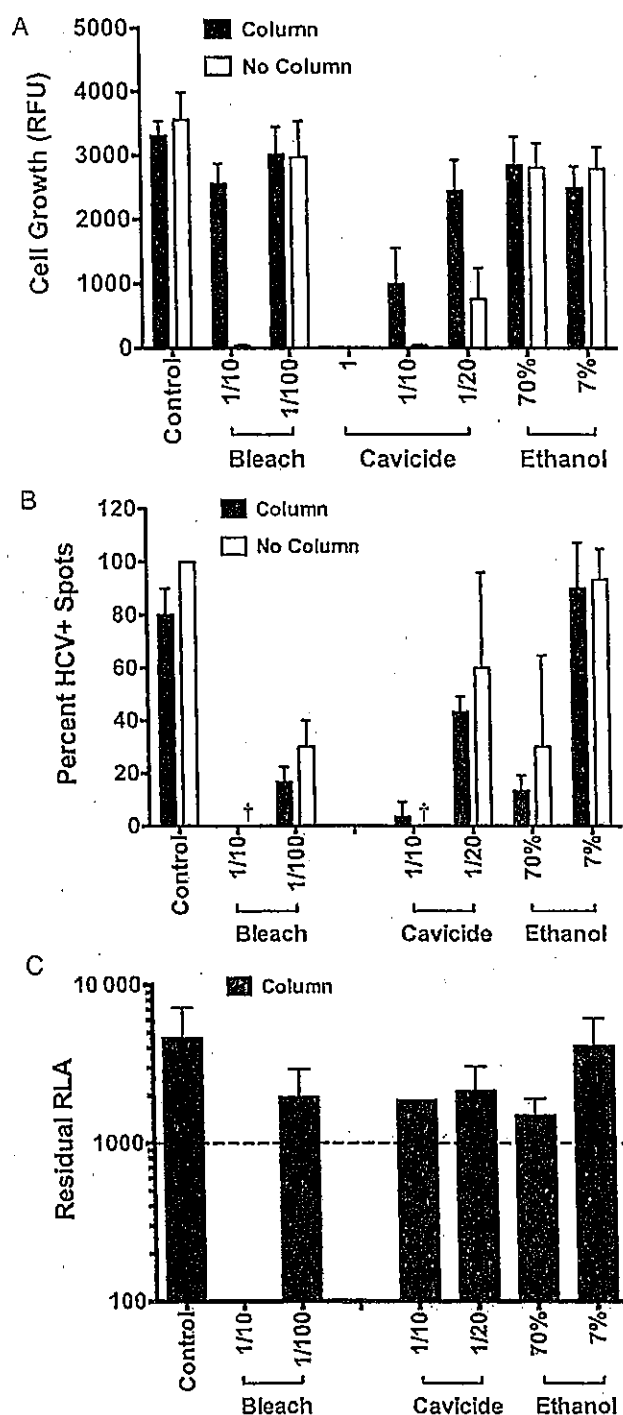


Figure 3. Effect of commercially available antiseptic on Huh-7.5 cell growth and hepatitis C virus (HCV) infectivity. Three antiseptics—bleach, cavicide, and ethanol—were tested for their ability to inactivate HCV at different concentrations. *A*, Effect of antiseptics on Huh-7.5 cell growth. Here 33 μ L of antiseptics were diluted 1:10 with cell culture medium and purified, or not, on S-400 HR columns and incubated on Huh-7.5 cells overnight. Cell growth was determined by alamarBlue assay. *B*, The percentage of HCV-positive dried spots after application of antiseptic. Here 33 μ L of HCV-spiked blood was spotted on 24-well plates and exposed to 33 μ L of antiseptics at different concentrations for 1 minute. The reaction was

respectively (Figure 3B). Further dilutions of bleach (1:100), cavicide (1:20), and ethanol (7%) resulted in $17\% \pm 6\%$, $43\% \pm 6\%$, and $90\% \pm 17\%$ positive spots, respectively. For certain viruses, passage through a MicroSpin column could reduce viral infectivity [30]. Therefore, we performed a control experiment comprising HCVcc without exposure to any antiseptic and with or without passage through a MicroSpin column prior to infection of Huh 7.5 cells. The infectivity was $80\% \pm 10\%$ and 100% for HCVcc with and without passage through MicroSpin column, respectively (Figure 3B). Next, we tested the infectivity of HCVcc without MicroSpin column after exposure to antiseptic at concentrations that are least cytotoxic. After 1 minute of exposure to bleach (1:100 dilution), cavicide (1:20), 70% ethanol, and 7% ethanol, the percentages of positive contaminated HCVcc spots were $30\% \pm 10\%$, $60\% \pm 36\%$, $30\% \pm 35\%$, and $93\% \pm 12\%$, respectively (Figure 3B). The infectivity of residual HCVcc after passage through MicroSpin column (Figure 3C) was correlated with the likelihood of recovery of viable HCVcc. RLA was highest for 7% ethanol (27 of 30 spots yielded viable HCVcc) and lowest for 1:10 cavicide (1 of 30 spots yielded viable HCV).

DISCUSSION

In our simulation of real-world risks of HCV transmission in settings conducive to exposure to HCV-contaminated fomites, we observed that HCVcc could maintain infectivity for up to 6 weeks at 4°C and 22°C . This finding supports our hypothesis that the increasing incidence of nosocomial HCV infections may be due to accidental contact with HCV-contaminated fomites and other hospital equipment, even after prolonged periods following their deposition. Moreover, we found that HCVcc infectivity was influenced by HCVcc viral titer and the temperature and humidity of the storage environment. Furthermore, the commercially available antiseptics reduced the infectivity of HCVcc on surfaces only when used at the recommended concentrations [25, 27] but not when further diluted.

Although there have been 2 previous studies on infectivity and stability of HCV on surfaces [28, 31], to our knowledge,

Figure 3. *Continued.* stopped by diluting the antiseptic 1:10 with cell culture media and purified with or without MicroSpin S-400 HR columns. The eluents were used to infect Huh-7.5 cells. HCV survival, a function of infectivity, was determined by the relative luciferase units after 3 days of culture. Dark and gray bars represent experiments with and without passage through MicroSpin column, respectively. The dagger (†) indicates experiments without columns were not done because of the cytotoxicity of the antiseptic at these concentrations. *C*, Residual infectivity of HCV-contaminated surface after application of antiseptics. Each value is mean $\pm$ standard deviation from at least 3 independent experiments.

this is the first study that closely simulates the natural events likely to cause transmission of HCV. Kamili et al reported that 100 μ L aliquots of chimpanzee plasma contaminated with HCV was still infectious when dried and stored at room temperature for up to 16 hours [31]. Transmission of infection did not occur after 16 hours to up 7 days of storage. More recently, Doerrbecker et al demonstrated that 50 μ L of cell culture-derived HCV dried on steel discs remains infectious for up to 5 days at room temperature [28]. The limitations of these previous studies include simulation of HCV transmission under artificial drying conditions. Furthermore, Doerrbecker et al found that the infectivity of the virus recovered from the carrier system was 10-fold lower than that stored in liquid media. Therefore, one can speculate that the duration of infectivity observed in their study could be an underestimation. Moreover, differences in the 3 assay systems (eg, in vivo vs in vitro assay; artificial vs passive desiccation) might account for the different durations of survival reported. Our study sought to overcome some of these limitations by determining the exact size of accidentally misplaced HCVcc-contaminated plasma and allowing the drops to dry under natural conditions. The fact that we found HCVcc to be infectious for up to 6 weeks under these conditions, which is consistent with our previous report that HCVcc survived in tuberculin syringes for up to 63 days [20], is of public health concern. Taken together, these studies show that HCVcc remains potentially infectious for prolonged periods of time, ranging from 16 hours to 6 weeks depending on the assay system. Previously, we reported on the biphasic decay rate of our genotype 2a HCVcc at room temperature; there was a rapid decline of infectivity within the first 6 hours followed by a second phase of relatively slow exponential decay [20]. This is consistent with a recent report on the thermostability of 7 genotypes including 2a genotype [32]. Such prolonged infectivity could contribute to the increasing incidence of nosocomially acquired HCV infections.

Relevant to infection control is the fact that all HCVcc-contaminated spots dried at room air temperature within 4 hours, becoming inconspicuous and therefore more likely to cause accidental exposures to HCV. HIV was also reported to dry at room temperature within 3 hours and to retain infectivity for up to 7 days [33, 34]. The infectivity of HCVcc and HIV when stored at room temperature for several days is consistent with that of other envelope viruses [35, 36]. The prolonged infectivity of these viruses has been attributed, in part, to their lipid envelope, which resists drying and protects the viral capsid from the deleterious effects of dehydration [37]. Hepatitis B virus, another lipid-enveloped hepatotropic virus, was reported to survive for up to 7 days at room temperature; further time points were not available due to a laboratory mishap [38]. The resilience of these viruses at room temperature raises the possibility of their being transmitted through fomites. Our findings support the surveillance data on the increasing incidence of

nosocomial transmissions of HCV in developed countries [13–17]. Interestingly, most of the patients who acquired HCV in the hospital had no surgeries or invasive procedures; their only risk was hospital admission [18, 19]. Fomites could, therefore, be an important vehicle for transmission of HCV in hospital and household settings.

Finally, given the infection control implications of our findings, we decided to determine if commonly used antiseptics are effective against HCV. We demonstrated that bleach, cavicide, and ethanol are effective at their recommended concentrations [25–27]. It is possible that the efficacy of cavicide at 1:10 is overestimated because the disinfectant itself reduced host cell viability by 70%. Further dilution of each antiseptic proved suboptimal (Figures 3B and 3C). The finding for ethanol paralleled that of Ciesek et al, who found that HCV titers decreased at concentrations of 30% and 40%, but complete inactivation did not occur at an exposure time of 5 minutes [29]. However, undiluted concentrations of several hand antiseptics (based on povidone-iodine, chlorhexidine digluconate, and triclosan) reduced HCV infectivity to undetectable levels [29]. Thus, there are several commercially available antiseptics that are effective against HCV.

Our study, which sought to improve upon prior studies, still has some limitations. First, the assay uses a genetically modified HCV laboratory clone derived from a genotype 2a virus that may not reflect survival characteristics of human isolates. However, the thermostability pattern of our virus is similar to that of other genotypes [32]. Second, the spiking of HCVcc-seronegative blood might not sufficiently replicate the biological factors present in the blood of HCV-infected individuals that could moderate HCV transmission and infectivity. However, the consistency of our results with those from previous in vitro studies and epidemiologic studies that reported on the transmission of HCV in healthcare settings and through sharing of injection paraphernalia [39–43] support our findings.

In conclusion, we have demonstrated that HCVcc can remain infectious at room temperature for up to 6 weeks. Our hypothesis of potential transmission from fomites was supported by the experimental results and provides the biological basis for recent observational studies that have reported an increasing incidence of nosocomial HCV infections and continued high incidence among people who inject drugs.

Notes

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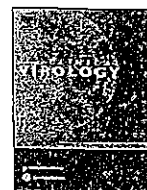
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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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 2014. 4. 5	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人赤血球液			公表国 ドイツ	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)	研究報告の公表状況	Novakova V, Hamprecht K, Müller AM, Arellano-Galindo J, Ehlen M, Horneff G. J Clin Virol. 2014 Apr;59(4):259-63. doi: 10.1016/j.jcv.2014.01.022. Epub 2014 Feb 4.		
研究報告の概要	○母乳を通じてサイトメガロウイルス(CMV)に感染した生後2カ月の満期産男児における、広範な結腸狭窄を伴う重症CMV腸炎CMV疾患は多くが早産児または栄養失調児において発生し、免疫不全のない乳児や幼児がCMVに感染しても、通常無症候性あるいは軽症であり、抗ウイルス剤治療を必要としない。 便、尿及び咽頭スワブ検体から比較的高いウイルス量が検出された播種性CMV感染に基づき、免疫組織学的に証明された、CMV疾患としては稀な症状である重症CMV腸炎の、生後2カ月の男児における1症例の報告である。ガングリオン投与により、下痢は治まり尿中のウイルス量は減少したが、男児は回復した。出生時の検体(血液)はPCR検査においてCMV陰性であったとした。これらの薬剤を併用することによって、男児は回復した。母乳の細胞成分及び無め先天性感染は除外され、また男児は帝王切開で産まれたため、経産道感染の可能性も否定された。母乳の細胞成分及び無脂肪乳清からCMV DNA約80,000コピー/mLを検出した。母乳、男児の結腸生検、尿及び咽頭スワブにおけるウイルス株のN末端CMV gO遺伝子領域の配列を決定したところ、全ての分離株がgO2bで、100%の同一性を示した。イムノブロット解析によりIgM抗体が検出され、急性CMV初感染であることが明らかとなった。従って、この症例におけるCMV感染の原因は母乳であると考えられた。 CMV感染による胃腸障害はこれまで認識されていたよりも一般的である可能性があり、標準的な治療で回復しない長期または重症の下痢や、通常の感染症検査で陰性となる場合は考慮されるべきである。				
報告企業の意見	母乳を介してサイトメガロウイルス(CMV)に感染した、生後2カ月の満期産児における、広範な結腸狭窄を伴う重症CMV腸炎の症例報告である。				
今後の対応	日本赤十字社では、保存前白血球除去した血液製剤のみを供給している。さらに、医療機関の要請に応じてCMV抗体(IgG及びIgM)が陰性であることを確認した血液製剤を供給している。今後もCMV感染に関する新たな知見等について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 赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 赤血球液-LR「日赤」 照射赤血球液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染、vCJD等の伝播のリスク				



Case Report

Severe postnatal CMV colitis with an extensive colonic stenosis in a 2-month-old male immunocompetent term infant infected via breast milk



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1. Why this case is important

CMV infections in immunocompetent infants/children are usually asymptomatic or mild and do not require antiviral treatment. CMV disease often occurs in preterm or dystrophic infants.

We report the case of a 2-month-old male immunocompetent infant with severe immunohistochemically proven CMV colitis, as a rare manifestation of CMV disease, based on a disseminated CMV infection with relatively high viral load and virus shedding into stool, urine and throat swab. With administration of intravenous ganciclovir the diarrhea subsided and the virus load in the urine decreased. Nevertheless the patient developed an extensive colonic stenosis and required anti-inflammatory treatment with corticosteroids. With the combined therapy he eventually got well. In the follow up one year later, there has been no sign of reactivation of the CMV infection. The infant is growing and developing normally.

Postnatally shedded viral strains including strains from breast milk, colon biopsy, urine and throat swabs were sequenced for the N-terminal CMV gO gene region. All isolates were gO2-subtype

and showed 100% identity between all viral isolates. Additionally immunoblot analysis revealed an acute CMV primary infection with low avidity.

To our knowledge this is the first reported case of severe postnatal CMV enterocolitis in an immunocompetent infant who received intravenous ganciclovir and nevertheless developed an extensive colonic stenosis which required steroids.

2. Case description

A 2-month-old infant was admitted with a 7 day history of decreased appetite, vomiting and bloody diarrhea. The infant was born at term after an uneventful pregnancy. Perinatal history was inconspicuous. He was breast fed.

On examination the baby was dehydrated. Blood results were unremarkable: blood count, electrolytes, liver and renal function were within normal range, C-reactive protein was negative. Stool cultures did not reveal any gastrointestinal pathogen. Sonography of the abdomen ruled out a pyloric stenosis and the upper gastrointestinal passage demonstrated a mild gastroesophageal reflux. All criteria suggesting a common gastroenteritis, a supportive care with intravenous rehydration was started.

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Table 1
Lymphocyte typing.

	In total [μl^{-1}]	Reference	% of lymphocytes	Reference
T-lymphocytes (CD3+)	9580	2300–7000	67	48–77
T-helper lymphocytes (CD4+)	2978	1000–5000	22	31–58
T-suppressor lymphocytes (CD8+)	6016	400–2200	44	9–28
Activated T-lymphocytes (HLA DR+)	3358	70–600	22	1–9
B-lymphocytes (CD19+)	1211	600–3000	09	13–39
Natural killer cells (CD3–/CD16+)	2983	100–1300	21	2–17

On day 3 of admission the infant instantly developed acute abdominal distress. Abdominal sonography failed to detect peristalsis. Plain radiograph demonstrated distended bowel loops with air fluid levels suggesting an intestinal obstruction. An emergency laparotomy did not detect any abnormalities, i.e. intussusception, volvulus or malrotation of the gut. A contrast enema confirmed the normal anatomy. Postoperatively the infant still presented with a sepsis-like-syndrome (CrP max 190 mg/l) and was transferred to the ICU where intravenous antibiotics and one erythrocyte transfusion (radiated, leukocyte depleted) were applied. Gastrointestinal complaints continued. Enteral feeding was impossible. Diarrhea became aggravated, leading to electrolyte imbalance and exsudative enteropathy and required correspondent therapy.

Search for infections in stool, blood and cerebrospinal fluid revealed negative results for more than 20 microorganisms. Detailed investigation ruled out an underlying primary immunodeficiency. Total immunoglobulin levels were within normal limits and granulocyte function was normal. Differentiation of the lymphocytes gave normal numbers, but showed a CD4/CD8 inversion and activated T-cells with increased CD3/HLA DR expression (Table 1) suggesting a viral infection. Hence, we ruled out HIV and EBV infection.

CMV DNA was first detected in the urine of the infant (439,000 copies/ml). Further analyses yielded a disseminated CMV infection with leuko- and plasma-DNAemia (25,300 copies/ml) and viral shedding from stool (237,000 copies/ml) and saliva. The CMV enterocolitis was documented by positive PCR in the biopsy material and by PCR of frozen cryosections of colon histology. The infant was able to raise specific antibodies with positive titers for IgM and IgG (1:1900). There was no involvement of the central nervous system or any ophthalmologic impairment. In the breast milk we found about 80,000 copies/ml of CMV DNA in cell and fat-free milk whey.

Congenital CMV infection can be excluded with relatively high probability since PCR from original Guthrie-card was negative. Recombinant CMV immunoblot analysis documents an acute primary infection with total absence of gB 1/2-IgG reactivity, low avidity IgG and bright IgM reactivity. Sequence analysis of the N-terminal hyper variable region of the gO gen region, which codes for viral glycoprotein O, was performed (Fig. 1a) to compare the different viral strains with that of the colon biopsy, as a gO2b variant. Results for pair wise identity are shown in comparison to gO2b reference strains (Fig. 1b) and for the patient strains (Fig. 1c). The results strongly suggest that CMV was transmitted from mother to infant postnatally by breastfeeding. The viral variant detected in the breast milk is identical to the one found in the colon biopsy material. Thus, we found molecular evidence for the following viral transmission cascade: postnatal maternal CMV reactivation and shedding of the virus strain into breast milk, initial gastroenteric viral uptake in the infant, dissemination via blood stream and end organ manifestation in stromal cells of the colon ulcer.

At colonoscopy, we discovered severe inflammation especially in the ascending colon which decreased distally. Histologically the specimens from the colon showed ulcer ground, swollen mucosa with epithelial necrosis, disrupted crypts and neutrophilic

infiltrate. At higher magnification typical large epithelial as well as stromal cells with intranuclear resp. cytoplasmic inclusions were observed. When stained with an antibody against CMV (DAKO, M0854; Clone: CCH2+DDG9; 1:50) these inclusions displayed a positive staining reaction (Fig. 2).

In summary, we diagnosed a postnatal CMV-disease, transmitted via breast milk, in an immunocompetent term infant, with colitis being the major manifestation.

