U.S. National Institutes of Health awards AI57158, AI070411, AI067549, the U.S. Department of Defense and Google.org. ICDDR,B acknowledges with gratitude the commitment of CDC, the U.S. National Institutes of Health, and the Government of Bangladesh to the Centre's research efforts.

DECLARATION OF INTEREST

None.

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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 2011年8月25日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②乾燥抗 HBs 人免疫グロブリン ③ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の公表状況	PLoS Pathogens 2011; 7(7)	公表国 アメリカ	
販売名（企業名）	①ヘプスブリン筋注用 200 単位　（ベネシス） ②ヘプスブリン筋注用 1000 単位　（ベネシス） ③ヘプスブリン IH 静注 1000 単位　（ベネシス）					
研究報告の概要	アデノウイルスはヒトとサルを含む多くの脊椎動物に感染し、ヒトで広範囲の臨床疾患を引き起こす。特定株からの感染は、従来、種特異的であると考えられてきた。 カリフォルニア国立霊長類研究所(CNPRC)で、新世界猿(titi: サル)の閉鎖集団で集団発生した致命的な新規アデノウイルス(TMAdV:titi サル・アデノウイルス)を特定した。建屋内の 65 匹のサルの間で、23 匹(34%)が劇症の肺炎と肝炎が進行した上気道症状を発症し、うち 19 匹が死亡、または人道的に安楽死させた。 このアデノウイルスは新種で、他のアデノウイルスから高度に分岐し、ヌクレオチド対の同一性は 57%未満であることが TMAdV の全ゲノム配列で分かった。 集団発生の開始時、サルと最も接触した研究者は急性呼吸器疾患を発症し、回復期血清検体は TMAdV により血清陽性であった。また、81 人のランダムな成人献血者のスクリーニングは 2 人(2.5%)に TMAdV-特異の中和抗体を検出した。TMAdV の発見は、新規アデノウイルスが異種間集団発生の潜在的な原因として密接に監視する必要があることを示唆している。					
報告企業の意見			今後の対応			
アデノウイルスは二重鎖直鎖状DNAウイルスで、カプシドは直径約80nmの正20面体の球形粒子をしており、エンベロープは持たない。万一、原料血漿にアデノウイルスが混入したとしても、BMCおよびCPVをモデルウイルスとしたウイルスクリアランス試験結果から、本剤の製造工程において十分に不活化・除去されると考えている。			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。			

使用上の注意記載状況・その他参考事項等

代表としてヘプスブリン IH 静注 1000 単位の記載を示す。

2. 重要な基本的注意

(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1, HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜による濾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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Cross-Species Transmission of a Novel Adenovirus Associated with a Fulminant Pneumonia Outbreak in a New World Monkey Colony

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Abstract

Adenoviruses are DNA viruses that naturally infect many vertebrates, including humans and monkeys, and cause a wide range of clinical illnesses in humans. Infection from individual strains has conventionally been thought to be species-specific. Here we applied the Virochip, a pan-viral microarray, to identify a novel adenovirus (TMAdV, titi monkey adenovirus) as the cause of a deadly outbreak in a closed colony of New World monkeys (titi monkeys; *Callicebus cupreus*) at the California National Primate Research Center (CNPRC). Among 65 titi monkeys housed in a building, 23 (34%) developed upper respiratory symptoms that progressed to fulminant pneumonia and hepatitis, and 19 of 23 monkeys, or 83% of those infected, died or were humanely euthanized. Whole-genome sequencing of TMAdV revealed that this adenovirus is a new species and highly divergent, sharing <57% pairwise nucleotide identity with other adenoviruses. Cultivation of TMAdV was successful in a human A549 lung adenocarcinoma cell line, but not in primary or established monkey kidney cells. At the onset of the outbreak, the researcher in closest contact with the monkeys developed an acute respiratory illness, with symptoms persisting for 4 weeks, and had a convalescent serum sample seropositive for TMAdV. A clinically ill family member, despite having no contact with the CNPRC, also tested positive, and screening of a set of 81 random adult blood donors from the Western United States detected TMAdV-specific neutralizing antibodies in 2 individuals (2/81, or 2.5%). These findings raise the possibility of zoonotic infection by TMAdV and human-to-human transmission of the virus in the population. Given the unusually high case fatality rate from the outbreak (83%), it is unlikely that titi monkeys are the native host species for TMAdV, and the natural reservoir of the virus is still unknown. The discovery of TMAdV, a novel adenovirus with the capacity to infect both monkeys and humans, suggests that adenoviruses should be monitored closely as potential causes of cross-species outbreaks.