Informed consent was obtained and treatment with intravenous ganciclovir (10 mg/kg/day) was started. Consecutively the infant tolerated feeding and diarrhea subsided. Simultaneously viral shedding in the urine decreased (Fig. 3). On day 14 of ganciclovir therapy the patient deteriorated instantly. The abdominal ultrasound revealed a massive colonic stenosis of almost the entire colon ascendens and transverse. The infant immunoglobulin G level had increased naturally at that point from 1.6 to 3.2 g/l within two weeks time. Knowing of the infant immunocompetency, intravenous immune globulins (400 mg/kg) were administered once and high dose corticosteroids (2 mg/kg/day) were started. With that regime a constant improvement was achieved. Ganciclovir therapy was continued for 6 weeks and corticosteroids for 4 weeks on tapered dosings. The infant was fully enteral fed and started to thrive (Fig. 4). CMV dissolved in the urine (Fig. 3).

In the follow up one year later, the patient is doing well, thriving without reactivation of the CMV infection and no signs of any sequelae or immunoincompetence.

3. Other similar and contrasting cases in the literature

The gastrointestinal system is an uncommon manifestation in CMV infection in immunocompetent individuals. To our knowledge just a few comparable cases have been published. One 5-week-old term infant that presented with intractable diarrhea, was diagnosed as CMV enterocolitis, treated with ganciclovir and not develop any sequelae [5]. A 2-month-old term infant presenting with bloody diarrhea and dehydration was diagnosed as CMV enterocolitis which resolved without treatment. Although the mucosa appeared macroscopically normal, histology revealed characteristic cytomegalovirus inclusions [6]. As complications of gastrointestinal involvement, ileal perforation in a 5-week-old infant [7] and colonic stricture [8] have been described. In both cases histopathologic examinations showed cytomegalic intranuclear inclusion bodies and it was not clear whether it was a congenital or perinatal infection. Only the latter case was treated with ganciclovir since chorioretinitis was also present.

The drug most commonly used is ganciclovir (GCV). With intravenous application we noticed a good clinical response and viral shedding from the urine decreased. GCV treatment did not have much effect on the colitis and did not promote thriving either (Fig. 4). In the literature there are three similar cases, where oral GCV (1–2 months courses at the dosage of 70 mg/kg) was more efficient than intravenous administration and the authors suggest that the higher local concentration of the drug in the infected enterocytes was causal [9]. In another case of gastrointestinal manifestation the infant was treated with oral valganciclovir and the

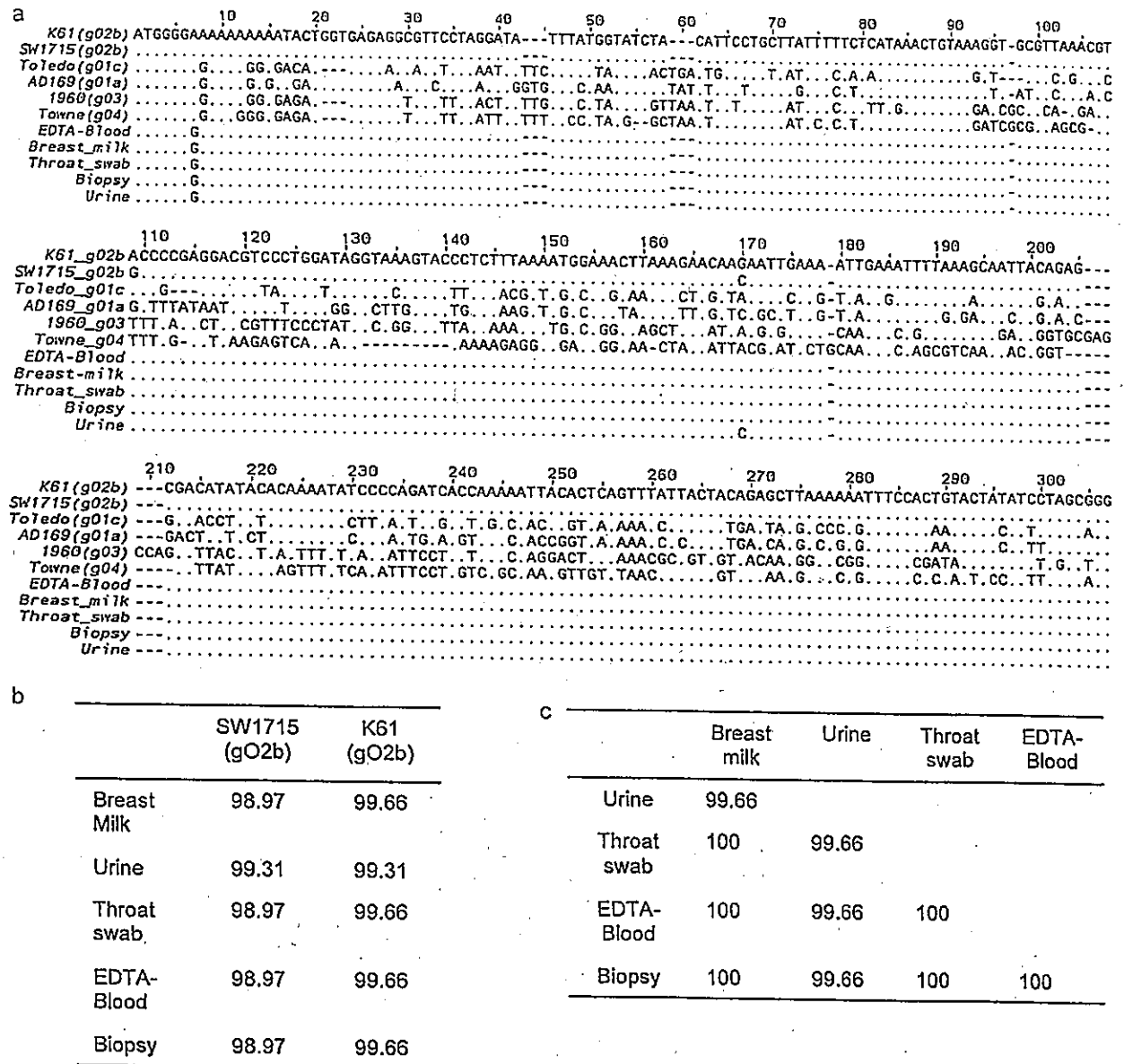


Fig. 1. Sequencing of 307 bp of the N-terminal hypervariable region on the gO glycoprotein [1]. (a) Multiple alignment of the isolates from the mother, the baby and references as well as representative strains for each gO genotype [2]. (b) Pairwise identities between the isolates from each sample from the mother (breast milk) and from the baby (urine, throat swab, EDTA-blood and colon biopsy) with representative strains reported previously as gO2b subtype (SW1715 and K61) [2,3]. (c) Pairwise identities between the isolates using the Smith Waterman algorithm with the software Jalview Version 2 (available online: www.jalview.org) [4].

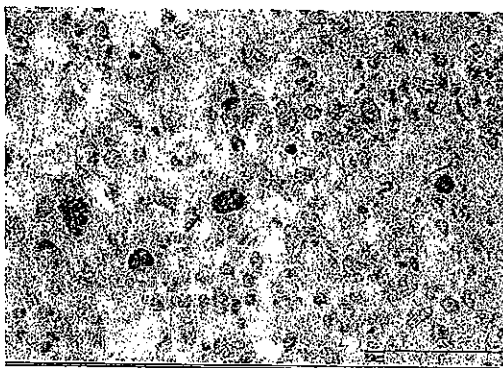


Fig. 2. Photo detail of the heavily ulcerated colonic mucosa, resp. ulcer ground, immunohistochemically stained with an antibody against CMV, revealing brown staining of the viral inclusions in stromal cells.

symptoms resolved. Valganciclovir is a prodrug and about 10 times more bioavailable than GCV [10].

4. Discussion

To our knowledge this is the first reported case of immunohistochemical and molecular proven severe postnatal CMV enterocolitis,

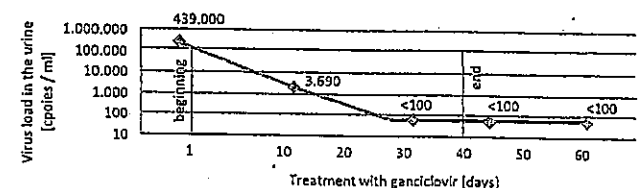


Fig. 3. Effect of GCV treatment on virus load in urine.

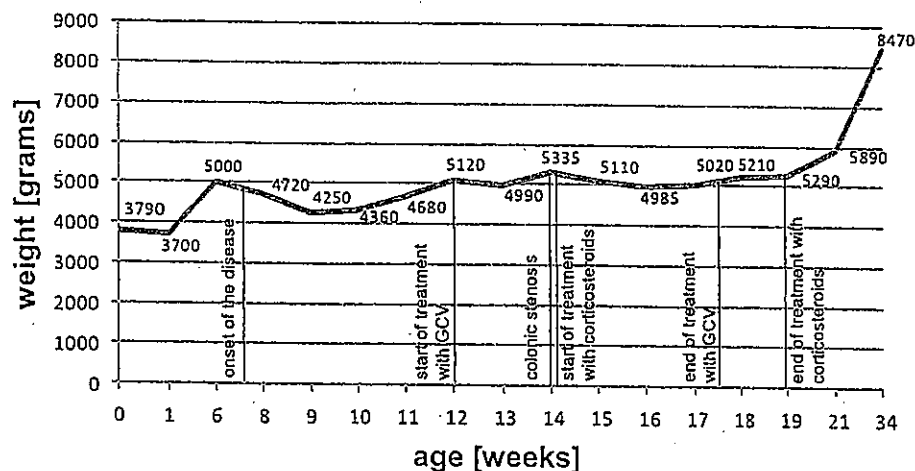


Fig. 4. Effect of GCV treatment on thriving.

transmitted through breast milk, in an immunocompetent term infant, who received intravenous ganciclovir and nevertheless developed an extensive colonic stenosis, which required steroids.

We were able to determine the infant's immunocompetency: there was no evidence for cellular or humoral immunodeficiency and he was able to raise antibodies in general and specific ones against CMV as well. We ruled out acquired immunodeficiency due to HIV. Congenital CMV infection could be excluded, since nested PCR from original Guthrie-card was negative [11].

Since he was born via cesarean section, transmission via maternal fluids during labor is less likely. Transfused blood is no longer likely to be a route of infection, as blood products for neonates are prepared from CMV negative donors and are leukocyte depleted, which was also the case in our transfusion.

Breastfeeding is a known acquisition route in preterm infants [12–14]. Hamprecht showed that almost every surveyed mother underwent a CMV reactivation during lactation and the cumulative transmission rate was 37% [13].

CMV reactivation during lactation shows a unimodal course with peak levels about 4–6 weeks post partum. Since we found about 80,000 copies/ml of CMV DNA in cell and fat-free milk whey (in week 11 after birth), we assume that the peak level of DNA lactia was still higher. This and the relatively long duration of DNA lactia demonstrates a high CMV exposure to the infant, with a plasma viral load about 25,000 copies/ml. CMV immunoblotting showed no gB1/2 reactivity, low avidity IgG and bright IgM reactivity against recombinant CMV antigens with high reactivity against rec p150 and rec CM2 (each very strong). This shows, together with recIE1 IgM reactivity, that the infection is a recent primary infection. It clearly proves a postnatally acquired CMV infection. gB-specific antibodies normally can be detected about 3 months after onset of primary infection [15].

The source of the severe disseminated CMV infection was elucidated by sequencing of the N-terminal hyper variable region of the gO glycoprotein. Multiple alignment and pair wise identities to gO2 genotype reference strains SW1715 and K61, as well as the viral isolates from our patient, revealed 100% of sequence identity. Therefore, the isolates from the breast milk were the same as those found in the urine, throat swab, blood and biopsy (Fig. 2). Breast milk was the source of CMV infection in our case.

In summary, we report the case of a 2-month-old immunocompetent term infant, with intractable diarrhea diagnosed as postnatal CMV disease, acquired via breastfeeding and treated with ganciclovir, who nevertheless developed an extensive colonic stenosis and required additional anti-inflammatory treatment. We consider

that gastrointestinal involvement in CMV infection may be relatively more common than previously recognized. Although an apparently uncommon cause of gastrointestinal disease in the immunocompetent infant, the diagnosis should be considered in any infant with prolonged or severe diarrhea that does not improve with standard management and is negative for the commonly screened microorganisms.

Funding

None.

Competing interests

None declared.

Ethical approval

Not required.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2014. 4. 7	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人赤血球液			公表国 WHO等	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)	研究報告の公表状況	ProMED 20140403.2379386		
研究報告の概要	○エボラウイルス疾患-西アフリカ ・西アフリカにおけるエボラウイルス疾患(EVD;以前はエボラ出血熱と呼ばれた)のアウトブレイクは、現在ザイールエボラウイルスと非常に高い相同性(98%)を持つ株に起因することが確定されている。西アフリカにおいてこの疾患が確認されたのはこれが初めてである。初症例はギニア南東部の森林地域から報告された。アウトブレイクは急速に拡大し、いくつかの地域及びコナクリにおいてEVD患者と死亡者が報告されている。疑い症例と死亡例が近隣諸国からも報告され、彼らは全員ギニアへの渡航歴があった。確定症例はギニア及びリベリアから報告された。 ・ギニア保健省は、2014年4月1日現在EVDの臨床適合症例は累計127症例で、そのうち検査確定症例は35症例であり、83人は死亡したと報告した(致死率65%)。22人の患者は隔離されている。報告された地域の最新臨床症例数は以下のとおり。 ・コナクリ(12症例、死亡4例含む)、ゲケドウ(79症例、死亡57例含む)、マセクタ(23症例、死亡14例含む)、キンドローグー(9症例、死亡5例含む)、ダボラとデインギラエ合算(4症例、死亡3例含む)				使用上の注意記載状況・ その他参考事項等 赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 赤血球液-LR「日赤」 照射赤血球液-LR「日赤」 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク
報告企業の意見	西アフリカで初めて、エボラウイルス疾患が流行しているとの報告である。				
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。今後も引き続き情報の収集に努める。				

7



Published Date: 2014-04-03 21:49:04

Subject: PRO/AH/EDR> Ebola virus disease - West Africa (08): WHO, miscellaneous reports

Archive Number: 20140403.2379386

EBOLA VIRUS DISEASE - WEST AFRICA (08): WHO, MISCELLANEOUS REPORTS

A ProMED-mail post

<http://www.promedmail.org>

ProMED-mail is a program of the

International Society for Infectious Diseases

<http://www.isid.org>

[1]

Date: 3 Apr 2014

Source: WHO Global Alert and Response (GAR), Disease Outbreak News [edited]

http://www.who.int/csr/don/2014_04_ebola/en

WHO is supporting the national authorities in the response to an outbreak of Ebola virus disease (EVD; formerly known as Ebola haemorrhagic fever). The outbreak is now confirmed to be caused by a strain of ebolavirus with very close homology (98 percent) to the Zaire ebolavirus. This is the 1st time the disease has been detected in West Africa. Cases were 1st reported from forested areas in southeastern Guinea. The outbreak has rapidly evolved, and several districts and Conakry have reported cases and deaths caused by EVD. A small number of suspected cases and deaths has also been reported from neighbouring countries with all of them having crossed from Guinea. Confirmed cases have been reported from Guinea and Liberia. (Read more at source URL).

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Communicated by:

ProMED-mail

<promed@promedmail.org>

[This is a good summary of the outbreak. - Mod.JW]

[2]

Date: 3 Apr 2014

Source: Business Standard, India [edited]

http://www.business-standard.com/article/news-ians/ebola-toll-rises-to-84-in-guinea-114040300723_1.html

Guinea's health authorities Thursday [3 Apr 2014] said 4 new ebolavirus disease cases have been reported in the country, bringing the total number of patients to 134, out of which 84 have succumbed to the viral disease. The disease is mostly widespread in the southern regional towns of Gueckedou, Macenta, Kissidougou and the capital Conakry, Xinhua reported.

To curb the spread of the epidemic, the government has taken certain measures that include setting up medical isolation centres in the affected regions and disinfection of homes with suspected cases or where ebolavirus disease patients have died. The government has also undertaken mobilisation of necessary resources to provide individual protective materials to the most affected zones. The UN Children's Fund (UNICEF) has equally proceeded to distribute hygiene kits to schools in areas most hit by the virus.

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[3]

Date: 2 Apr 2014

Source: WHO/AFRO EPR - Outbreak News [edited]

<http://www.afro.who.int/en/clusters-a-programmes/dpc/epidemic-a-pandemic-alert-and-response/outbreak-news.html>

As of 1 Apr 2014, the Ministry of Health of Guinea has reported a cumulative total of 127 clinically compatible cases of EVD, of which 35 are laboratory confirmed by PCR. The total number includes 83 deaths (CRF 65 percent). These figures correspond to 5 new clinical cases and 3 new deaths since the last update on 31 Mar 2014; 22 patients remain in isolation.

The current numbers of clinical cases by place of report are:

- Conakry (12 cases, including 4 deaths),
- Guekedou (79 cases/57 deaths),
- Macenta (23 cases/14 deaths),
- Kissidougou (9 cases/5 deaths), and
- Dabola and Djingaraye combined (4 cases/3 deaths).

11 of the cases in Conakry have been laboratory confirmed for Ebola virus disease (EVD); 14 health care workers have also been laboratory confirmed for EVD, 8 of whom have died. Case investigation and contact tracing are continuing, with 375 contacts under medical follow-up at present.

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[4]

Date: April 2014

Source: WHO Features [edited]

<http://www.who.int/features/2014/guinea-ebola/en>

When more than 3.5 tons of protection material arrived in Conakry, the capital of Guinea, on 30 Mar 2014, WHO immediately started distributing it to health facilities in different locations dealing with the outbreak of Ebola virus disease (EVD).

The supplies include single-use personal protection equipment and disinfection and secure burial material. Half of the infected persons in Conakry are health workers. Providing them with adequate training and necessary equipment is crucial for infection control. As there is no treatment or vaccine available, prevention is the most effective way to stop the transmission.

"These supplies are essential, as we were not able to be in contact with sick persons who could have Ebola. With protection equipment, we feel reassured and can do our job to help patients," said Dr Lansana Kourouma, head of the emergency section of the Chinese-Guinean Friendship hospital, where 5 patients are currently under observation.

The material was also sent to the Donka national teaching hospital in Conakry, where an isolation ward has been set up to provide care to infected persons. The ward is located in a separate building. The Institut Pasteur, Dakar, Senegal has dispatched technicians to the hospital so it can carry out on-site rapid testing.

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<promed@promedmail.org>

[But see below. - Mod.JW]

[5]

Date: 13 Feb 2014 [relevant to report above]

Source: Pulitzer Center on Crisis Reporting [edited]

<http://pulitzercenter.org/reporting/africa-guinea-maternal-health-infant-mortality>

"The biggest problems at Donka are no electricity, no water, no equipment, no sanitation, and very high rates of infection," said Bintu Cisse, adjunct midwife supervisor, who has worked at Donka National Hospital for 20 years.

Donka, the largest medical center in Guinea, is run by the government and maintains short and long term partnerships with international aid organizations such as UNICEF and MSF (Medecins sans Frontieres or Doctors Without Borders). External support provides some operational assistance, but Donka lacks basic facilities due to the inefficiency of Guinea's under-performing infrastructure.

Inside the maternity ward operating room, Cisse pointed out that the equipment did not work, and doctors used suspended basins of water and a mixture of chlorine to sanitize. The main light sources were open windows. Outside, garbage was burning.

[Byline: Brandice Camara, for the Pulitzer Center]

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Communicated by:

ProMED-mail Rapporteur Joe Dudley

[See more below. - Mod.JW]

[6]

Date: 2 Apr 2014

Source: Canadian Manufacturing online, 27 Jan 2014 [edited]

<http://www.canadianmanufacturing.com/manufacturing/kallo-signs-200m-medical-software-deal-with-republic-of-guinea-130898>

Medical software and technology company Kallo has signed a USD 200 million medical software deal with the Republic Of Guinea.

The technology will aid healthcare delivery and bring a higher standard of health services to the developing country's rural population.

[Read more at source URL.]

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Communicated by:

ProMED-mail Rapporteur Joe Dudley

[Without improved infrastructure, this system will be useless.

Joe points out that USD 200 million would provide clean water to a good number of government hospitals. - Mod.JW]

[7]

Date: 2 Apr 2014

Source: Journal du Mali [in French, summarized, edited]

<http://www.journaldumali.com/article.php?aid=8119>

[In summary, a young man from the frontier with Guinea was spotted at the bus station in Bamako, capital of Mali, and suspected to be a case of ebolavirus disease. He was taken to a hospital to be quarantined, but his relatives took him away by taxi.

Later in the day, 2 more suspect cases were spotted. One was also from the Guinea frontier, arriving with severe diarrhea and vomiting.

The entire busload of passengers was quarantined for 17 hours, while blood specimens were taken for testing. It is emphasized that there is no confirmation that they were cases of ebolavirus disease, but there is considerable anxiety in the capital.

A map of Mali is at:

http://upload.wikimedia.org/wikipedia/commons/6/66/Mali_Map.jpg. - Mod.JW]

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[8]

Date: 2 Apr 2014

Source: RTE News Ireland [edited]

<http://www.rte.ie/news/2014/0402/606281-ebola-death-toll-hits-83-in-guinea>

Foreign mining firms have locked down operations in Guinea and pulled out some international staff as the death toll from suspected cases of Ebola there hit 83.

The epicentre of Guinea's 2-month old outbreak has been in the southeast, close to its main iron ore reserves. The country is also the world's top exporter of Bauxite, the raw material used in aluminium production, and has rich deposits of gold.

"Everyone is practicing precautionary strict hygiene, but there has been no real impact on production so far," a senior executive at a mining company told Reuters. The executive said he had been placed on extended leave, while other companies were preventing people from entering or leaving their mines. "Firms are more concerned by what is happening in the densely populated capital Conakry than in remote mining sites in the interior, where controls are easier to put in place," he added.

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[9]

Date: Thu 3 Apr 2014

Source: Agence France Presse [edited]

<http://www.rappler.com/world/regions/africa/54627-ebola-liberia-epidemic>

Liberia said Thursday [3 Apr 2014] that it was dealing with its 1st suspected Ebola case thought to be unconnected to the epidemic raging in Guinea and to have originated separately within its borders.

If confirmed, the case in the eastern town of Tapeta would mark a worrying development in the fight against Ebola, as cases so far have been attributed to people returning with the infection from neighbouring Guinea, where 84 people have died.

"We have a case in Tapeta where a hunter who has not had any contact with anyone coming from Guinea got sick," chief medical officer Bernice Dahn said. "He was rushed to the hospital and died 30 minutes later. He never

had any interaction with someone suspected to be a carrier of the virus, and he has never gone to Guinea. This is an isolated case."

The fruit bat, thought to be the host of the highly contagious Ebola virus, is a delicacy in the region straddling Guinea, Liberia and Sierra Leone, and experts suspect hunters may be the source of the outbreak.

Tapeta, a small town in the eastern county of Nimba, is 400 km (250 miles) from the epicentre of the Ebola outbreak in southern Guinea, at least a 5-hour drive and much further from the border than other suspected cases.

"The huntsman has 500 traps in the forest. He felt sick in the forest and was rushed to the hospital," Dahn told AFP, adding that 7 new patients brought the total suspected Ebola cases in Liberia to 14; 6 people have died, she said, since Liberia reported its 1st cases of haemorrhagic fever last month [March 2014], raising the previous toll by 2.

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[No mention of whether any specimens were taken for laboratory confirmation. It is not stated whether any of his traps were for bats. - Mod.JW]

A HealthMap/ProMED-mail map can be accessed at: <http://healthmap.org/promed/p/45.>

See Also

Ebola virus disease - West Africa (07) [20140402.2376459](#)
Ebola virus disease - West Africa (06) [20140402.2373940](#)
Ebola virus disease - West Africa (05): Guinea, Liberia, Sierra Leone WHO update [20140401.2373662](#)
Ebola virus disease - West Africa (04): Guinea, Liberia [20140331.2371054](#)
Ebola virus disease - West Africa (03): Liberia, timeline [20140331.2369368](#)
Ebola virus disease - West Africa: Guinea (02): WHO update [20140331.2368463](#)
Ebola virus disease - Guinea (05): update [20140329.2366532](#)
Ebola virus disease - Guinea (04): WHO update, Conakry conf., alerts [20140328.2364547](#)
Ebola virus disease - Liberia ex Guinea: susp. alert, RFI [20140326.2360265](#)
Ebola virus disease - Guinea (03): WHO update, travel health advisories [20140326.2359361](#)
Ebola virus disease - Guinea (02): bat eating banned [20140326.2359281](#)
Ebola virus disease - Guinea: not Conakry [20140324.2354606](#)
Ebola virus disease - West Africa: Guinea, Zaire ebolavirus suspected [20140322.2349865](#)
Ebola - Sierra Leone: susp. alert, RFI [20140322.2349697](#)
Undiagnosed viral hemorrhagic fever - Guinea (02): Ebola conf. [20140322.2349696](#)
.....jw/msp/dk

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2014年6月25日	該当なし。	
一般的名称	別紙のとおり。	研究報告の公表状況	MMWR.2014;63:548-551	公表国 ギニア		
販売名(企業名)	別紙のとおり。					
問題点：西アフリカで過去最大のエボラウイルス感染症のアウトブレイクが報告された。 2014年3月21日にギニアの保健当局は49名のエボラウイルス感染症のアウトブレイクを報告した。パスツール研究所で検査された20例のうち、15例の標本がPCRによりエボラウイルスに対し陽性とされ、ウイルスシーケンセスによりザイールエボラウイルスが特定された。 3月にリベリア、5月にシエラレオネでも症例が報告され、6月18日時点でこれまでに確認された最大のアウトブレイクとなっており、これら西アフリカ3か国で合計528例(検査確定例364例、可能性例99例、疑い例65例を含む)の感染可能性が認められ、その内337例の死亡が報告されている。 今回のアウトブレイクは、首都圏で初めてエボラウイルスの伝播が報告されたという点で特徴的である。						
研究報告の概要		使用上の注意記載状況・ その他参考事項等 記載なし。				
報告企業の意見		今後の対応				
別紙のとおり。		今後とも関連情報の収集に努め、本剤の安全性の確保を図ってきたい。				

8

[illegible]

*：現在製造を行っていない

INF2014-003



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Morbidity and Mortality Weekly Report (MMWR)

Ebola Viral Disease Outbreak — West Africa, 2014

On June 24, 2014, this report was posted as an MMWR Early Release on the MMWR website (<http://www.cdc.gov/mmwr>).

Weekly

June 27, 2014 / 63(25);548-551

Meredith G. Dixon, MD^{1,2}, Ilana J. Schafer, DVM^{1,2} (Author affiliations at end of text)

On March 21, 2014, the Guinea Ministry of Health reported the outbreak of an illness characterized by fever, severe diarrhea, vomiting, and a high case-fatality rate (59%) among 49 persons (1). Specimens from 15 of 20 persons tested at Institut Pasteur in Lyon, France, were positive for an Ebola virus by polymerase chain reaction (2). Viral sequencing identified Ebola virus (species *Zaire ebolavirus*), one of five viruses in the genus *Ebolavirus*, as the cause (2). Cases of Ebola viral disease (EVD) were initially reported in three southeastern districts (Gueckedou, Macenta, and Kissidougou) of Guinea and in the capital city of Conakry. By March 30, cases had been reported in Foya district in neighboring Liberia (1), and in May, the first cases identified in Sierra Leone were reported. As of June 18, the outbreak was the largest EVD outbreak ever documented, with a combined total of 528 cases (including laboratory-confirmed, probable, and suspected cases) and 337 deaths (case-fatality rate = 64%) reported in the three countries. The largest previous outbreak occurred in Uganda during 2000–2001, when 425 cases were reported with 224 deaths (case-fatality rate = 53%) (3). The current outbreak also represents the first outbreak of EVD in West Africa (a single case caused by Tai Forest virus was reported in Côte d'Ivoire in 1994 [3]) and marks the first time that Ebola virus transmission has been reported in a capital city.

Characteristics of EVD

EVD is characterized by the sudden onset of fever and malaise, accompanied by other nonspecific signs and symptoms such as myalgia, headache, vomiting, and diarrhea. Among EVD patients, 30%–50% experience hemorrhagic symptoms (4). In severe and fatal forms, multiorgan dysfunction, including hepatic damage, renal failure, and central nervous system involvement occur, leading to shock and death. The first two *Ebolavirus* species were initially recognized in 1976 during simultaneous outbreaks in Sudan (*Sudan ebolavirus*) and Zaire (now Democratic Republic of the Congo) (*Zaire ebolavirus*) (5). Since 1976, there have been more than 20 EVD outbreaks across Central Africa, with the majority caused by Ebola virus (species *Zaire ebolavirus*), which historically has demonstrated the highest case-fatality rate (up to 90%) (3).

The wildlife reservoir has not been definitively ascertained; however, evidence supports fruit bats as one reservoir (6). The virus initially is spread to the human population after contact with infected wildlife and is then spread person-to-person through direct contact with body fluids such as, but not limited to, blood, urine, sweat, semen, and breast milk. The incubation period is 2–21 days. Patients can transmit the virus while febrile and through later stages of disease, as well as postmortem, when persons contact the body during funeral preparations. Additionally, the virus has been isolated in semen for as many as 61 days after illness onset.

Diagnosis is made most commonly through detection of Ebola virus RNA or Ebola virus antibodies in blood (5). Testing in this outbreak is being performed by Institut Pasteur, the European Mobile Laboratory, and CDC in Guinea; by the Kenema Government Hospital Viral Hemorrhagic Fever Laboratory in Sierra Leone; and by the Liberia Institute of Biomedical Research. Patient care is supportive; there is no approved treatment known to be effective against Ebola virus. Clinical support consists of aggressive volume and electrolyte management, oral and intravenous nutrition, and medications to control fever and gastrointestinal distress, as well as to treat pain, anxiety, and agitation (4,5). Diagnosis and treatment of concomitant infections and superinfections, including malaria and typhoid, also are important aspects of patient care (4).

Keys to controlling EVD outbreaks include 1) active case identification and isolation of patients from the community to prevent continued virus spread; 2) identifying contacts of ill or deceased persons and tracking the contacts daily for the entire incubation period of 21 days; 3) investigation of retrospective and current cases to document all historic and ongoing chains of virus transmission; 4) identifying deaths in the community and using safe burial practices; and 5) daily reporting of cases (4,7,8). Education of health-care workers regarding safe infection-control practices, including appropriate use of personal protective equipment, is essential to protect them and their patients because health-care-associated transmission has played a part in transmission during previous outbreaks (4,9).

Efforts to Control the Current Outbreak

To implement prevention and control measures in both Guinea and Liberia, ministries of health with assistance from Médecins Sans Frontières, the World Health Organization, and others, put in place Ebola treatment centers to provide better patient care and interrupt virus transmission. Teams from CDC traveled to Guinea and Liberia at the end of March as part of a response by the Global Outbreak Alert and Response Network to assist the respective ministries of health in characterizing and controlling the outbreak through collection of case reports, interviewing of patients and family members, coordination of contact tracing, and consolidation of data into centralized databases. Cases are categorized into one of three case definitions: suspected (alive or dead person with fever and at least three additional symptoms, or fever and a history of contact with a person with hemorrhagic fever or a dead or sick animal, or unexplained bleeding); probable (meets the suspected case definition and has an epidemiologic link to a confirmed or probable case); confirmed (suspected or probable case that also has laboratory confirmation).*

In late April, it appeared that the outbreak was slowing when Liberia did not report new cases for several weeks after April 9, and the number of new reported cases in Guinea decreased to nine for the week of April 27 (Figure 1). Since then, however, the EVD outbreak has resurged, with neighboring Sierra Leone reporting its first laboratory-confirmed case on May 24, Liberia reporting a new case on May 29 that originated in Sierra Leone, and Guinea reporting a new high of 38 cases for the week of May 25.

As of June 18, the total EVD case count reported for all three countries combined was 528, including 364 laboratory-confirmed, 99 probable, and 65 suspected cases, with 337 deaths (case-fatality rate = 64%). Guinea had reported 398 cases (254 laboratory-confirmed, 88 probable, and 56 suspected) with 264 deaths (case-fatality rate = 66%) across nine districts (Figure 1). Sierra Leone had reported 97 cases (92 laboratory-confirmed, three probable, and two suspected) with 49 deaths (case-fatality rate = 51%) across five districts and the capital, Freetown. Liberia had reported 33 cases (18 confirmed, eight probable, and seven suspected) with 24 deaths (case-fatality rate = 73%) across four districts.

Major challenges faced by all partners in the efforts to control the outbreak include its wide geographic spread (Figure 2), weak health-care infrastructures, and community mistrust and resistance (10). Retrospective case investigation has indicated that the first case of EVD might

have occurred as early as December 2013 (Figure 1) (2). To control the outbreak, additional strategies such as involving community leaders in response efforts are needed to alleviate concerns of hesitant and fearful populations so that health-care workers can care for patients in treatment centers and thorough contact tracing can be performed. Enhancing communication across borders with respect to disease surveillance will assist in the control and prevention of more cases in this EVD outbreak.

In June 2014, the World Health Organization, via the Global Outbreak Alert and Response Network, requested additional support from CDC and other partners, necessitating the deployment of additional staff members to Guinea and Sierra Leone to further coordinate efforts aimed at halting and preventing virus transmission. Persistence of the outbreak necessitates high-level, regional and international coordination to bolster response efforts among involved and neighboring nations and other response partners in order to expeditiously end this outbreak.

Acknowledgments

The West Africa Ebola national and international response teams, including the ministries of health of Guinea, Liberia, and Sierra Leone; the World Health Organization; Médecins Sans Frontières; CDC response teams; the United Nations Children's Fund; the International Federation of Red Cross; Institut Pasteur; the European Mobile Laboratory; the Kenema Government Hospital Viral Hemorrhagic Fever Laboratory; the Liberia Institute of Biomedical Research; African Field Epidemiology Network; Elizabeth Ervin, Viral Special Pathogens Branch, National Center for Emerging and Zoonotic Infectious Diseases, CDC.

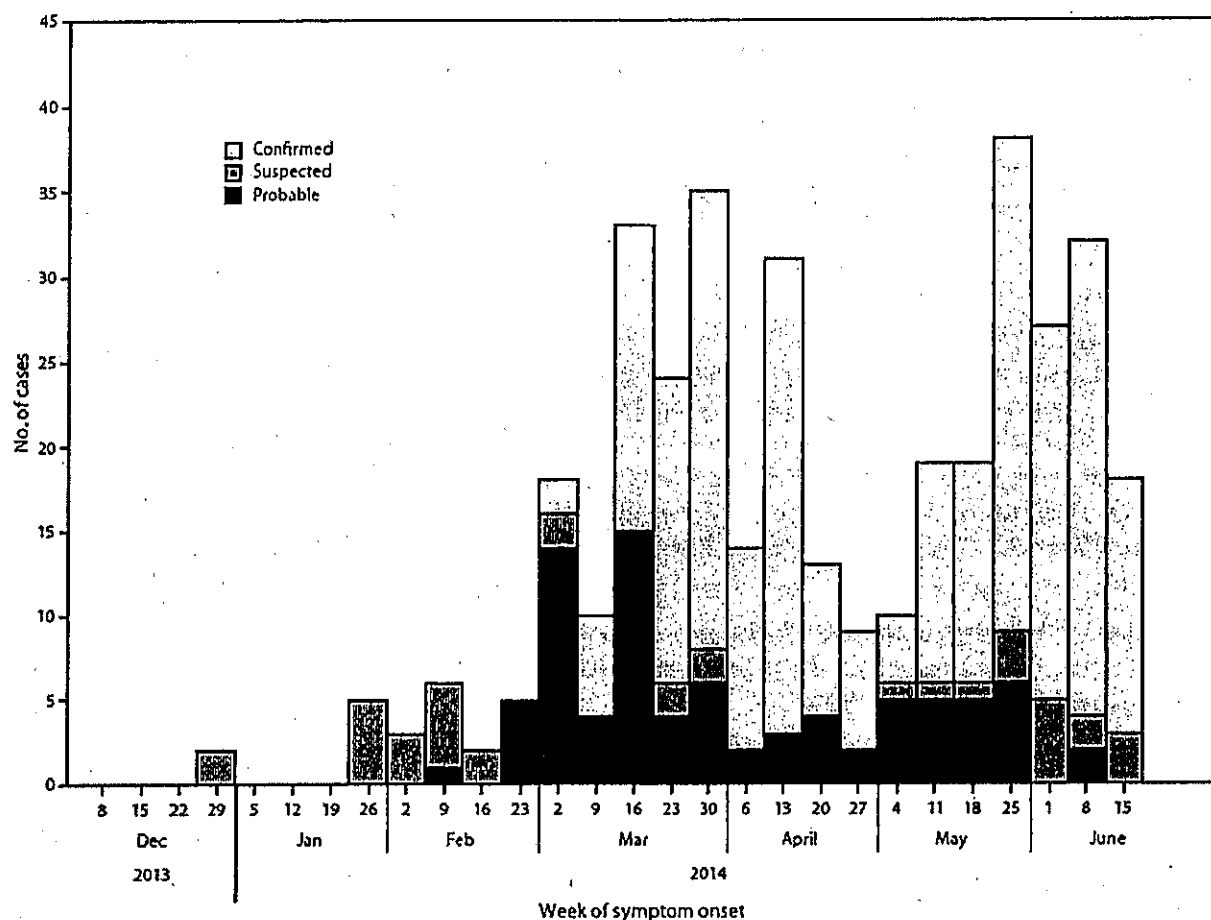
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* Case definitions were modified from those available at <http://who.int/csr/resources/publications/ebola/ebola-case-definition-contact-en.pdf>.