Citation: Chen EC, Yagi S, Kelly KR, Mendoza SP, Maninger N, et al. (2011) Cross-Species Transmission of a Novel Adenovirus Associated with a Fulminant Pneumonia Outbreak in a New World Monkey Colony. PLoS Pathog 7(7): e1002155. doi:10.1371/journal.ppat.1002155

Editor: Glen R. Nemerow, The Scripps Research Institute, United States of America

Received January 20, 2011; **Accepted** May 23, 2011; **Published** July 14, 2011

Funding: This work is supported in part by CNPRC NIH grant NCRR P51-RR000169, NIH grant HD053555 (to KLB, SPM, and WAM), NIH grants K08-AI074913 and R56-AI089532 (to CYC), and an Abbott Viral Discovery Award (to CYC). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors received a viral discovery award from Abbott Diagnostics (to CYC). The University of California, San Francisco (UCSF) has also filed a patent application related to TMAdV. This does not alter the authors' adherence to all PloS Pathogens policies on sharing data and materials.

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Introduction

Adenoviruses, first isolated in the 1950s from explanted adenoid tissue, are double-stranded nonenveloped DNA viruses that naturally infect many vertebrates, including humans and nonhuman primates. The human adenoviruses in the *Mastadenovirus* genus, comprised of all mammalian adenoviruses, are classified into 7 species A-G, and at least 51 different serotypes (and 5 proposed types, HAdV-52 to HAdV-56) have been described to date [1,2]. Adenoviruses are the cause of an estimated 5–10% of febrile illnesses in children worldwide [3]. Some serotypes, such as human adenovirus type 14 (HAdV-14), have been associated with severe and potentially fatal outbreaks of pneumonia in residential facilities and military bases [4]. Adenoviruses have also been associated with other clinical syndromes including conjunctivitis, hepatitis, and diarrhea [5]. In nonhuman primates, most epidemiologic studies of adenoviruses have focused on their identification in fecal samples from asymptomatic animals [6,7,8]. Overt respiratory disease associated with simian adenoviruses has also been observed [9]. Although adenoviruses are significant pathogens, genetically modified strains are being actively explored as potential vectors for vaccines and gene therapy [10].

Infection by adenoviruses has generally been thought to be species-specific. Human adenoviruses do not usually replicate in monkey cells in the absence of helper viruses [11], and do not productively infect rodents (and vice versa) [12]. Studies of sera from animal handlers and zoo workers exposed to chimpanzees in captivity fail to detect antibodies to chimpanzee adenoviruses [13,14]. However, recent serological surveys have found antibodies to New World and Old World monkey adenoviruses in donor human sera from regions where the monkeys are endemic [14,15]. In addition, phylogenetic analyses of adenoviruses from greater apes reveal that they fall precisely into "human" adenoviral species B, C, and E [7]. The high degree of sequence relatedness within members of each species suggests that at least some adenoviral strains may be capable of infecting both nonhuman primates and humans.

Beginning in May of 2009, a deadly outbreak of fulminant pneumonia and hepatitis occurred in a closed colony of New World titi monkeys of the *Callicebus* genus at the California National Primate Research Center (CNPRC). Routine microbiological testing for an infectious etiology was negative. We previously developed the Virochip (University of California, San Francisco) as a broad-spectrum surveillance assay for identifying viral causes of unknown acute and chronic illnesses [16,17,18,19,20,21,22]. The Virochip, a pan-viral microarray containing ~19,000 probes derived from all viral species in GenBank (n~2500) [21,23], has been previously successful in detection of novel outbreak viruses such as the SARS coronavirus [22,24] and the 2009 pandemic H1N1 influenza virus [23]. Here we apply the Virochip to identify a novel and highly divergent adenovirus as the cause of the titi monkey outbreak. In addition, we present clinical and serological evidence that this virus may have infected a researcher at the CNPRC and a family member, thus demonstrating for the first time the potential for cross-species infection by adenoviruses.

Author Summary

Infection from adenoviruses, viruses that cause a variety of illnesses in humans, monkeys, and other animals, has conventionally been thought to be species-specific. We used the Virochip, a microarray designed to detect all viruses, to identify a new species of adenovirus (TMAdV, or titi monkey adenovirus) that caused a deadly outbreak in a colony of New World titi monkeys at the California National Primate Research Center (CNPRC), and also infected a human researcher. One-third of the monkeys developed pneumonia and liver inflammation, and 19 of 23 monkeys died or were humanely euthanized. The unusually high death rate (83%) makes titi monkeys unlikely to be natural hosts for TMAdV, and the genomic sequence of TMAdV revealed that it is very different from any other known adenovirus. The researcher developed an acute respiratory illness at the onset of the outbreak, and was found to be infected by TMAdV by subsequent antibody testing. A clinically ill family member with no prior contact with the CNPRC also tested positive. Further investigation is needed to identify whether TMAdV originated from humans, monkeys, or another animal. The discovery of TMAdV suggests that adenoviruses should be monitored closely as potential causes of cross-species outbreaks.