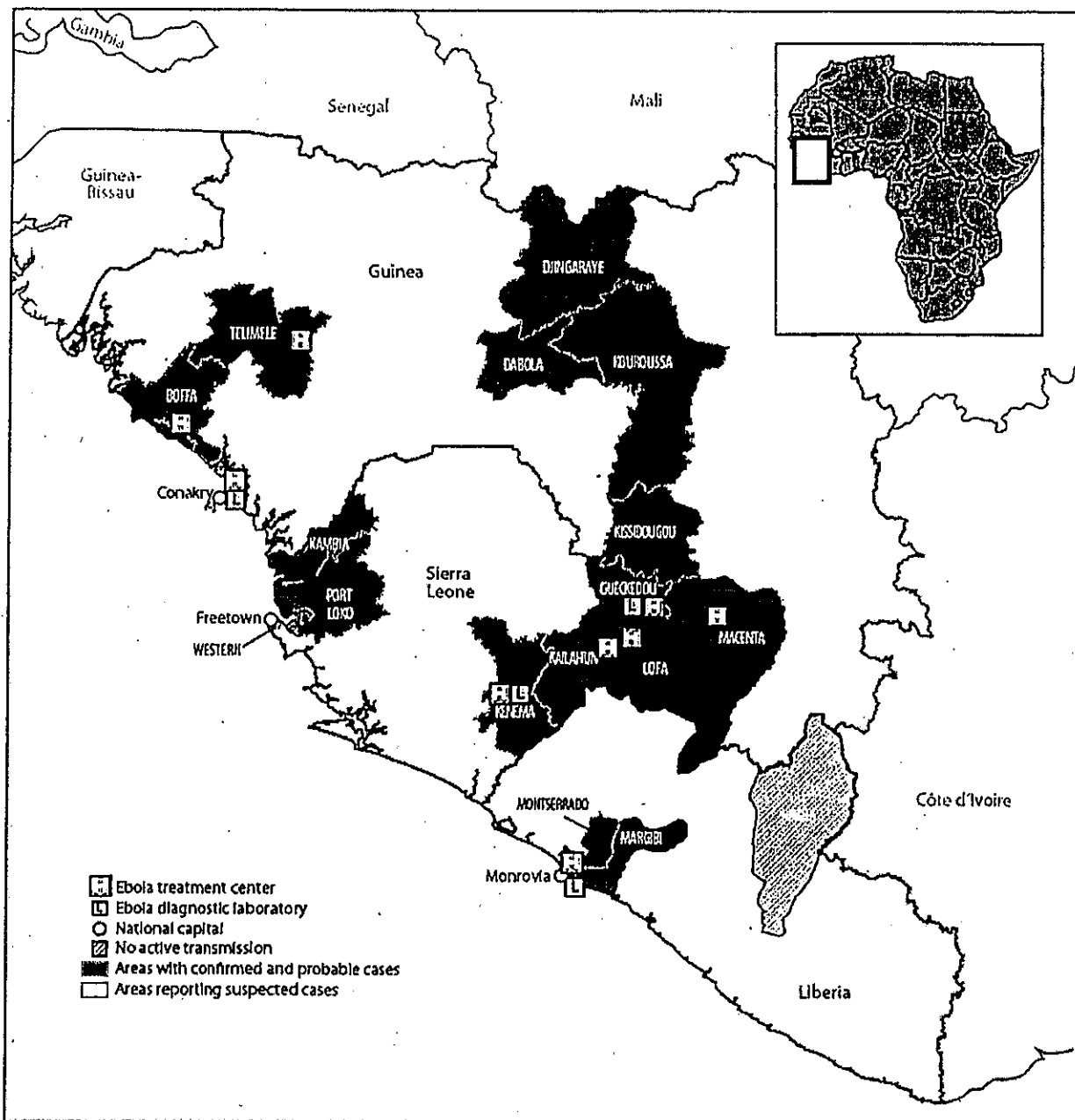
FIGURE 1. Number of cases of Ebola viral disease (n = 398*), by week of symptom onset — Guinea, 2014



* Cases reported as of June 18, 2014.

Alternate Text: The figure above is a bar chart showing the number of cases of Ebola viral disease (EVD) in the ongoing outbreak that were reported from Guinea, by week of symptom onset in during 2014. Although cases also were reported from Liberia and Sierra Leone, as of June 18, 2014, the majority (398) of the 528 total EVD cases had been reported from Guinea.

FIGURE 2. Location of cases of Ebola viral disease* — West Africa, 2014



* Cases reported as of June 18, 2014.

Alternate Text: The figure above is a map of West Africa, showing the wide geographic spread of cases of Ebola viral disease during the ongoing outbreak. As of June 18, 2014, a total of 528 cases, including 337 deaths, had been reported from Guinea, Liberia, and Sierra Leone.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2014. 3. 4	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	解凍人赤血球液	研究報告の公表状況	Tappe D, Rissland J, Gabriel M, Emmerich P, Gunther S, Held G, Smola S, Schmidt-Chanasit J. Euro Surveill. 2014 Jan 30;19(4). pii: 20685.	公表国 ドイツ	使用上の注意記載状況・ その他参考事項等 解凍赤血球液-LR「日赤」 照射解凍赤血球液-LR「日赤」 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク
販売名(企業名)	解凍赤血球液-LR「日赤」(日本赤十字社) 照射解凍赤血球液-LR「日赤」(日本赤十字社)	○ヨーロッパで初めて確認されたジカウイルス(ZIKV)感染症の輸入症例 50歳代前半のドイツ人男性が、タイ旅行から帰国した後の2013年11月22日に、デング熱様症状を呈し、第三次救急病院を受診した。デングウイルス(DENV)感染症が疑われ、抗体検査を行ったところ、DENV-IgM抗体は陽性であったが、IgG抗体、NS-1抗原検査では陰性であったため、他のフラビウイルス(日本脳炎ウイルス、ウエストナイルウイルス、黄熱ウイルス、ダニ媒介脳炎ウイルス、ZIKV)及びチクングニヤウイルスに対する血清学的検査を行った。その結果、患者の血液からZIKV-IgM抗体、IgG抗体が検出され、またZIKV特異的中和抗体の存在も確認されたため、ZIKV感染症と診断された。 ZIKVの媒介昆虫であるネッタイシマカ及びヒトスジシマカが存在する地域では、ウイルス血症状態の旅行者によりZIKVが国内に持ち込まれ、国内感染リスクが増大する可能性がある。			
研究報告の概要					
報告企業の意見		今後の対応			
タイ旅行から帰国したドイツ人旅行者に、ヨーロッパで初の輸入ZIKV感染症が確認されたとの報告である。		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、発熱などの体調不良者を献血不適としている。今後引き続き、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。			

9

RAPID COMMUNICATIONS

First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013

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In November 2013, an acute Zika virus (ZIKV) infection was diagnosed in a German traveller returning from Thailand. The patient reported a clinical picture resembling dengue fever. Serological investigations revealed anti-ZIKV-IgM and -IgG, as well as ZIKV-specific neutralising antibodies in the patient's blood. In Europe, viraemic travellers may become a source of local transmission of ZIKV, because *Aedes albopictus* (Skuse) and *Ae. aegypti* (Linnaeus) are invasive mosquitoes and competent vectors for ZIKV.

We report the clinical and laboratory findings of a Zika virus (ZIKV) infection imported into Europe by a German traveller from Thailand, in winter of 2013.

Case description

A previously healthy German traveller in his early 50s was seen at a tertiary hospital, Germany, on 22 November 2013, after returning from a vacation in Thailand. During the patient's three-week round trip (in early November) which included visits to Phuket, Krabi, Ko Jum, and Ko Lanta, he developed joint pain and swelling of his left ankle and foot on 12 days after entering the country. Pain and swelling was followed by a maculopapular rash on his back and chest that later spread to the face, arms, and legs over a period of four days before fading. Concomitantly, the patient suffered from malaise, fever (self-reported), and chills. Fever and shivering were treated by self-medication with non-steroidal anti-inflammatory drugs and only lasted for one day. The patient had noted several mosquito bites previously, despite using insect repellents regularly. He had sought pre-travel advice and his travel partner did not have any symptoms and also did not develop any.

Upon return to Germany, the patient was asymptomatic except for the subjective complaint of ongoing exhaustion. Physical examination was normal and no particular treatment was initiated. Laboratory parameters 10 days after disease onset revealed a slightly increased

C-reactive protein level (5.9 mg/L; normal value <5.0), a normal leucocyte count of 8,200 g/μL (45% lymphocytes, 5% monocytes, and a mildly decreased relative neutrophil count of 47% (normal range: 50–75%)). Platelet count was normal with 238,000 g/μL. Lactate dehydrogenase levels were elevated (311 U/L; normal <262 U/L), with an increased plasma fibrinogen concentration (422 mg/dL; normal range: 180–400 mg/dL) and serum ferritin concentration (486 ng/mL; normal range: 30–400). Serum electrophoresis, clotting tests, kidney and liver function tests were normal except for an increased gamma-glutamyltransferase activity of 81 U/L (normal <60 U/L).

A serum sample from the same day (10 days after symptom onset) showed a positive result for anti-dengue virus (DENV)-IgM in both the indirect immunofluorescence assay (IIFA), according to [1–3]) and rapid test (SD BIOLINE Dengue Duo NS1 Ag + Ab Combo). However, anti-DENV-IgG was not detected in either test. Testing for DENV nonstructural protein-1 (NS1) antigen (tested by enzyme-linked immunosorbent assay (ELISA): Bio-Rad Platelia Dengue NS1 Ag) and rapid test (SD BIOLINE Dengue Duo NS1 Ag + Ab Combo) were also negative. The detection of isolated anti-DENV-IgM prompted us to investigate a probable flavivirus etiology other than DENV of the patient's illness. Serological tests for Japanese encephalitis virus (JEV), West Nile virus (WNV), yellow fever virus (YFV), tick-borne encephalitis virus (TBEV), and ZIKV were performed according to [1–3] and the IIFAs showed only positive results for anti-ZIKV-IgM and -IgG antibodies (Table), demonstrating an acute or recent ZIKV-infection of the patient. Serological tests for chikungunya virus (CHIKV) were negative (Table).

ZIKV-specific real-time reverse transcription-polymerase chain reaction (RT-PCR) (in-house) with primers ZIKAF (5'-TGGAGATGAGTACATGTATG-3'), ZIKAR (5'-GGTAGATGTTGCAAGAAG-3'), probe – labeled with 6-carboxyfluorescein (FAM) and black hole quencher 1

TABLE

Serological results of a case of Zika virus infection from Thailand imported into Germany, November 2013

Antibody or antigen tested	Serum samples taken after symptom onset (days)		
	10	31	67
Anti-ZIKV-IgG ^a	1:5,120	1:2,560	1:2,560
Anti-ZIKV-IgM ^a	1:10,240	1:2,560	1:320
Anti-DENV-IgG ^a	<1:20	1:80	1:160
Anti-DENV-IgM ^a	1:40	<1:20	<1:20
DENV NS1 ^b	Negative (0.1 arbitrary units)	Negative (0.2 arbitrary units)	Negative (0.1 arbitrary units)
Anti-JEV-IgG ^a	<1:20	1:40	1:20
Anti-JEV-IgM ^a	<1:20	<1:20	<1:20
Anti-WNV-IgG ^a	<1:20	1:20	1:80
Anti-WNV-IgM ^a	<1:20	<1:20	<1:20
Anti-YFV-IgG ^a	<1:20	<1:20	1:20
Anti-YFV-IgM ^a	<1:20	<1:20	<1:20
Anti-CHIKV-IgG ^a	<1:20	<1:20	<1:20
Anti-CHIKV-IgM ^a	<1:20	<1:20	<1:20

CHIKV: chikungunya virus; DENV: dengue virus; JEV: Japanese encephalitis virus; NS1: nonstructural protein-1; WNV: West Nile virus; YFV: yellow fever virus; ZIKV: Zika virus.

^a Indirect immunofluorescence assay (IIFA) titres <1:20 for serum were considered negative [1-3].

^b SD BIOLINE Dengue Duo NS1 Ag + Ab Combo and Bio-Rad Platelia Dengue NS1 Ag.

(BHQ-1) – ZIKAp (5'-FAM-CTGATGAAGGCCATGCACACTG-BHQ1-3') was negative on serum. Generic flavivirus real-time RT-PCR [4] was negative as well on serum. A significant 5-fold anti-ZIKV-IgM titre decrease in the IIFA was demonstrated in the third serum sample collected 67 days after disease onset (Table). The presence of ZIKV-specific neutralising antibodies in the third serum sample was confirmed by a virus neutralisation assay. No laboratory investigation was conducted with the travel partner.

Background

ZIKV is a mosquito-borne RNA virus of the *Flaviviridae* family causing a dengue fever-like syndrome in humans. The virus was first isolated in 1947 from a febrile sentinel rhesus monkey in the Zika Forest of Uganda [5]. ZIKV virus is thought to be maintained in a sylvatic cycle involving non-human primates and several *Aedes* species (*Ae. africanus*, *Ae. aegypti*, and others) as mosquito vectors [6-8]. Human infection is acquired after an infective mosquito bite in endemic countries. However, the possibility of a secondary sexual transmission has been reported recently [9]. The virus is endemic in Africa and south-east Asia [8], and phylogenetic analysis suggested that African and Asian strains emerged as two distinct lineages [10-11]. ZIKV has caused an outbreak involving 49 confirmed and 59 probable cases on Yap Island, Federated States of Micronesia, in 2007 [12]. This outbreak highlighted the potential of the virus as an emerging pathogen [9], and epidemiological and phylogenetic studies provided

evidence that the outbreak strain has been introduced from south-east Asia [10].

The most common signs and symptoms of ZIKV infection are rash, fever, arthralgia, myalgia, headache, and conjunctivitis. The rash is most often maculopapular. Occasionally, oedema, sore throat, cough, vomiting, and loose bowels are reported [11-13]. ZIKV infection can easily be confused with dengue and might be misdiagnosed during local dengue outbreaks [8]. ZIKV-associated illness may thus be underreported or misdiagnosed [9].

In contrast to acute dengue cases, our patient neither showed elevated aspartate amino transferase (AST) or alanine amino transferase (ALT) levels, nor thrombocytopenia. It is unclear whether these test results may help in differentiating ZIKV from dengue cases, as information about laboratory data during ZIKV infection is very scarce. An Australian case [11] did not show thrombocytopenia or elevated liver function tests either. It was reported recently that a low platelet count is a key variable distinguishing between dengue versus chikungunya [14], the latter being another mosquito-borne virus infection with similar clinical presentation and geographical distribution. Chikungunya is thus also an important differential diagnosis for ZIKV disease and future studies might address this issue for ZIKV.

Despite the virus endemicity in many geographical areas and its potential to cause outbreaks, imported cases to non-endemic areas are rarely reported. In 2013, one imported case from Indonesia to Australia and one imported case from Thailand to Canada were diagnosed in travellers [11,15]. Also in the Australian and Canadian cases, anti-DENV-IgM was positive and DENV NS1 antigen testing was negative. In both cases, ZIKV infection was diagnosed after sequencing of a positive generic flavivirus RT-PCR amplicon. Four further cases of imported ZIKV to temperate regions have been reported in American scientists who had returned from Senegal and in Japanese travellers who returned from French Polynesia, where a ZIKV outbreak is currently ongoing [16,17]. A secondary infection in the wife of one of the American patients was assumed to be due to sexual contact [9]. The ZIKV outbreak in French Polynesia so far comprises more than 361 laboratory-confirmed cases [18]. The first indigenous infection in New Caledonia was recently reported suggesting the spread of ZIKV, as 26 imported cases of ZIKV infection from French Polynesia have been observed in this territory [19].

Conclusions

This report constitutes, to the best of our knowledge, the first laboratory-confirmed case of a ZIKV infection imported into Europe. The case highlights that unusual DENV serology results might be caused by a flavivirus different than DENV despite a similar clinical picture. A serological study after the Yap outbreak indicated that ZIKV-infected patients can be positive in anti-DENV-IgM assays [20], as also experienced in our case. This cross-reaction in the Yap outbreak was seen especially if ZIKV was a secondary flavivirus infection. These findings underscore the importance of a careful diagnostic investigation in travellers suspected with dengue, and the well-known serological cross-reactions in the flavivirus group. Thus, the rate at which seemingly imported dengue cases among travellers from endemic areas in the recent years were actually ZIKV infections remains a question.

In all published cases of imported ZIKV infections, in outbreak and sporadic endemic cases, the symptoms were dengue-like. Clinicians, virologists, and public health authorities should thus be aware of this emerging flavivirus infection. As the local transmission of DENV by previously introduced competent vectors in non-endemic countries has recently been reported from Croatia, France and Madeira [2,21,22], there might be the risk of a similar establishment in Europe of ZIKV, after import by viraemic travellers, in particular in areas where ZIKV competent vectors *Ae. albopictus* and *Ae. aegypti* are present.

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Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: JSC, SG, DT, JR, SS, GH; performed laboratory or epidemiological investigations: JSC, PE, MG, JR, GH, DT; performed data analysis: JSC, PE, JR, GH.

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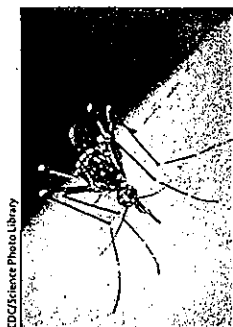
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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2014. 3. 6	新医薬品等の区分 該当なし		総合機構処理欄 10
一般的名称		解凍人赤血球液		公表国 米国		
販売名(企業名)		解凍赤血球液-LR「日赤」(日本赤十字社) 照射解凍赤血球液-LR「日赤」(日本赤十字社)		研究報告の公表状況 Leparc-Goffart I, Nougairède A, Cassadou S, Prat C, de Lamballerie X. Lancet. 2014 Feb 8;383(9916):514. doi: 10.1016/S0140-6736(14)60185-9.		
研究報告の概要		使用上の注意記載状況・ その他参考事項等 解凍赤血球液-LR「日赤」 照射解凍赤血球液-LR「日赤」 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク				
報告企業の意見		今後の対応				
西インド諸島においてチクングニヤウイルス感染症が広が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。				

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there is long-standing evidence for this intentional, unprovoked violence.³ The assault might fulfil certain psychological needs for the assailant, despite the random victim selection.⁴ There is additional concern that some attacks are targeted hate crimes, which has prompted community leaders and anti-crime groups to convene and discuss comprehensive mitigation strategies.⁵ Public awareness of the knockout game and further understanding of assailants' motives are crucial to prevent future assaults, injuries, and deaths.

We declare that we have no conflicts of interest.

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See Online for appendix

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Chikungunya in the Americas

See Editorial page 488

Chikungunya is an arthropod-borne disease transmitted by aedes mosquitoes, characterised by febrile arthralgia and responsible for devastating outbreaks. No licensed

vaccine or specific treatment is currently available. Since 2005, the East Central South African (ECSA) genotype of chikungunya virus (genus Alphavirus, family Togaviridae) has been responsible for more than 1 million human cases in the Indian Ocean islands, the Indian subcontinent, southeast Asia, and Africa, and some autochthonous cases in Europe.¹ The virus, previously thought to be transmitted by *Aedes aegypti* mosquitoes, has repeatedly been associated with a new vector, *Aedes albopictus*. This vector change represents a unique example of evolutionary convergence: in independent instances the virus acquired an adaptive mutation in its envelope gene that favoured replication in *A. albopictus*.^{2,3}

On Dec 5, 2013, the French National Reference Centre for arboviruses confirmed autochthonous chikungunya cases on Saint Martin Island, French West Indies (appendix). On Dec 20, about 50 confirmed cases were reported with additional evidence for autochthonous transmission on Martinique island. In January, 2014, autochthonous cases have been reported also from Guadeloupe, Saint Barthelemy, Dominica, and the British Virgin Islands.

A. aegypti is the unique potential vector locally. The full-length viral RNA genome was characterised directly from the serum of two viraemic patients using next-generation sequencing methods. The virus does not belong to the ECSA genotype but to the Asian genotype, identified in late 1950s in southeast Asian countries. It is phylogenetically related to strains recently identified in Asia (in Indonesia in 2007, in China in 2012, and in the Philippines in 2013), most of them sharing a specific four-aminoacid deletion in the NSP3 gene.⁴ The virus is only distantly related to the Asian genotype virus that recently

circulated in New Caledonia, which harbours different aminoacid deletion in the NSP3 gene.

This episode represents the first evidence for the emergence of autochthonous chikungunya cases in the Americas. It is likely that the chikungunya epidemic will extend to other Caribbean islands, and it also has substantial potential for spreading from this region visited yearly by millions of tourists to the American mainland where *A. aegypti* is endemic. Assuming that this strain will be transmitted efficiently by *A. albopictus* mosquitoes, its persistence in the Caribbean would also represent, as a consequence of seasonal synchronicity,⁵ a great threat for southern European countries where the mosquito has recently dispersed. This situation warrants reinforced epidemiological surveillance and specific preparedness.

We declare that we have no conflicts of interest.

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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 2014年05月09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	人ハプトグロビン		研究報告の 公表状況		公表国 アメリカ	11
販売名 (企業名)	ハプトグロビン静注2000単位「ベネシス」 (日本血液製剤機構)		www.cdc.gov/mmwr/preview/mmwr.html/2014/05/09			
第1期および第2期梅毒—米国、2005～2013年						
2014年4月28日時点で報告されたデータによれば、2013年に米国で報告された第1期および第2期梅毒の罹患率は人口10万人当たり5.3例であり、罹患率もほとんど低かった2000年の2.1例の2倍を超えていた。CDCは、米国における梅毒の最近の疫学的特徴を明らかにするために、全国届出疾病監視システム（NNDSS）から得られた第1期および第2期梅毒の2005～2013年に診断された症例に関するデータを分析したが、ここでは同性愛者、同性愛者および男性と性交渉があったその他の男性（MSMと総称される）の間で報告された梅毒を説明するために、2009～2012年中の性交渉相手の性別が報告されている州に注目した。2005～2013年の間、第1期および第2期梅毒の罹患率は米国全域において全ての年齢および人種/民族の間で上昇していた。ここ数年、症例数は加速的に増加しているが、増加が最も顕著なのはMSMである。女性については、罹患率は2005～2008年は上昇し、2009～2013年は低下したが、その傾向は人種/民族群の間で異なっている。報告された梅毒における人種/民族間の不均衡は2005～2013年を通じて続いていたが、これはコミュニティにおける梅毒の負荷の一因となりうる社会経済的状況といった健康の社会的決定因子を反映したものである可能性が高い。これらの所見は、MSMに対して継続的な梅毒予防策が必要であることを強く示している。 CDCでは、2005～2013年の間に診断された症例について全国の保健局からNNDSSに報告された梅毒症例に関する届出疾病調査データを、これには患者の地理的背景および梅毒の病期（即ち、第1期および第2期、早期潜伏、晩期潜伏および先天性）も含めて分析した。1年毎の第1期及び第2期梅毒（ごく最近になって被った感染であることを示す）の傾向を2005～2013年について分析した。米国勢調査地域、年齢群およびNNDSSの人種/民族カテゴリー（白人、黒人、ヒスパニック、アジア/太平洋諸国およびアメリカンインディアン/アラスカ原住民、何れの人種群も非ヒスパニック系）別に地理的傾向を分析した。更に、MSM間の梅毒を説明するために、男性の第1期および第2期梅毒症例の70%以上で性交渉相手の性別が報告されている（CDCは性交渉相手の性別に関するデータの収集を2005年に開始した）34の州およびコロンビア特別区から報告された2009～2012年の間のMSMの第1期および第2期梅毒の1年間の症例について検討した。罹患率の計算には、米国勢調査局から得た人口を分母として用いた。 2005～2013年で、米国で各年に報告された第1期および第2期梅毒の症例数は8,724例から16,663例へと2倍近くまで増加した；年間の罹患率は人口10万人当たり2.9例から5.3例へと上昇した（表）。症例の増加は男性であり、2013年における第1期および第2期梅毒の症例全体の91.1%を占めていた。男性における罹患率は、2005年の5.1例から2013年には9.8例へ上昇している（図1）。増加は全ての年齢および人種/民族の男性で認められたが、2009年には人種/民族のシフトが発生した。2005～2009年では、罹患率の上昇は黒人男性（104.1%、2005年の14.6例から2009年の29.8例）でヒスパニック系男性（52.0%、5.0～7.6）および白人男性（19.4%、3.1～3.7）よりも大きかった。2009～2013年には、罹患率はヒスパニック系男性（52.6%、2009年の7.6例～2013年の11.6例）および白人男性（45.9%、3.7～5.4）で上昇したが、黒人男性（6.4%、29.8～27.9）ではわずかに低下した。2005～2009年では年齢20～24歳の男性（149.4%、8.1～20.2）で、また2009～2013年には年齢25～29歳の男性（ほぼ同じころ出生した集団）で（48.4%、18.2～27.0）、罹患率もほとんど大きく上昇した（表）。2012年には、男性症例の70%以上について性交渉相手の性別を報告した35地域における第1期および第2期梅毒の症例数が米国全体の症例の83.7%（13,113例）を占めていた。これらの地域では、MSMの第1期および第2期梅毒症例が男性症例全体を占める比率が2009年の77.0%（6,366）から2012年には83.9%（8,701）まで上昇していた。罹患率の上昇は全ての地域において、全ての年齢および人種/民族MSM間で認め						
研究報告の概要						
使用上の注意記載状況・その他参考事項等						
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人、ハプトグロビンを濃縮・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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認められた。黒人 (21.2%、2009 年の 2,267 例から 2012 年の 2,747 例) に比べて症例数の増加率が最も大きかったのはヒスパニック (53.4%、1,291~1,980) および白人 (38.1%、2,449~3,381) であった (図 2)。年齢群別にみると、増加率が最も高かったのは年齢 25~29 歳の MSM であった (53.2%、1,073~1,644)。 女性の第 1 期および第 2 期梅毒の報告率は、2005~2008 年の間は毎年、人口 10 万人当たり 0.9 から 1.5 例まで上昇したが、2013 年には 0.9 まで低下した。この傾向はすべての年齢群の女性に認められた。白人およびヒスパニック系女性の罹患率は安定を保っていた；女性全体の傾向は、概ね黒人女性における変化を反映したものであった (2005~2009 年間に 4.2 から 7.9 まで上昇、2013 年に 4.0 まで低下) (図 1)。梅毒における人種/民族の不均衡は持続的なものであった。2013 年における黒人男性間の第 1 期および第 2 期梅毒の罹患率は白人男性の 5.2 倍であった (人口 10 万人当たり 27.9 例に対して 5.4 例)；黒人女性間の罹患率は白人女性の 13.3 倍であった (4.0 に対して 0.3)。ヒスパニック系男性の罹患率は白人男性の 2.1 倍 (11.6 に対して 5.4)、またヒスパニック系女性の罹患率は白人女性の 2.7 倍 (0.8 に対して 0.3) であった。このような不均衡は 2005 年に認められたものと同様であった (表)。 米国の 4 つの国勢調査地域全てを通じて、第 1 期および第 2 期梅毒の罹患率は 2005 年よりも 2013 年のほうが高かった。2013 年において梅毒全体の罹患率が最も高かったのは西部地域であった (人口 10 万人当たり 6.5 例)。2013 年において南部地域は梅毒全体の罹患率が最も高い地域とならなかったが、これは過去 50 年以上で初めてのことであった。人種/民族別の男性および女性における地域的な傾向は、2009~2013 年の間に黒人男性の罹患率が低下しなかった西部地域を除けば、国全体の傾向を反映していた。西部地域のすべての人種/民族の女性で、報告率は 2005~2010 年の間は低下し、2011~2013 年の間は上昇した (表)。	考 察 米国において、梅毒は 2000 年に根絶目前となった後、患者が再び増加した。2005~2013 年の間は、第 1 期および第 2 期梅毒の罹患率が全体的に上昇を続けた；罹患率は 2009~2010 年間は安定していたが、2011 年以降は上昇した。上昇が認められたのは主に男性、特に 2009~2012 年には第 1 期および第 2 期梅毒の男性症例の大部分を占めていた MSM であった。 MSM も含めた男性における梅毒の疫学は 2009 年以降にシフトし、ヒスパニック系男性および白人男性において大きく増加した。このような増加に関わらず、黒人男性とその他の人種/民族群間の第 1 期および第 2 期梅毒における不均衡は大きなままである。保健局への報告の遅延、医師が性交渉相手への通知を患者に依存すること、また MSM は異性愛者に比べて性交渉相手に通知する比率が低い傾向があることなど、性交渉相手に接触し、性交渉相手を治療することには多くの障壁が存在している。 これらの分析は、全ての人種/民族の MSM に対する梅毒の予防策を米国全体で強化する必要があることを示している。MSM の第 1 期および第 2 期梅毒のさきわめて多くの症例が個人病院の医師から報告されているため、このことは個人的な医療従事者とともに取り組むことで達成可能と考えられる。更に、個人および公共の医療従事者は梅毒の再流行を意識し、梅毒の徴候および症状を認識し、リスク評価を実施し、また性的に活動的な MSM については血清学的検査による梅毒のスクリーニングを年に 1 回以上、必要であれば確定検査を併せて実施することが可能でなければならない。複数または不特定の性交渉相手を有する MSM については、より頻繁なスクリーニング検査 (すなわち 3~6 カ月おき) が勧められる。一部の MSM については、性行為の開示が困難なままである；従って、医療従事者は患者の性的前歴を性的指向、性別認識および性交渉相手の性別など、文化的に適切な方法で引き出すことが勧められる。その他の情報源および達成のための訓練法は、オンラインで閲覧可能である。 MSM 間での梅毒の増加は公衆衛生上大きな問題であるが、それは特に梅毒と梅毒に感染する行動がヒト免疫不全ウイルス (HIV) の感染と伝播の増加に関連するためである。第 1 期および第 2 期梅毒に感染した MSM における HIV 重感染率は 50~70%、また第 1 期および第 2 期梅毒感染後は HIV のセロコンバージョン率が高いことが報告されている。梅毒の再流行は、それが HIV と強く関係していることと併せて、予防プログラムの必要性、また医療従事者が 1) より安全な性行為を促すこと (例えば性交渉相手の数を減らす、ラテックス・コンドームの使用、性感染症の検査結果が陰性の相手と長期にわたり互いに 1 対 1 の関係を持つこと)；2) 梅毒に対する認知を促進し、淋菌、クラミジアおよび HIV 感染の適切なスクリーニング検査に加えて梅毒のスクリーニング検査を促進すること；3) 性交渉相手に通知し、性交渉相手を治療することの必要性を強く示している。 公衆衛生当局は、性交渉相手の性別に関するデータの品質を改善し、国内の法律と規則に即して各地域の MSM、性感染症および HIV に関するデータを医療従事者と共有して、医療従事者の地域における梅毒の負担に対する認識を高め、また医療従事者が梅毒の症状を認識できるよ
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うにしなければならぬ。CDCの2つの共同契約が地方および国の参加者に対し、国内の疫学に基づいてMSMを優先集団および最も必要な領域の直接の情報源とすることを推奨している。CDCは州および地域の協力者、医療従事者およびMSM向けの組織と協力し、MSM間の梅毒に関するリスク因子の認識を向上させ、MSM集団へのより良い教育と奉仕を目指した良好なケアモデルを開発し、MSMが検査を受け、また適切な治療を受けているかを評価し、MSMにおいて梅毒の診断と治療を受ける上でどんな抵抗を感じているかを判断するための研究に取り組んでいる。 2008年以降、黒人女性の間で第1期および第2期梅毒が減少し続けていることは明らかな話題であり、特定の集団において梅毒を抑制するための目的を絞った努力の成功を示すものと考えられる。現時点では、第1期および第2期梅毒は主にMSMで流行しているが、子宮に感染した梅毒は死産など重篤な結果をもたらすことから、異性愛者の男性および女性において梅毒予防の努力が続けられていることが大切である。 本報告書における所見には少なくとも2つの欠点がある。第一に、第1期および第2期梅毒の症例報告書のデータは、診断された症例の過少申告、感染しても受診しない患者がいること、誤診、また第1期および第2期梅毒の症例が2012年において報告された梅毒症例全体の31.4%に過ぎないという事実などのために、米国における梅毒感染患者数を実際より少なく見積もっている可能性が高い。第二に、MSMに関する所見には、男性の第1期および第2期梅毒症例の70%以上で性交渉相手の性別が報告されている34の州およびコロンビア特別区からのデータのみが含まれていた。これら35の報告地域の症例の12%については、性交渉相手の性別が不明であった。 米国では1990年代後半に第1期および第2期梅毒の報告率が低下したが、最近の症例の再増加はこの難題がまだ消えていないという事実、またMSM間での増加が特に問題であることを強く示している。公衆衛生担当者は臨床医との関係を強化し、症例の同定と報告、性交渉相手への通知プログラムおよびMSMへの支援の改善を目指した努力に重点を置くべきと考えられる。	
報告企業の意見	
梅毒トレポネーマ (Treponema pallidum) はスピロヘータ科 (Spirochaetaceae) トレポネーマ属 (Treponema) の一種で、直径：0.1～0.2μm、長さ：6～20μmの6～14旋回の螺旋状をした細菌。低温保管や凍結乾燥、加熱処理により死滅するとされている。そのため、万一原料血漿に梅毒トレポネーマが混入したとしても、製造工程において滅菌または除菌されると考えている。	今後の対応 本報告は本剤の安全性に影響を与えないと考えられるので、特段の措置はとらない。

Primary and Secondary Syphilis — United States, 2005–2013

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In 2013, based on data reported as of April 28, 2014, the rate of reported primary and secondary syphilis in the United States was 5.3 cases per 100,000 population, more than double the lowest-ever rate of 2.1 in 2000. To characterize the recent epidemiology of syphilis in the United States, CDC analyzed data from the National Notifiable Diseases Surveillance System (NNDSS) for cases of primary and secondary syphilis diagnosed during 2005–2013 with a focus on states that reported the sex of sex partners during 2009–2012 to describe reported syphilis among gay, bisexual, and other men who have sex with men (collectively referred to as MSM). During 2005–2013, primary and secondary syphilis rates increased among men of all ages and races/ethnicities across all regions of the United States. Recent years have shown an accelerated increase in the number of cases, with the largest increases occurring among MSM. Among women, rates increased during 2005–2008 and decreased during 2009–2013, with different trends among different racial/ethnic groups. Racial/ethnic disparities in reported syphilis persisted during 2005–2013, likely reflecting social determinants of health, such as socioeconomic status, that might contribute to the burden of syphilis in a community (1). These findings underscore the need for continued syphilis prevention measures among MSM.

CDC analyzed notifiable disease surveillance data on reported syphilis, including patient demographics and stage of syphilis (i.e., primary and secondary, early latent, late, late latent, and congenital*) reported by health departments to NNDSS nationwide for cases diagnosed during 2005–2013. Trends in annual primary and secondary syphilis (representing more recently acquired infections, which usually are infectious) were analyzed for 2005–2013. Geographic trends were analyzed by U.S. census region, age group, and NNDSS racial/ethnic categories (white, black, Hispanic, Asian/Pacific Islander, and American Indian/Alaska Native, for which all racial groups were non-Hispanic). In addition, to describe syphilis among MSM, annual cases of primary and secondary syphilis among MSM that were reported during 2009–2012 were reviewed from 34 states and the District of Columbia, where the sex of sex partners was reported for ≥70% of male primary and secondary syphilis cases (CDC began collecting

data on the sex of sex partners in 2005). Rates were calculated using population denominators from the U.S. Census Bureau.

During 2005–2013, the number of primary and secondary syphilis cases reported each year in the United States nearly doubled, from 8,724 to 16,663; the annual rate increased from 2.9 to 5.3 cases per 100,000 population (Table). Men contributed an increasing proportion of cases, accounting for 91.1% of all primary and secondary syphilis cases in 2013. The rate among men increased from 5.1 in 2005 to 9.8 in 2013 (Figure 1). Increases occurred among men of all ages and races/ethnicities, but race/ethnicity shifts occurred in 2009. During 2005–2009, rate increases were greatest among black men (104.1%, from 14.6 in 2005 to 29.8 in 2009) compared with Hispanic men (52.0%, 5.0 to 7.6) and white men (19.4%, 3.1 to 3.7). During 2009–2013, rates increased among Hispanic men (52.6%, from 7.6 in 2009 to 11.6 in 2013) and white men (45.9%, 3.7 to 5.4), but decreased slightly among black men (6.4%, 29.8 to 27.9). From 2005 to 2009, men aged 20–24 years had the greatest percentage increase (149.4%, 8.1 to 20.2), and from 2009 to 2013, men aged 25–29 years (the same approximate birth cohort) had the greatest increase (48.4%, 18.2 to 27.0) (Table).

In 2012, primary and secondary syphilis cases in the 35 reporting areas that reported the sex of sex partners for ≥70% of male cases comprised 83.7% (13,113) of all nationwide cases. In those areas, the proportion of male primary and secondary syphilis cases attributed to MSM increased from 77.0% (6,366) in 2009 to 83.9% (8,701) in 2012. Increases in incidence occurred among MSM of all ages and races/ethnicities from all regions. The greatest percentage increases in cases occurred among Hispanics (53.4%, from 1,291 in 2009 to 1,980 in 2012) and whites (38.1%, 2,449 to 3,381), when compared with blacks (21.2%, 2,267 to 2,747) (Figure 2). By age group, the greatest percentage increases occurred among MSM aged 25–29 (53.2%, 1,073 to 1,644).

Among women, the reported primary and secondary syphilis rate increased from 0.9 to 1.5 per 100,000 population per year during 2005–2008 and decreased to 0.9 in 2013. This trend occurred among women in all age groups. Rates among white and Hispanic women remained stable; the trend among all women mostly reflected changes in rates among black women (from 4.2 to 7.9 during 2005–2009, decreasing to 4.0 in 2013) (Figure 1).

* Additional information available at <http://wwwn.cdc.gov/nndss/script/casedef.aspx?condyrid=941&datepub=1/1/2014>.

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TABLE. Number and rate* of primary and secondary syphilis cases, by race/ethnicity, U.S. Census region,[†] age group, and sex — National Notifiable Diseases Surveillance System, United States, 2005–2013