Results

An outbreak of fulminant pneumonia in a titi monkey colony

In early 2009, the CNPRC housed 65 titi monkeys in one quadrant of an animal building. The index case, a healthy adult titi monkey, presented on May 14, 2009 with cough, lethargy, and decreased appetite (Fig. 1A, T1). Despite aggressive treatment with intravenous fluids and antibiotics, the animal developed severe respiratory distress and was humanely euthanized 5 days later. A second case presented 4 weeks later near the entrance to the building (Fig. 1A, T54). In the interim period, 3 healthy titi monkeys had been relocated from a separate building (Fig. 1A, T2, T3, and T19), with 2 of the 3 monkeys placed into the cage formerly occupied by the index case, reflecting a total at-risk population of 68. Over the ensuing 2 months, 21 additional monkeys, including one of the relocated monkeys, presented with clinical signs similar to those shown by the index case (attack rate = 23/68, or 34%) (Figs. 1A and 1B). Clinical signs in affected animals included cough, lethargy, poor appetite, tachypnea, and abdominal breathing. These symptoms progressed to overt respiratory distress and death or humane euthanasia within an average of 8 days. Chest radiographs typically revealed diffuse interstitial pulmonary changes and bronchoalveolar consolidation indicative of pneumonia, with right middle lobe predominance (Fig. 1C). Animals displaying clinical signs were quarantined and aggressively treated by veterinarians with supplemental oxygen, anti-inflammatory medications, bronchodilators (nebulized albuterol), broad-spectrum antibiotics, and antivirals (oseltamivir and/or ribavirin). In total, 19 animals died or were euthanized due to the illness during the outbreak (case fatality rate = 19/23, or 83%). Only 4 monkeys survived, even though the majority of sick animals (17/23, or 74%) consisted of apparently healthy adults and juveniles. Interestingly, none of the 133 rhesus macaques (*Macaca mulatta*) housed in the same building became sick during the outbreak, and neither did any of the Old World monkeys from surrounding outdoor colonies of rhesus and cynomolgus macaques (*Macaca fascicularis*).

Gross necropsy findings were similar in all titi monkeys and were characterized primarily by diffuse, consolidated pneumonias, with occasional evidence of fibrinous pleuritis, pericardial/pleural edema, and hemorrhage (Fig. 1D-1). Some livers, spleens, and lymph nodes were found to be abnormally enlarged. Hepatic necrosis and hemorrhage, along with ascites, were occasionally appreciated. On histologic examination, the normal cellular architecture of the lung and trachea was destroyed, and prominent intranuclear inclusion bodies were observed in the liver, lung, and trachea (Figs. 1D-2 and 1D-3).

A routine microbiological workup for infectious causes of the outbreak, including bacterial, mycoplasma, and fungal cultures, was negative. Respiratory viral testing failed to detect evidence of respiratory syncytial virus, adenovirus, influenza virus A and B, human metapneumovirus, and parainfluenza virus types 1, 2, and 3.

Virochip identification, PCR screening and electron microscopic (EM) confirmation of TMAdV

Given the clinical presentation of a severe acute viral respiratory illness and the appearance of intranuclear inclusion bodies on histological examination, we strongly suspected that a virus that had eluded detection by conventional assays was the cause of the titi monkey outbreak. Nasal, lung, and liver swab samples collected during necropsy were analyzed using the Virochip [21,23]. Microarrays were analyzed using ranked Z-scores to assess the highest-intensity viral probes [18]. From a lung swab sample from an affected monkey, 4 of the top 80 probes on the Virochip corresponded to adenoviruses. Other viruses or viral families with ≥4 probes among the top 80, including chimpanzee herpesvirus (*Herpesviridae*), bovine viral diarrhea virus (*Flaviviridae*), and endogenous retroviruses (*Retroviridae*), were regarded as less likely to cause fulminant pneumonia and hepatitis, so were not pursued any further. The 4 adenovirus probes mapped to 2 different gene regions corresponding to the DNA polymerase and penton base