Characteristic	2005		2006		2007		2008		2009		2010		2011		2012		2013 [§]	
	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate
Males																		
Race/ Ethnicity																		
White, non-Hispanic	3,049	3.1	3,249	3.3	3,456	3.5	3,789	3.8	3,702	3.7	3,973	4.0	4,321	4.4	4,931	5.0	5,293	5.4
Black, non-Hispanic	2,607	14.6	3,086	17.1	3,952	21.7	4,876	26.7	5,535	29.8	5,236	27.8	5,074	26.6	5,369	27.8	5,383	27.9
Hispanic	1,111	5.0	1,304	5.7	1,627	6.9	1,844	7.6	1,904	7.6	2,119	8.3	2,182	8.3	2,889	10.7	3,118	11.6
A/PI	136	2.1	154	2.3	155	2.2	201	2.9	203	2.8	190	2.5	139	3.0	318	3.9	381	4.6
AI/AN	37	3.1	53	4.3	49	4.0	38	3.0	47	3.7	55	4.4	63	4.9	57	4.4	61	4.7
Region																		
Northeast	1,171	4.4	1,314	4.9	1,741	6.5	1,924	7.2	1,946	7.2	2,068	7.7	1,987	7.4	2,292	8.4	2,466	9.1
Midwest	1,059	3.3	1,020	3.1	1,113	3.4	1,485	4.5	1,638	5.0	1,944	5.9	1,926	5.8	1,987	6.0	2,311	7.0
South	3,170	6.0	3,619	6.8	4,378	8.1	5,008	9.1	5,657	10.2	5,029	9.0	5,164	9.1	5,904	10.3	5,933	10.3
West	1,983	5.8	2,340	6.7	2,537	7.2	2,838	8.0	2,523	7.0	2,940	8.2	3,376	9.3	4,007	10.9	4,465	12.2
Age group (yrs)[‡]																		
15–19	251	2.3	332	3.0	416	3.8	585	5.3	661	6.0	617	5.5	606	5.5	640	5.8	663	6.0
20–24	875	8.1	1,080	9.9	1,461	13.5	1,877	17.3	2,242	20.2	2,429	22.1	2,582	22.8	2,859	24.8	3,042	26.3
25–29	1,007	9.8	1,330	12.6	1,574	14.6	1,851	16.9	2,027	18.2	2,131	20.0	2,277	21.2	2,641	24.4	2,925	27.0
30–34	1,178	11.6	1,056	10.6	1,303	13.2	1,489	15.0	1,571	15.5	1,597	16.0	1,657	16.1	2,023	19.3	2,179	20.8
35–39	1,394	13.2	1,426	13.4	1,529	14.4	1,568	14.8	1,409	13.6	1,313	13.1	1,265	13.0	1,443	14.9	1,597	16.4
40–44	1,253	11.0	1,362	12.2	1,551	14.1	1,573	14.6	1,476	14.1	1,448	13.9	1,408	13.5	1,544	14.8	1,515	14.5
45–54	1,080	5.2	1,277	6.0	1,463	6.8	1,790	8.2	1,815	8.3	1,877	8.5	1,999	9.1	2,310	10.6	1,398	11.0
55–64	283	1.9	340	2.2	379	2.4	412	2.5	475	2.8	457	2.6	510	2.8	586	3.2	682	3.7
≥65	59	0.4	87	0.6	86	0.5	102	0.6	84	0.5	105	0.6	137	0.8	138	0.7	159	0.8
Total	7,383	5.1	8,293	5.6	9,769	6.6	11,255	7.5	11,764	7.8	11,981	7.9	12,453	8.1	14,190	9.2	15,175	9.8
Females																		
Race/ Ethnicity																		
White, non-Hispanic	263	0.3	295	0.3	370	0.4	474	0.5	418	0.4	299	0.3	261	0.3	274	0.3	293	0.3
Black, non-Hispanic	828	4.2	942	4.8	1,075	5.4	1,478	7.4	1,605	7.9	1,296	6.3	1,041	5.0	931	4.4	852	4.0
Hispanic	183	0.9	158	0.7	163	0.7	209	0.9	144	0.6	118	0.5	142	0.6	193	0.7	209	0.8
A/PI	11	0.2	10	0.1	7	0.1	9	0.1	15	0.2	11	0.1	12	0.1	14	0.2	21	0.2
AI/AN	19	1.5	22	1.7	32	2.5	18	1.4	12	0.9	9	0.7	6	0.5	8	0.6	13	1.0
Region																		
Northeast	91	0.3	86	0.3	96	0.3	100	0.4	130	0.5	125	0.4	116	0.4	125	0.4	115	0.4
Midwest	137	0.4	156	0.5	147	0.4	237	0.7	212	0.6	309	0.9	249	0.7	251	0.7	239	0.7
South	884	1.6	984	1.8	1,228	2.2	1,697	3.0	1,756	3.0	1,225	2.1	997	1.7	915	1.5	818	1.4
West	227	0.7	232	0.7	221	0.6	208	0.6	134	0.4	121	0.3	139	0.4	167	0.5	299	0.8
Age group (yrs)[‡]																		
15–19	192	1.9	233	2.2	248	2.4	318	3.0	344	3.3	313	2.9	258	2.5	238	2.3	202	1.9
20–24	305	3.0	299	2.9	356	3.5	520	5.1	570	5.5	474	4.5	403	3.7	417	3.8	429	3.9
25–29	205	2.1	241	2.4	265	2.6	404	3.9	377	3.6	322	3.1	268	2.5	266	2.5	272	2.6
30–34	150	1.5	163	1.7	193	2.0	244	2.5	286	2.9	197	2.0	187	1.8	182	1.7	164	1.6
35–39	179	1.7	154	1.5	191	1.8	241	2.3	203	2.0	140	1.4	115	1.2	120	1.2	121	1.2
40–44	164	1.4	153	1.4	192	1.7	202	1.9	167	1.6	104	1.0	91	0.9	70	0.7	101	1.0
45–54	111	0.5	165	0.8	200	0.9	236	1.0	218	1.0	176	0.8	120	0.5	128	0.6	122	0.5
55–64	20	0.1	35	0.2	30	0.2	46	0.3	42	0.2	36	0.2	43	0.2	27	0.1	36	0.2
≥65	5	0.0	2	0.0	9	0.0	9	0.0	8	0.0	6	0.0	3	0.0	5	0.0	12	0.0
Total	1,339	0.9	1,458	1.0	1,692	1.1	2,242	1.5	2,232	1.4	1,780	1.1	1,501	0.9	1,458	0.9	1,471	0.9
Overall total**	8,724	2.9	9,756	3.3	11,466	3.8	13,500	4.4	13,997	4.6	13,774	4.5	13,970	4.5	15,667	5.0	16,663	5.3

Abbreviations: A/PI = Asian/Pacific Islander; AI/AN = American Indian/Alaska Native.

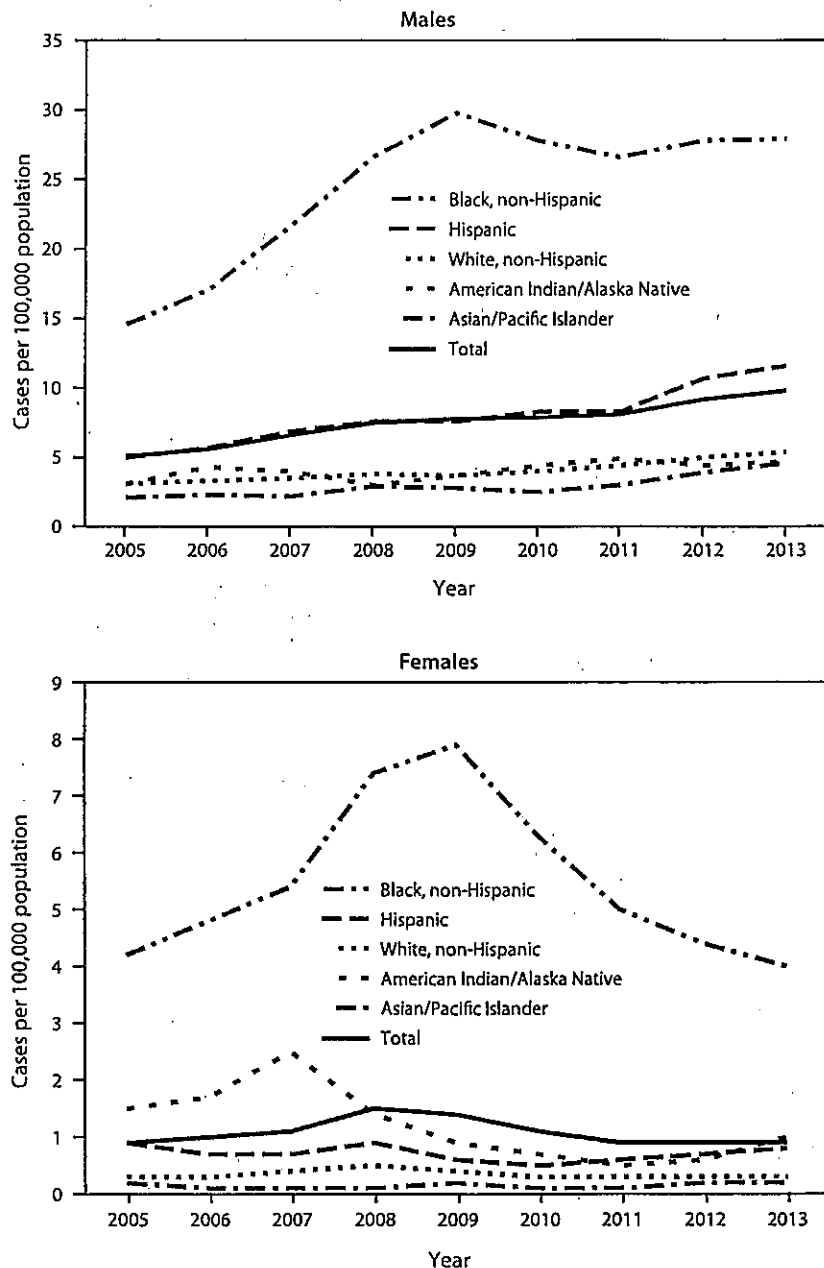
* Per 100,000 population.

[†] *Northeast*: Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, and Vermont; *Midwest*: Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin; *South*: Alabama, Arkansas, Delaware, District of Columbia, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, and West Virginia; *West*: Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming.

[§] Data are as of April 28, 2014.[‡] Includes persons aged ≥15 years.

** Cases among persons aged ≤14 years not shown.

FIGURE 1. Annual rate of primary and secondary syphilis cases among males and females, by race/ethnicity — National Notifiable Diseases Surveillance System, United States, 2005–2013



Racial/ethnic disparities in syphilis persisted. In 2013, the primary and secondary syphilis rate among black men was 5.2 times that among white men (27.9 versus 5.4 cases per 100,000 population); the rate among black women was 13.3 times that among white women (4.0 versus 0.3). The rate among Hispanic men was 2.1 times that among white men

(11.6 versus 5.4), and the rate among Hispanic women was 2.7 times that among white women (0.8 versus 0.3). These disparities were similar to disparities observed in 2005 (Table).

Across all four U.S. Census regions, primary and secondary syphilis rates were greater in 2013 than in 2005. In 2013, the highest overall regional rate (6.5 cases per 100,000 population) was in the West region. In 2013, for the first time in at least 50 years, the South did not have the highest overall syphilis rate among regions. Regional trends among men and women by race/ethnicity mirrored national trends except in the West region, where there was no decrease among black men during 2009–2013. Among women of all races/ethnicities in the West region, rates declined during 2005–2010 and increased during 2011–2013 (Table).

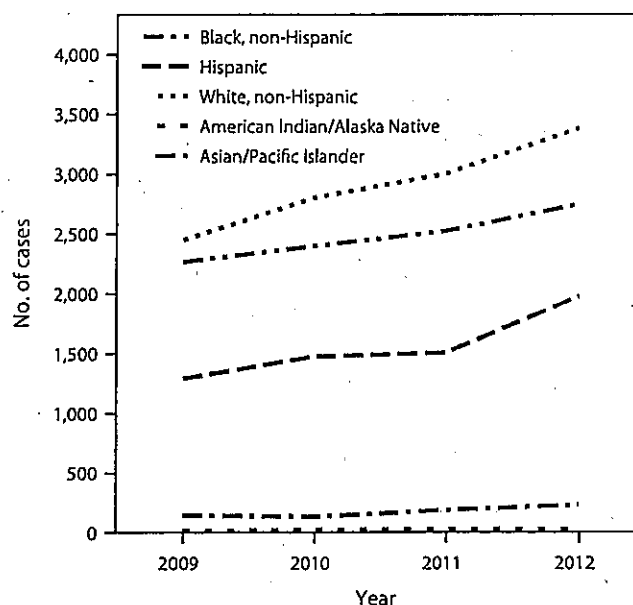
Discussion

After being on the verge of elimination in 2000 in the United States, syphilis cases have rebounded. Rates of primary and secondary syphilis continued to increase overall during 2005–2013; although rates stabilized during 2009–2010, rates have increased since 2011. Increases have occurred primarily among men, and particularly among MSM, who contributed the vast majority of male primary and secondary syphilis cases during 2009–2012.

The epidemiology of syphilis among men, including MSM, has shifted since 2009, with larger increases occurring among Hispanic and white men. Despite this increase, disparities in primary and secondary syphilis between black men and other racial/ethnic groups remain large. Many barriers to contacting and treating sex partners exist, including delays in reporting cases to the health department, anonymous partners, physicians who rely on patients to notify their partners (2), and the observed tendency of MSM to notify a smaller proportion of their sex partners than do heterosexuals (3).

These analyses indicate that syphilis prevention measures for MSM of all races/ethnicities need to be strengthened throughout the United States. This could be accomplished by working with private health-care providers because a substantial number of primary and secondary syphilis cases among MSM are reported by private physicians (1). Further, both private and

FIGURE 2. Number of primary and secondary syphilis cases among men who have sex with men, by race/ethnicity — National Notifiable Diseases Surveillance System, 34 states* and the District of Columbia with complete sex partner data,† 2009–2012



* Arkansas, California, Colorado, Connecticut, Florida, Hawaii, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Nevada, New Hampshire, New Jersey, New York, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Vermont, Virginia, Washington, and West Virginia.

† Sex of partners reported for ≥70% of cases of primary and secondary syphilis in males aged ≥15 years during 2009–2012.

public providers should be aware of the resurgence in syphilis and should be able to recognize the signs and symptoms of syphilis, conduct risk assessments, and screen all sexually active MSM for syphilis at least annually with syphilis serologic tests with confirmatory testing where indicated (4). More frequent screening (i.e., at 3–6 month intervals) is recommended for MSM who have multiple or anonymous sex partners. Disclosure of sexual practices remains difficult for some MSM (5); therefore, providers are encouraged to elicit sexual histories of their patients in a culturally appropriate manner, including recognition of sexual orientation, gender identity, and the sex of patients' sex partners. Additional resources and training for accomplishing this are available online.†

The increase in syphilis among MSM is a major public health concern, particularly because syphilis and the behaviors associated with acquiring it increase the likelihood of acquiring and transmitting human immunodeficiency virus (HIV) (6). There are reported rates of 50%–70% HIV coinfection

† Available at <http://www2a.cdc.gov/stdtraining/self-study/syphilis/default.htm>, <http://www.cdc.gov/std/syphilis/stdfact-msm-syphilis.htm>, and <http://nnptc.org/resourcecats/sexual-history>.

What is already known on this topic

Rates of reported primary and secondary syphilis in the United States have increased since reaching historic lows in 2000. Cases of primary and secondary syphilis increasingly are among males, particularly men who have sex with men (MSM).

What is added by this report?

Primary and secondary syphilis rates increased among men of all ages and race/ethnicities during 2005–2013, from 5.1 cases per 100,000 population in 2005 to 9.4 in 2013, when men accounted for 91.1% of all cases reported in the United States. Although rates remain higher among black men (23.1), recent increases were greatest among Hispanic and white men. Currently, syphilis is predominantly an MSM epidemic.

What are the implications for public health practice?

Syphilis prevention measures for MSM of all race/ethnicities should be strengthened throughout the United States, including 1) encouraging safer sexual practices (e.g., reducing the number of sex partners, using latex condoms, and having a long-term mutually monogamous relationship with a partner who has negative test results for sexually transmitted diseases);

2) promoting syphilis awareness and screening as well as appropriate screening for gonorrhea, chlamydia, and human immunodeficiency virus infection; and 3) notifying and treating sex partners. In addition, efforts to prevent and treat syphilis among the general male and female population should continue in order to prevent congenital syphilis.

among MSM infected with primary and secondary syphilis (7) and high HIV seroconversion rates following primary and secondary syphilis infection (8). The resurgence of syphilis, coupled with its strong link with HIV, underscores the need for programs and providers to 1) urge safer sexual practices (e.g., reduce the number of sex partners, use latex condoms, and have a long-term mutually monogamous relationship with a partner who has negative test results for sexually transmitted diseases); 2) promote syphilis awareness and screening as well as appropriate screening for gonorrhea, chlamydia, and HIV infection; and 3) notify and treat sex partners.

Public health officials should seek to improve the quality of data regarding the sex of sex partners, share local MSM, sexually transmitted disease, and HIV data consistent with local laws and regulations with medical providers to increase their awareness of disease burden in their communities, and ensure that providers can recognize syphilis symptoms. Two CDC cooperative agreements are encouraging local and state participants to make MSM a priority population and direct resources to areas of greatest need based on local epidemiology (9,10). CDC, in collaboration with state and local partners, health-care providers, and MSM-oriented organizations, is also engaged in research to better understand risk factors for syphilis among MSM, develop improved care models to better reach

and serve MSM populations, assess whether MSM are being tested and treated appropriately, and determine what barriers exist in the diagnosis and treatment of syphilis among MSM.

The continued decline of primary and secondary syphilis rates among black women since 2008 is encouraging and might suggest that targeted efforts to reduce syphilis among certain populations have had some success. Although primary and secondary syphilis is currently a predominantly MSM epidemic, it is important that efforts to prevent syphilis among heterosexual men and women continue, especially given the severe consequences of syphilis infection acquired in utero, including stillbirths.

The findings in this report are subject to at least two limitations. First, primary and secondary syphilis case-report data likely underestimate the true number of syphilis infections in the United States because of underreporting of diagnosed cases, infected persons not accessing health care, misdiagnosis, and the fact that primary and secondary syphilis cases amounted to only 31.4% of all syphilis cases reported in 2012. Second, the findings for MSM included only data from 34 states and the District of Columbia, where the sex of sex partners was reported for 70% or more of male primary and secondary syphilis cases. For 12% of cases in these 35 reporting areas, the sex of sex partners was unknown.

Despite decreasing rates of primary and secondary syphilis in the late 1990s in the United States, the resurgence of cases in recent years highlights the fact that challenges remain, and the increases among MSM are particularly concerning. Public health practitioners might want to consider focusing on efforts to strengthen linkages with practicing physicians to improve case identification and reporting, partner-notification programs, and outreach to MSM.

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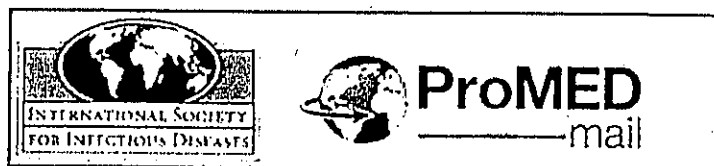
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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
			2014年5月19日	該当なし。	
一般的名称	別紙のとおり。	研究報告の公表状況	ProMED-mail 20140509.2451125	公表国 中国	使用上の注意記載状況・ その他参考事項等 記載なし。
販売名(企業名)	別紙のとおり。				
問題点：中国で、トリインフルエンザA (H5N6) ウイルスの人感染例が初めて報告された。 四川省、南充市において、重度急性肺炎を患っていた男性1名(49歳)が死亡し、China Disease Prevention and Controlの検査で、採取された咽頭スワブがトリインフルエンザウイルスH5N6核酸陽性であることが判明した。この男性には、同市の南部郡において、病気で死亡した家禽への曝露歴があった。この男性と密接な接触があった者の中に健康の異常を示す者は無く、専門家は拡大の危険性は低いとした。2014年4月23日以降、四川省獣医当局は南充市、南部郡においてトリインフルエンザに対し陽性であることが判明した病気の農場従事者1例の所有するニワトリの調査を行っており、2014年5月3日に家禽から採取したサンプルからトリインフルエンザウイルスサブタイプH5N6が分離された。					
研究報告の概要					
報告企業の意見		今後の対応			
別紙のとおり。		今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。			

*：現在製造を行っていない

INF2014-002



Published Date: 2014-05-05 20:13:11

Subject: PRO/AH/EDR> Avian Influenza (59): China (SC) LPAI H5N6, human, 1st case, poultry

Archive Number: 20140505.2451125

AVIAN INFLUENZA (59): CHINA (SICHUAN) LPAI H5N6, HUMAN, FIRST CASE, POULTRY

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 posting:

- [1] A fatal human case, H5N6
- [2] Avian influenza virus H5N6 in poultry

[1] A fatal human case, H5N6

Date: Sun 4 May 2014

Source: Sichuan Province Health and Family Planning Commission of Sichuan Province [In Chinese, machine trans., edited]

<http://www.scwst.gov.cn/index.php/2012-07-24-12-47-36/9425-1h5n6>

Nanbu: a human case of severe pneumonia by avian influenza virus H5N6

In recent years Sichuan province has intensified and improved the sensitivity of its surveillance addressing influenza-like illness and unexplained cases of pneumonia in human patients. Recently, a throat swab sampled from a case of severe pneumonia in Nanchong city, was found positive for nucleic acid of avian influenza virus H5N6. This was confirmed by the China Disease Prevention and Control Center.

The patient, a 49-year-old male, clinically diagnosed by a joint group of experts of the provincial and municipal rescue team as suffering acute severe pneumonia, died. He had a history of exposure to sick and dead poultry in Nanbu County, Nanchong City. All his close contacts did not show abnormal health signs. Experts consider this case as a human infection with avian influenza virus. They consider the spread risk as low.

To prevent respiratory diseases, experts advise the public to provide for satisfactory indoor ventilation, reasonable rest, reducing stays in crowded air spaces, routinely wash hands, cover mouth and nose when coughing and sneezing, cater for general personal hygiene, and refrain from coming in contact with dead poultry or consuming produce from such animals. It is recommended to buy poultry meat from freshly slaughtered healthy animals. If frozen poultry is purchased, one should look for the accompanying veterinary certificates of origin. People having any symptoms of influenza should seek immediate medical attention.

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[2] Avian influenza virus H5N6 in poultry

Date: Sun 4 May 2014

Source: Ministry of Agriculture, Beijing, China [In Chinese, machine trans., edited]

http://www.moa.gov.cn/sjzz/syj/dwyqdt/qt/201405/t20140504_3891926.htm

Avian influenza virus subtype H5N6 detected in poultry in Nanbu County, Nanchong City

 Since 23 Apr 2014, the Sichuan veterinary department has been investigating chickens of a sick farmer who was found positive for avian influenza in Nanbu County, Nanchong City. Samples from the poultry were submitted to the National Avian Influenza Reference Laboratory. On 3 May 2014, the reference laboratory isolated avian influenza virus subtype H5N6 from the samples.

Following the detection of the positive samples in Sichuan Province, the local emergency response, plan, and control team, has performed the prescribed control measures in accordance with the official specifications. The group of positive birds were culled and disinfection applied. Surveillance and monitoring activities in the area surrounding the farm are being carried out; so far, no abnormalities have been found.

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[To the best of our knowledge, this is the 1st human case of H5N6; correction, if available, will be welcomed. Since no information on clinical disease in the chickens has been reported, it may be assumed that the causative agent is a low pathogenic avian influenza (LPAI) strain. LPAI H5N6 strains have been recorded in wild birds in several continents in the past. For example, on 15 Dec 2004 Taiwan reported 2 strains of avian flu in migratory birds in the northern part of the island, the milder H5N2 strain and also the H5N6-strain (see ProMED-mail post [20051010.2949](#)).

H5N6 virus has been used as a poultry vaccine strain; similarly to H5N2, it was used as a DIVA or "differentiating infected from vaccinated animals." It was also shown that cats can be protected against a lethal high-dose challenge infection with HPAI H5N1 by an inactivated, adjuvanted heterologous H5N6 avian influenza virus vaccine (<http://jgv.sgmjournals.org/content/89/4/968.full>).

A preliminary report on the human case described in China was published on 23 Apr 2014. The title in the Chinese newspaper was "An official Nanbu microbiologist said a farmer suffering from severe pneumonia is suspected of bird flu"; see http://region.scdaily.cn/jrsz/content/2014-04/23/content_7761608.htm?node=3538 [Chinese].

The detection of LPAI H5N6 in poultry in Nanbu, Nanchong, Sichuan has been officially notified today, 5 May 2014, to the OIE. See at http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=15200.

Maps of China can be seen at <http://www.sacu.org/maps/provmap.png> and <http://healthmap.org/promed/p/38938>. Nanbu County is under the administration of Nanchong city, a prefecture-level city in the northeast of Sichuan province. ~ Mod.AS]

See Also

2005

 Avian influenza, human - East Asia (143) [20051010.2949](#)
arn/mj/jw

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
			2014年5月19日	該当なし。	
一般的名称	別紙のとおり。	研究報告の公表状況	CID RAP, 2014; May 21	公表国 米国	
販売名(企業名)	別紙のとおり。				
問題点：米国で、新規なオルソポックスウイルス科のウイルスが確認された。 米国Center for Disease Control and Prevention (CDC) によると、グルジアにおいて、2名の牛飼いを含み3名から新規なオルソポックスウイルス (orthopoxvirus) 科のウイルスが検出された。天然痘の予防接種を受けていないウシ飼ひ2名は2013年夏に病気のウシに接触をした後、具合が悪くなった。CDCによる検査の結果、3名はこれまでに知られていないオルソポックスウイルス科のウイルスに感染したものと結論付けられた。また、CDCによる調査の結果、グルジアの他の地域で、元々は2010年に旋痘に罹患したものと推測されていた別の患者が特定された。この新規なウイルスは、発熱、手や腕に痛みを伴う水泡、リンパ節の腫脹等を引き起こすが、上記の患者は感染から回復している。					
研究報告の概要					
使用上の注意記載状況・その他参考事項等 記載なし。					
報告企業の意見			今後の対応		
別紙のとおり。			今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。		

*: 現在製造を行っていない

INF2014-001

News Scan for May 01, 2014

New orthopoxvirus; Smallpox virus destruction

Filed Under: [Orthopoxvirus \(free-tagging/orthopoxvirus\)](#); [Smallpox \(infectious-disease-topics/smallpox\)](#)

CDC reports 3 human cases caused by new orthopoxvirus

Scientists with the US Centers for Disease Control and Prevention (CDC) have detected a new orthopoxvirus—the family of viruses that includes smallpox and cowpox—in three people in the nation of Georgia, including two herdsmen, the CDC reported in a news release yesterday.

The finding was announced this week at the CDC's 63rd Annual Epidemic Intelligence Service (EIS) Conference, which highlights the work of CDC's EIS officers, or "disease detectives."

When the two herdsmen, neither of whom was vaccinated against smallpox, became ill after contact with sick cattle last summer, the CDC investigators determined through extensive testing that they were infected with a previously unknown orthopoxvirus.

The CDC team then interviewed 55 people who had contact with the herdsmen or with cattle. They found that, of 9 interviewees born after routine smallpox vaccination had stopped in 1980 because of eradication of the disease, 5 had orthopoxvirus antibodies. They also discovered an additional case in a different region of Georgia in a person who was originally suspected of having anthrax in 2010.

The new virus causes painful blisters on the hands and arms, as well as fever, swollen lymph nodes, and weakness, according to a story today from NPR. The patients survived their infections, the CDC said.

The CDC researchers also found evidence that some of the herdsmen's cows had previously been exposed to orthopoxviruses and that viruses also circulated among rodents in the area, the agency said.

The researchers conclude, "Orthopoxviruses are anticipated to emerge in the absence of routine smallpox vaccination and should be considered in persons who experience cutaneous lesions after animal contact."

EIS conference abstract (<http://www.cdc.gov/media/dpk/2014/docs/eis/novel-Orthopoxvirus.pdf>)

Apr 30 CDC news release (<http://www.cdc.gov/media/releases/2014/p0430-2014eis-conference.html>)

May 1 NPR story (<http://www.npr.org/blogs/health/2014/05/01/308357520/new-virus-related-to-smallpox-is-found-in-republic-of-georgia>)

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 該当なし	総合機構処理欄				
		2014. 4. 5						
一般的名称	人赤血球液	研究報告の公表状況	公表国 トルコ					
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社) 赤血球液-LR「日赤」(日本赤十字社) 照射赤血球液-LR「日赤」(日本赤十字社)	Ergonul O, Battal I. Jpn J Infect Dis. 2014;67(2):137-8.						
研究報告の概要	○性感染の可能性があるクリミア・コンゴ出血熱(CCHF)症例 CCHFウイルスはダニ咬、感染急性期中中のCCHF患者との接触やウイルス血症の家畜の血液及び組織への接触によりヒトに感染するとされている。性的接触による感染については、エボラ、マールブルグ及びラッサウイルスでは感染原因の一つとされているが、CCHFウイルスでは報告されていない。 これは回復期患者との性交渉を介したCCHFウイルス感染の可能性が示唆される初の報告である。 2008年6月23日、トルコの中央アナトリア地方で畜産に従事する68歳男性が、頭痛、倦怠感、悪心を呈し、4日前にダニに咬まれていることが判明した。退院11日後、男性の60歳の妻が、男性よりも軽い同様の症状を呈して同じ病院に入院した。彼女にダニ咬歴はなかったが、男性が退院した3、4日後に性交渉があったことを報告した。彼女はウシなどの動物との接触歴もあり、糖尿病でインスリン治療を受けていた。二人からはCCHFウイルス IgM抗体及びRNAが検出され、CCHFが確認された。 これまでCCHFウイルスの感染源として精液は報告されておらず、性交渉はCCHF感染経路として考慮されていなかった。過小評価されていた潜在的な感染経路に注意を払う必要がある。							
報告企業の意見	クリミア・コンゴ出血熱が性的接触により感染したと示唆される初の報告である。							
今後の対応	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。							
使用上の注意記載状況・ その他参考事項等								
赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 赤血球液-LR「日赤」 照射赤血球液-LR「日赤」 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク								

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Laboratory and Epidemiology Communications

Potential Sexual Transmission of Crimean-Congo Hemorrhagic Fever Infection

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Communicated by Masayuki Saijo

(Accepted October 31, 2013)

Crimean-Congo hemorrhagic fever (CCHF) is a potentially fatal viral infection in certain regions of Africa, Asia, Eastern Europe, and the Middle East (1). The causative virus, CCHF virus (CCHFV), belongs to the genus *Nairovirus* of the *Bunyaviridae* family and causes severe disease, with a fatality rate of 3-30% among infected humans (2). Human infections occur through tick bites, contact with a patient with CCHF during the acute phase of infection, or by contact with blood or tissues from viremic livestock (2-4). Approximately 8,000 confirmed cases of CCHF have been reported within the last 10 years in Turkey. However, sexual transmission as a route of infection has not been reported, although this route has been reported for other viral hemorrhagic fevers caused by Ebola (5,6), Marburg (7), and Lassa (8) viruses. In the present report, we describe the characteristics of a couple infected with CCHFV by probable sexual transmission. To our knowledge, this report is the first to emphasize the risk of CCHFV transmission through sexual intercourse with a convalescing patient.

A 68-year-old man, who was involved with livestock husbandry in a village in the central Anatolia region of Turkey, was admitted to the Department of Infectious Diseases of Karabuk Sirinevler State Hospital with suspected CCHF infection on June 23, 2008. His primary complaints were headache, malaise, and nausea. He became aware of tick bite on his body 4 days ago. On physical examination, fever and maculopapular eruption on the trunk were noted. On admission, his leukocyte count was 4,490/mm³ and platelet count (PLT) was 157,000/mm³. On the 2nd day of hospitalization, his leukocyte count dropped to 3,240/mm³ and PLT to 115,000/mm³. On the 8th day of hospitalization, which was the 12 days after the tick bite, his leukocyte count and PLT further declined to minimum levels of 650/mm³ and 76,000/mm³, respectively. The maximum levels of aspartate aminotransferase and alanine aminotransferase were 59 IU and 49 IU, respectively. The maximum levels of lactate dehydrogenase and creatine phosphokinase were 320 IU and 187 IU, respectively. On admission, oral ribavirin therapy was started and steroid therapy was administered on hospitalization day 6. On hospitalization day 9, his biochemical test

results and complete blood counts returned to within normal limits.

Eleven days after discharge, his 60-year-old wife was admitted to the same hospital with similar but milder complaints of headache, malaise, and diarrhea. Her diarrhea lasted 1 day. She had no history of tick bite, although she reported sexual intercourse with her husband 3 or 4 days after discharge of her husband from the hospital. She also had a multiyear history of contact with animals (such as cows) and had active diabetes mellitus, for which she was receiving insulin therapy. She was administered oral ribavirin on admission. After 5 days, she achieved complete recovery and was discharged. Diagnoses of both cases were confirmed by CCHFV IgM seropositivity through ELISA and detection of CCHFV RNA by reverse transcription (RT)-PCR performed at the Refik Saydam Public Health Institute in Ankara.

Viral RNA in hemorrhagic fever is detectable in serum several days after disease onset, and can be detected on average for 2 weeks afterward (9). Some reports have cited cases in which viral RNA was detectable more than 2 weeks after infection (1). Therefore, convalescent patients continue to pose a risk of disease transmission. The presence of filoviruses and arenaviruses in seminal fluid for approximately 3 months has been well documented in some studies (5,6). In one such study after the 1995 Ebola outbreak in the Democratic Republic of Congo, virus persistence was examined in body fluids from 12 convalescent patients by virus isolation and RT-PCR (5). Viral RNA could be detected for up to 33 days in vaginal, rectal, and conjunctival swabs of 1 patient and up to 101 days in the seminal fluid of 4 patients. Infectious viruses were detected in 1 seminal fluid sample obtained 82 days after disease onset (5). In a second study after the same Ebola outbreak, a cohort of convalescent Ebola hemorrhagic fever (EHF) patients and their household contacts (HHCs) were prospectively examined to determine if convalescent body fluids contain Ebola virus and if secondary transmission occurs during convalescence (6). In this study, 29 EHF convalescents and 152 HHCs were monitored for up to 21 months. Evidence of Ebola virus was detected by RT-PCR in semen specimens up to 91 days after disease onset, but no direct evidence of convalescent-to-HHC transmission of EHF was found, although the semen of convalescents could have been infectious (6). Marburg virus was isolated 83 days after disease onset from the seminal fluid of a patient who may have sexually transmitted the disease to his spouse

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(10). Lassa virus could be detected in semen up to 3 months after acute infection (8).

The leading transmission routes of CCHFV are reportedly through tick bite, contact with infected blood and body fluids, and contact with viremic animals (2). However, semen has not been previously reported as a potential infectious material for transmission of CCHFV. Accordingly, sexual intercourse is not currently considered a potential route of CCHFV transmission. By reporting this case, we wish to bring attention to this underestimated route of transmission.

At the beginning of the CCHFV outbreak in Turkey, only 60% of infected patients reported a history of tick bite (11). The proportion of patients reporting tick bites increased because of increased public awareness. However, the transmission route has remained obscure in a significant proportion of infections. An Iranian study reported that infectivity of CCHFV through usual routine contact among household members was low (12). Therefore, further studies are necessary to investigate the routes of CCHFV infection, particularly human to human transmission. Aksoy et al. (13) reported a CCHF case with epididymo-orchitis, which suggested that the virus could replicate in the male genital organs and be present in semen. This case offers indirect evidence of the virus transmission from a male patient to his partner. Therefore, sexual transmission of viral infections may be underreported.

The present report on potential sexual transmission of CCHF has two major limitations. One is the failure to demonstrate the virus in seminal fluid. This route could be confirmed because of the acute and severe nature of the disease. Although the presence of filoviruses and arenaviruses has been demonstrated in semen (5,6), these studies have not demonstrated sexual transmission of the viruses. The second limitation of this study is related to the source of infection. It is possible that the infection acquired by our patient's wife may have been due to direct contact with a viremic animal or tick bite, although the woman reported no history of a tick bite (4). However, she may have been exposed to these risk factors for an extended period of time, but did not acquire an infection. Thus, her infection may have coincided with the infection of her husband.

To prevent an unnecessary impact on public health resources, it is recommended that patients convalescing from filoviral or arenaviral infections should refrain from sexual activity for 3 months after clinical recovery

(14). Hence, the potential of sexual transmission should be evaluated in survivors of CCHFV infection as well.

In conclusion, the findings of this report suggest the likelihood of CCHFV transmission through sexual intercourse. Therefore, clinicians and public health authorities should consider the possible risk of CCHFV transmission through sexual intercourse.

Conflict of interest None to declare.

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研究報告 調査報告書

番号 12

15

識別番号・報告回数		報告日	第一報入手日 2014年03月24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 乾燥濃縮人アンチトロンビンIII		研究報告の 公表状況		公表国 中国		使用上の注意記載状況・ その他参考事項等 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人アプテロニンIII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。
販売名 (企業名) ①ノイアート静注用500単位 (日本血液製剤機構) ②ノイアート静注用1500単位 (日本血液製剤機構)		研究報告の 公表状況		wwwnc.cdc.gov/eid/article/20/6/13-1022_article.htm/2014/03/14		
Volume 20, Number 6—2014年6月 レター ラットにおける新規ヘニパ様ウイルス Mojiang パラミクソウイルス、2012年中国 本論文に関して示唆される文献 (#suggestedcitation) 編集者様：ヘニパウイルス属 (パラミクソウイルス科) には3種の確立されたウイルス種 (ヘンドラウイルス、ニパウイルスおよびCedarウイルス) と、全長配列が明らかでないウイルスである Bat Paramyxovirus Fildhel/GH-M74a/GHA/2009 を含む19種の新たに確認されたウイルス種が含まれている。人獣共通のヘンドラウイルスは、ヒト、ウマおよびブタにおける致死性の神経疾患または呼吸器疾患と関連付けて考えられてきた。既知のヘニパウイルスの自然リザーバーはフルーゾウモリである；これらのウイルスは、他の野生動物では報告されていない。我々は中国のラット (<i>Rattus flavipectus</i>) における新規ヘニパ様ウイルスである Mojiang パラミクソウイルス (MoJV) について報告する。 2012年6月、中国雲南省の Mojiang Hani 自治県において、廃坑で働いていた3例が原因不明の重症肺炎と診断された；患者3例すべてが死亡した。その半年後、我々はこの洞窟で自然宿主内の新たな人獣共通病原体の存在を検討した。この研究のために、坑道でコウモリ (<i>Rhinolophus ferrumequinum</i>) 20匹、ラット (<i>R. flavipectus</i>) 9匹およびジャコウネズミ (<i>Crocidura dracula</i>) 5匹から肛門スワブサンプルを採取し、生体内ウイルス叢の分析を実施した。 ウイルス粒子保護核酸精製法、次いで抽出された RNA および DNA の配列非依存 PCR 増幅法を用いて、全てのサンプルを処理した。そして、81bp のシングルリード用の Illumina Genome Analyzer II (Illumina Trading, Beijing, China) を用いて、増幅されたウイルス核酸ライブラリの配列を決定した。リードを既報の手順でフィルタリング後)、BLASTx (http://blast.ncbi.nlm.nih.gov/Blast.cgi)、(http://www.cdc.gov/other/disclaimer.html)) を用いて、全ての生リードを国立生物工学情報センター (www.ncbi.nlm.nih.gov/RefSeq/) の非冗長タンパク質データベースにアラインした。MEGAN4 (MetaGenome Analyzer) を用いて、アラインしたリードを分類した。 非冗長タンパク質アラインメントの結果を基に、我々はヘニパウイルス種として分類される38の配列リードを同定した。しかしこのような配列の核酸およびアミノ酸の、既知のヘニパウイルスとの同一性は低かった。リードに基づいてPCRを実行し、このウイルスの一部のゲノムを同定した。残りのゲノム配列はゲノムウォーキングを用いて決定した。5' および 3' 未翻訳領域は、既報の手順で特異的プライマーとヘニパウイルス特異的変性プライマーを組み合わせた nested PCR によって決定し、5' および 3' ゲノム末端の正確な配列を cDNA 末端の迅速増幅によって決定した。 MoJV は既知のヘニパウイルスと共通の特性を有している。このウイルスのゲノム長は18,404ntであり (GenBank に登録番号 KF278639 で申請)、ヘニパウイルス遺伝子に特徴的な配列を有している：3'-ヌクレオカプシド (N) タンパク質 (539aa)；P/V/W/C タンパク質 (リンタンパク質：694aa, 464aa, 434aa, 177aa)；基質タンパク質 (340aa)；融合タンパク質 (545aa)；付着糖タンパク質 (625aa)；および大型 (L) タンパク質 (2,277aa) -5' (Technical Appendix Figure [PDF-31KB-1 ページ] (/eid/article/20/6/13-1022-techappl.pdf))。						