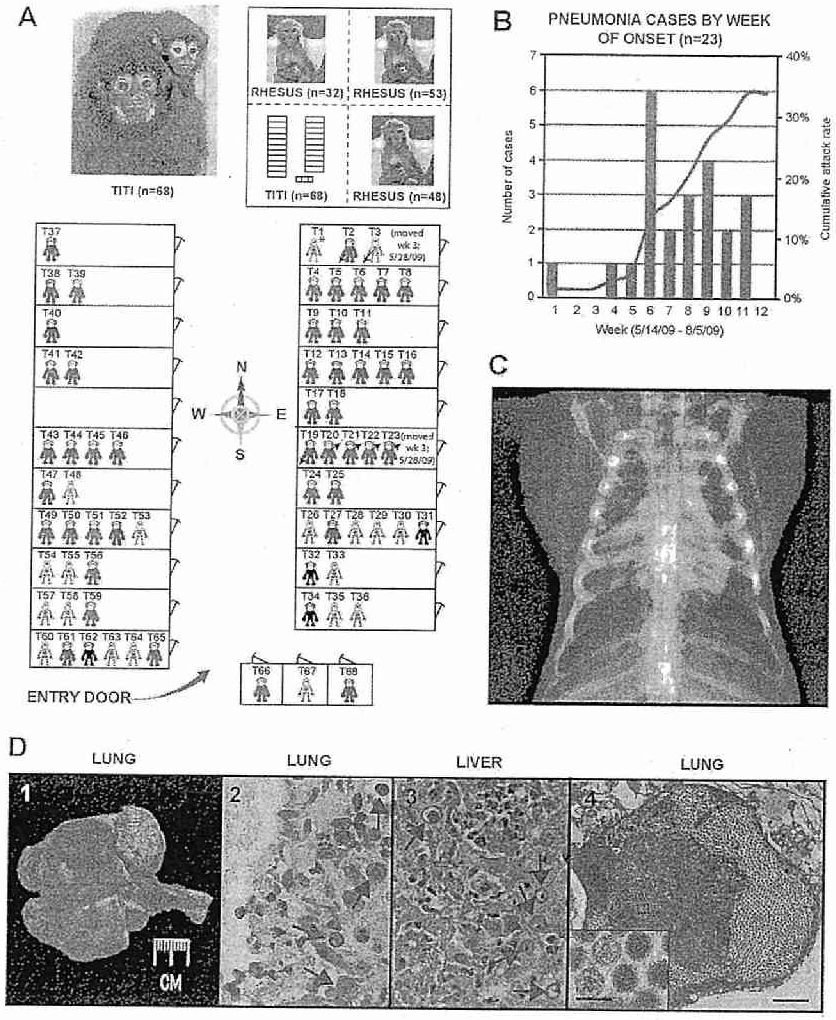

Figure 1. Clinical and epidemiologic features of the titi monkey outbreak. (**A**) Map of the titi monkey cages situated in one quadrant of a building, showing the locations of asymptomatic, at-risk monkeys (brown or green), affected surviving monkeys (black), and monkeys who died from their illness (skeleton). 3 monkeys were moved into the building (arrows pointing down and to the left) and 4 monkeys out of the building (arrows pointing up and to the right) during the 3rd week of the outbreak. The upper left photograph shows an image of an adult male titi monkey and his infant. The upper right inset shows the location of the titi monkey cages relative to other rhesus monkey cages in the building. Asymptomatic monkeys with positive serum antibody titers to TMAdV 4 months after the outbreak are shown in green. (**B**) Epidemic curve of the outbreak, with the number of cases in blue and cumulative attack rate in red. (**C**) Anteroposterior chest radiograph of an affected titi monkey, showing bilateral basilar infiltrates and a prominent right middle lobe consolidation. (**D**) *1* – gross photograph of lungs at necropsy; the lungs failed to fully collapse upon opening the chest, and a single ~1.5 cm focus of dark red discoloration (hemorrhage) can be seen in the left caudal lobe. *2* – photomicrograph of H&E stained lung tissue showing a severe diffuse necrotizing bronchopneumonia characterized by the presence of hemorrhage and intranuclear inclusions (arrows). *3* – photomicrograph of H&E stained liver tissue showing a multifocal necrotizing hepatitis with numerous intranuclear inclusions (arrows). *4* – transmission electron micrograph of an affected lung alveolus (scale bar = 1 μm) filled with adenovirus-like particles (inset, scale bar = 0.1 μm).

doi:10.1371/journal.ppat.1002155.g001

(Fig. 2A). Interestingly, the 4 viral probes were derived from 2 different *Adenoviridae* genera (SAdV-23, simian adenovirus 23, PAdV-A, porcine adenovirus A, and HAdV-5, human adenovirus 5, in the *Mastadenovirus* genus; FAdV-D, fowl adenovirus D, in the *Aviadenovirus* genus), suggesting the presence of a divergent adenovirus that was not a member of any previously known species.