研究報告の概要

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遺伝子間で保存されていることが予測される配列は、ヘニパウイルスの特徴を示していた (Technical Appendix Table [PDF-31KB-1 ページ] (leid/article/20/6/13-1022-techappl.pdf))。N タンパク質のセントラルドメインには、全てのパラミクソウイルスに共通の保存された 3 つのモチーフが含まれていた: QXW[L/V]X ₂ K[A/C]XT、FX ₂ T[L/L] [R/K] Φ [G/A] [L/I/V]XT および FX ₂ XP ₂ ΦSΦAMG、ただし Φ は芳香族アミノ酸である)。さらに、ヘンドラウイルスおよびニパウイルスのリンタンパク質遺伝子配列において保存されている V および W タンパク質を加工するための RNA 編集部位 (AAAAGG)、またモノネガウイルス目の L タンパク質内に存在する 6 つの保存領域が) Mojv L タンパク質内で見出された。	
予測される Mojv 遺伝子のヌクレオチドの特性は、既知のヘニパウイルスの遺伝子に類似していた: N (53.0%~57.0%の同一性)、リンタンパク質 (37.8%~43.0%の同一性)、基質 (59.5%~63.4%の同一性)、融合 (47.5%~51.4%の同一性)、付着糖タンパク質 (36.6%~41.8%の同一性) および L (55.9%~58.6%の同一性) 遺伝子。我々は MEGA5 を使い、N および L タンパク質に基づく系統樹で Mojv とパラミクソウイルス科のメンバーの間の進化的関係を説明した (Figure 1)。Mojv はヘニパウイルス属の 4 つのメンバーに集中していた。したがって、Mojv と他のヘニパウイルスとの間でゲノムの特性が類似していることを考慮し、我々は Mojv がヘニパウイルス種に極めて強く関連した新しいウイルス種であることを確認した。	
Mojv の L 遺伝子を標的とした特異的 nested プライマーセットをデザインし、34 本の肛門スワブサンプルといくつかの組織サンプルを個別に再評価した。R. flavipictus ラット由来の 9 本のスワブサンプル中 3 本が Mojv 陽性であり、Mojv 陽性ラット 3 匹中 1 匹に由来する組織サンプルも Mojv 陽性であった (他の 2 匹のラットからは組織を採取しなかった)。R. ferrumequinum コウモリ由来の 20 本全てのサンプル、また C. dracula ジャコウネズミ由来の 5 本全てのサンプルが Mojv 陰性であった。3 本の Mojv 陽性肛門スワブサンプルを Vero E6、Hep2 および BHK21 細胞内で培養してウイルスを分離した; 試験内容を伏せて 2 回継代培養後、細胞変性作用またはウイルス複製は検出されなかった。	
今回の研究から、中国にげっ歯類由来のヘニパウイルスである Mojv が存在することが明らかとなった。R. flavipictus ラットが Mojv の自然リザーバーである。この所見およびその背景は、ヘニパウイルス種のウイルスが過去に認識されていた以上に哺乳類宿主に感染しやすい可能性、またコウモリがヘニパウイルスの唯一の宿主ではない可能性を示している。	
新しいヘニパ様ウイルスである墨江 (Mojiang) パラミクソウイルスは、モノネガウイルス目 (Mononegavirales)、パラミクソウイルス科 (Paramyxoviridae) に属する。パラミクソウイルス科のウイルスは球形のエンベロープを有する 1 本鎖 RNA ウイルスで、直径は 100nm~200nm までの幅がある。万一原料血漿に新規パラミクソウイルスが混入したとしても、Human immunodeficiency virus-1 (HIV-1)、或いは Bovine viral diarrhoea virus (BVDV) をモデルウイルスとした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。	
報告企業の意見	
今後の対応	
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。	



Volume 20, Number 6—June 2014

Letter

Novel Henipa-like Virus, Mojiang Paramyxovirus, in
 Rats, China, 2012

Suggested citation for this article (#suggestedcitation)

To the Editor: The genus *Henipavirus* (family *Paramyxoviridae*) contains 3 established species (*Hendra virus*, *Nipah virus*, and *Cedar virus*) and 19 newly identified species, including 1 full-length sequenced virus, Bat Paramyxovirus Eidhel/GH-M74a/GHA/2009 (1 (#r1), 2 (#r2)). The zoonotic pathogens *Hendra virus* and *Nipah virus* have been associated with lethal neurologic and respiratory diseases in humans, horses, and pigs (3 (#r3)–5 (#r5)). The known natural reservoirs of henipaviruses are fruit bats (1 (#r1), 3 (#r3)); these viruses have not been reported in other wild animals. We report on a novel henipa-like virus, Mojiang paramyxovirus (MojV), in rats (*Rattus flavipectus*) in China.

In June 2012, in Mojiang Hani Autonomous County, Yunnan Province, China, severe pneumonia without a known cause was diagnosed in 3 persons who had been working in an abandoned mine; all 3 patients died. Half a year later, we investigated the presence of novel zoonotic pathogens in natural hosts in this cave. For the investigation, we collected anal swab samples from 20 bats (*Rhinolophus ferrumequinum*), 9 rats (*R. flavipectus*), and 5 musk shrews (*Crocidura dracula*) from the mine for virome analysis.

All samples were processed by using a virus particle-protected nucleic acid purification method, followed by sequence-independent PCR amplification of extracted RNA and DNA (6 (#r6)). The amplified viral nucleic acid libraries were then sequenced by using an Illumina Genome Analyzer II (Illumina Trading, Beijing, China) for a single read of 81 bp. All raw reads were then aligned to the nonredundant protein database of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/RefSeq/) (www.ncbi.nlm.nih.gov/RefSeq/) by using BLASTx (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) & (<http://www.cdc.gov/Other/disclaimer.html>) after filtering reads as described (6 (#r6)). The taxonomy of the aligned reads was parsed by using the MEGAN4 MetaGenome Analyzer (7 (#r7)).

On the basis of the nonredundant protein alignment results, we identified 38 sequence reads that were classified as *Henipavirus* spp. However, the sequences shared low nucleotide and amino acid identities with known henipaviruses. The reads were then used for reads-based PCR to identify the partial genome of this virus. The remaining genomic sequences were determined by using genome walking. The 5' and 3' untranslated regions were obtained by nested PCR with combined specific primers and henipavirus-specific

degenerate primers as described (8 (#r8)), and the exact sequences of the 5' and 3' genome termini were determined by rapid amplification of cDNA ends.

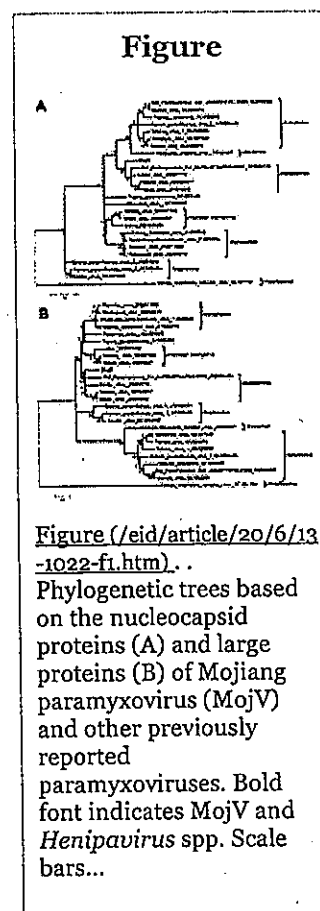
MojV shares similar features with known henipaviruses. The virus has a genome length of 18,404 nt (submitted to GenBank under accession no. KF278639), and has the characteristic henipavirus gene order: 3'-nucleocapsid (N) protein (539 aa); P/V/W/C proteins (phosphoprotein; 694 aa, 464 aa, 434 aa, 177 aa); matrix protein (340 aa); fusion protein (545 aa); attachment glycoprotein (625 aa); and large (L) protein (2,277 aa)-5' (Technical Appendix Figure 7 [PDF - 31 KB - 1 page] (/eid/article/20/6/13-1022-techapp1.pdf)). The predicted conserved sequences between genes showed features characteristic of henipaviruses (Technical Appendix Table 1 [PDF - 31 KB - 1 page] (/eid/article/20/6/13-1022-techapp1.pdf)). The central domain of the N protein contains 3 conserved motifs common in all paramyxoviruses: QXW [I/V] X₃K [A/C] XT, FX₂T [I/L] [R/K]Φ [G/A] [L/I/V]XT, and FX₄YPX₂ΦSΦAMG, where Φ is an aromatic amino acid (9 (#r9)). In addition, the RNA editing site (AAAAGG) for the processing of V and W proteins conserved in the phosphoprotein gene sequences of Hendra virus and Nipah virus was found, and 6 conserved domains within the L proteins of the order *Mononegavirales* (8 (#r8)) were found in the MojV L protein.

The nucleotide identities of predicted MojV genes exhibited similarity with genes of known henipaviruses: N (53.0%–57.0% identity), phosphoprotein (37.8%–43.0% identity), matrix (59.5%–63.4% identity), fusion (47.5%–51.4% identity), attachment glycoprotein (36.6%–41.8% identity), and L (55.9%–58.6% identity) genes. Using MEGA5 (10 (#r10)), we used the phylogenetic trees based on N and L proteins to describe the evolutionary relationships between MojV and members of the family *Paramyxoviridae* (Figure 1 (#tnF1)). MojV clustered with the 4 members of the genus *Henipavirus* and was distant from other clusters. Thus, considering the similar genome features between MojV and other henipaviruses, we confirmed that MojV could be classified as a new species closely related to *Henipavirus* spp.

Specific nested primer sets targeting the L gene of MojV were designed to separately re-evaluate the 34 anal swab samples and some tissue samples. Of 9 anal swab samples from the *R. flavipectus* rats, 3 were positive for MojV, and a tissue sample from 1 of the 3 MojV-positive rats was also MojV positive (tissue was not collected from the other 2 rats). All 20 samples from *R. ferrumequinum* bats and all 5 samples from *C. dracula* musk shrews were MojV negative. The 3 MojV-positive anal swab samples were cultured in Vero E6, Hep2, and BHK21 cells for virus isolation; no cytopathic effects or viral replication was detected after 2 blind subculture passages.

Our study showed the presence of a rodent-origin, henipa-like virus, MojV, in China. *R. flavipectus* rats are the natural reservoir of MojV. This finding and its context indicate that *Henipavirus* spp. viruses might infect more mammalian hosts than previously thought and that bats may not be the only hosts of henipaviruses.

Zhiqiang Wu¹(#fm), Li Yang¹(#fm), Fan Yang¹(#fm), Xianwen Ren¹(#fm), Jinyong Jiang, Jie Dong, Lilian Sun, Yafang Zhu, Hongning Zhou, and Qi Jin [✉] (/eid/article/20/6/13-1022_article.htm#comment)



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Figure

Figure. . Phylogenetic trees based on the nucleocapsid proteins (A) and large proteins (B) of Mojiang paramyxovirus (MojV) and other previously reported paramyxoviruses. Bold font indicates MojV and *Henipavirus* spp. Scale... (</eid/article/20/6/13-1022-f1.htm>)

Technical Appendix

Technical Appendix. . Genomic organization and nucleotide sequences for gene start and stop and the intergenic region of Mojiang paramyxovirus. 31 KB  (</eid/article/20/6/13-1022-techapp1.pdf>)

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		2014. 3. 4	該当なし	
一般的名称	解凍人赤血球液		公表国	
販売名(企業名)	解凍赤血球液-LR「日赤」(日本赤十字社) 照射解凍赤血球液-LR「日赤」(日本赤十字社)		Gaowa, Yoshikawa Y, Ohashi N, Wu D, Kawamori F, Ikegaya A, Watanabe T, Saitoh K, Takechi D, Murakami Y, Shichi D, Aso K, Ando S. Emerg Infect Dis. 2014 Mar;20(3):508-9. doi: 10.3201/eid2003.131337.	
研究報告の概要	○日本におけるヒトの <i>Anaplasma phagocytophilum</i> 感染、2010-2011年 ヒト顆粒球アナプラズマ症(HGA)は <i>A. phagocytophilum</i> を原因とする新興のダニ媒介性感染症であり、最近、後方視的研究により日本において2症例が確認された。HGAの血清学的診断にはHL60細胞で培養した <i>A. phagocytophilum</i> を抗原として用いるが、日本の2症例の血清は、間接蛍光抗体法(IFA)においてHL60細胞よりもむしろTHP-1細胞で培養した抗原に反応した。 これまでの研究で、THP-1細胞とHL60細胞で培養した <i>A. phagocytophilum</i> はそれぞれ血清学的に異なることが示唆されている。2010-2011年に静岡県でリケッチア様症状を呈した9患者は、日本紅斑熱またはツツガムシ病を疑われたがIFAで陰性となったため、HGAについてIFAを行ったところ、9人中4人がTHP-1及びHL60細胞で培養した抗原を用いたIFAによりIgM抗体あるいはIgG抗体が陽性となった。またウエススタンブロット法により、THP-1及びHL60細胞で培養された <i>A. phagocytophilum</i> のP44タンパク質抗原に対する特異的反応及び血清サンプルにおける組換え型P44-1タンパク質に対する反応性を確認した。 日本において、<i>A. phagocytophilum</i> は2005年に中部地方のダニに初めて検出されて以来、いくつかの地域のダニにも検出されているが、血清学的診断法に用いる細胞培養系の選択肢が乏しいため、ヒトへの感染についてはほとんど不明であった。この研究において、THP-1またはHL60細胞で培養した <i>A. phagocytophilum</i> におけるP44タンパク質特異抗体の存在が示され、HGAの血清学的診断法の標的タンパク質抗原として適当であることが示唆された。			使用上の注意記載状況・ その他参考事項等 解凍赤血球液-LR「日赤」 照射解凍赤血球液-LR「日赤」 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク
報告企業の意見	今後の対応 今後もし引き続き、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。			
HGAの血清学的診断には、抗原として <i>A. phagocytophilum</i> のP44タンパク質が有用であるとの報告である。				

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LETTERS

Anaplasma phagocytophilum Antibodies in Humans, Japan, 2010–2011

To the Editor: Human granulocytic anaplasmosis (HGA) is an emerging tick-borne infectious disease caused by *Anaplasma phagocytophilum*, an obligatory intracellular bacterium (1). Recently, 2 cases of HGA were identified by a retrospective study in Japan (2). For serodiagnosis of HGA, *A. phagocytophilum* propagated in HL60 cells is usually used as an antigen, especially by indirect immunofluorescent assay (IFA) (3). However, the serum from these 2 patients in Japan reacted with antigens of *A. phagocytophilum* cultured in THP-1 cells rather than in HL60 cells in IFA (2). In *A. phagocytophilum*, a *p44/msp2* multigene family encoding multiple 44-kDa immunodominant major outer membrane protein species (so-called P44) exists on the genome, and these multigenes are similar, but not identical, to each other, and the bacterium generates antigenic variations because of gene conversion (4). The previous studies showed that *A. phagocytophilum* expresses predominantly 2 species of *p44/msp2* transcripts in THP-1 cells, but it produces

the variation of P44 protein species in HL60 cells (2,5). This finding strongly suggested that *A. phagocytophilum* grown in THP-1 cells differs serologically from that in HL60 cells. Our serologic analysis found 4 recent cases of HGA in Japan by using infected THP-1 and HL60 cells as antigens, and some P44 immunoreactive protein species of *A. phagocytophilum* that were associated with the respective cell line cultures, binding to antibodies from the 4 patients' serum, also were identified.

In 2010 and 2011, nine patients in Shizuoka Prefecture, Japan, who had rickettsiosis-like symptoms, were suspected to have Japanese spotted fever or scrub typhus, but they were serologically negative by IFA. Therefore, IFA for HGA was conducted. In 4 of the patients, antibodies to *A. phagocytophilum* were detected in serum by using *A. phagocytophilum* cultured in THP-1 and HL60 cells as antigens (Table). In IFA tests for HGA, IgM and/or IgG from the patients' serum samples reacted with *A. phagocytophilum* cultured in THP-1, HL60, or both, and the seroconversions were observed in convalescent-phase serum from all patients. The clinical manifestation and laboratory findings for the 4 patients are summarized in the online Technical Appendix Table, (wwwnc.cdc.gov/EID/article/20/3/13-1337-Techapp1.pdf). Western blot analysis

further confirmed the specific reaction to P44 protein antigens (P44s) of *A. phagocytophilum* cultured in THP-1 and HL60 and to recombinant P44-1 protein (rP44-1) in the serum samples (online Technical Appendix Figures 1 and 2), supporting the IFA results in the Table.

To identify P44 immunodominant protein species binding to antibodies from the patients' serum, we selected P44-47E and P44-60 proteins that are dominantly expressed by *A. phagocytophilum* propagated in THP-1 cells (2) and P44-18ES protein that frequently predominates by *A. phagocytophilum* cultured in HL60 cells (6) as representatives for the preparation of recombinant proteins. The central hypervariable regions of the respective P44 proteins (online Technical Appendix Figure 3) were produced as recombinant proteins in vitro by insect cell-free protein synthesis system (Transdirect Insect Cell Kit; Shimadzu Co., Kyoto, Japan) (7) to avoid the strong nonspecific reaction with human serum that occurs in the *Escherichia coli* expression system. In Western blot analyses using these 3 recombinant P44 proteins (rP44-60 and rP44-47E for THP-1 and rP44-18ES for HL60) as antigens, most of the serum from the patients was reactive with *A. phagocytophilum* cultured in THP-1 cells in IFA bound to either rP44-60 or rP44-47E, whereas the

Table. Immunofluorescence antibody titers to *Anaplasma phagocytophilum* in serum from 4 patients with human granulocytic anaplasmosis and reactive rP44 protein species, Japan, 2010–2011*

Patient no.	Time after illness onset, d	Antigen			
		<i>A. phagocytophilum</i> propagated in THP-1 cells (rP44 species)		<i>A. phagocytophilum</i> propagated in HL60 cells (rP44 species)	
		IgM	IgG	IgM	IgG
1	1	80 (r60)	<20	80 (r18ES)	<20
	15	160 (r60)	<20	160 (r18ES)	<20
	30	320 (r60)	20 (r60)	320 (r18ES)	<20
2	13	40	40 (r47E)	<20	20
3	3	40	80 (r60)	<20	20 (r18ES)
	7	40	80 (r60)	<20	20 (r18ES)
	24	80 (r60)	160 (r60)	<20	40 (r18ES)
4	4	160 (r47E)	40	<20	<20
	15	160 (r47E)	80	<20	<20

*Three recombinant P44 (rP44) protein species (r18ES, r47E, r60) were prepared and either one bound to antibodies in each serum sample from 4 patients in Western blot analyses (online Technical Appendix Figure 4, wwwnc.cdc.gov/EID/article/20/3/13-1337-Techapp1.pdf). r18ES represents rP44-18ES immunoreactive outer membrane protein that is known to predominate in *A. phagocytophilum* cultured in HL60 cells (6). r47E and r60 show rP44-47E and rP44-60 proteins, respectively, that are dominantly transcribed in *A. phagocytophilum* propagated in THP-1 cells (2).

patients' serum reactive with *A. phagocytophilum* cultured in HL60 cells in IFA bound to rP44-18ES (online Technical Appendix Figure 4; Table). This finding strongly supports the results of IFA and Western blot analyses with the infected THP-1 and HL60 cells.

In Japan, rickettsioses such as Japanese spotted fever and scrub typhus, caused by *Rickettsia japonica* and *Orientia tsutsugamushi*, respectively, occur frequently. However, fever of unknown cause and rickettsiosis-like symptoms still occur in some patients. Detection of *A. phagocytophilum* in ticks was first reported in 2005 in central Japan (8). Since then, DNA of *A. phagocytophilum* has been detected in ticks inhabiting several places of Japan (9,10). However, little was known about human infection with *A. phagocytophilum* for many years, probably because of the poor selection of the culture cell line used as infected cell antigens for serodiagnosis. Our previous study first documented HGA in Japan and recommended that *A. phagocytophilum* propagated in THP-1 and in HL60 cells be used as antigens to avoid misdiagnosing cases of HGA. Our current study demonstrates the presence of specific antibodies against the central hyper-variable regions of P44-47E, P44-60, or P44-18ES proteins that predominate in infected THP-1 or HL60 cells, probably being suitable as protein antigens for serodiagnosis of HGA. The rP44-1 protein whose recombinant plasmid had previously been constructed for *E. coli* expression system may be available as well. Thus, our study provides substantial information about the usefulness of suitable P44 immunoreactive protein species of *A. phagocytophilum* as antigens for serodiagnosis of HGA.

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