To confirm the Virochip finding of an adenovirus, we used consensus primers to amplify a 301 bp fragment from the hexon gene by PCR [25]. The fragment shared ~86% nucleotide identity with its closest phylogenetic relatives in GenBank, SAdV-18, an Old World vervet monkey adenovirus, and the human species D adenoviruses. The newly identified adenovirus was designated TMAdV, or titi monkey adenovirus. Specific PCR for TMAdV was then used to screen body fluids and tissues from affected monkeys (Table 1). PCR results were positive from post-necropsy liver and lung tissues as well as from sera, conjunctival swabs, oral swabs, and nasal swabs collected at time of quarantine in 8 different affected monkeys, but were negative from a throat swab from an asymptomatic animal whose other 5 cage mates had become sick. In addition, nasal swabs were negative in 3 asymptomatic, minimal-risk titi monkeys housed in a separate building. To confirm the presence of virus in diseased tissues, we examined lung tissue from affected monkeys by transmission electron microscopy, revealing abundant icosahedral particles characteristic of adenovirus filling the alveoli (Fig. 1D-4).

Next, to assess persistent subclinical infection from TMAdV, we analyzed serum samples from at-risk asymptomatic or affected surviving monkeys 2 months after the outbreak (n = 41). All post-outbreak serum samples were negative for TMAdV by PCR (Table 1). To assess potential TMAdV shedding, stool samples collected from all cages housing titi monkeys 2 months post-outbreak were analyzed by PCR (n = 27), and were found to be negative. In addition, we checked for TMAdV in rectal swab samples from rhesus macaques housed in the same building as the titi monkeys (n = 26) and in pooled droppings from wild rodents (n = 2) living near the titi monkey cages. All macaque and rodent fecal samples were negative for TMAdV by PCR.

We also sought to determine whether PCR assays commonly used to detect human adenoviruses in clinical or public health settings could detect TMAdV. Adenovirus PCR was performed on a TMAdV-positive clinical sample, a TMAdV culture, and a human adenovirus B culture (as a positive control) using an additional 5 pairs of primers, according to previously published protocols [26,27,28] Three of the 5 primer pairs, designed to detect human respiratory adenoviruses B, C, and E, failed to amplify TMAdV [27]. The remaining 2 pairs of primers, both derived from highly conserved sequences in the hexon gene [26,28], were able to detect TMAdV in culture as well as directly from clinical material.

Whole-genome sequencing, features, and phylogenetic analysis of TMAdV

To facilitate whole-genome sequencing of TMAdV, deep sequencing of a lung swab from one affected titi monkey and lung tissue from another affected monkey was performed. Out of ~11.9 million high-quality reads, 2,782 reads and 3,767 reads aligned to the SAdV-18 genome by BLASTN (Fig. 2B, blue) and TBLASTX (Fig. 2B, transparent blue), respectively, with reads mapping to sites across the genome. *De novo* assembly of the complete TMAdV genome from reads that aligned to SAdV-18 was not possible due to insufficient sequence coverage (<46%). The poor apparent coverage was the result of high sequence divergence of the TMAdV genome from SAdV-18, which hindered the identification of most of the 16,524 actual deep sequencing reads derived from TMAdV (Fig. 2B, red). Thus, after partial assembly of TMAdV using overlapping reads aligning to the SAdV-18 genome, remaining gaps were closed by specific PCR. The complete genome of TMAdV was found to be 36,842 base pairs in length, with a base composition of 20.8% A, 29.8% C, 29.8% G, and 19.6% T, and a GC content of 59.6%, comparable to that of adenoviral species Groups C, D, and E in the *Mastadenovirus* genus. The deduced genomic structure of TMAdV was also similar to that of other mastadenoviruses and consists of 34 open reading frames (Fig. 2C).

Whole-genome phylogenetic analysis placed TMAdV in an independent species group separate from the known human adenoviral species A–G (Fig. 3). Among all 95 fully-sequenced adenovirus genomes in GenBank, the closest simian adenoviral relatives to TMAdV were SAdV-3, SAdV-18, and SAdV-21, with pairwise nucleotide identities ranging from 54.0% to 56.3% (Fig. 4). The closest human adenoviral relatives were the species D adenoviruses, which share 54.3% to 55.1% identity to TMAdV, with human adenoviruses of other species slightly less similar (51.1%–54.6%). The placement of TMAdV into a separate group by phylogenetic analysis was also observed when looking individually at the hexon, polymerase, penton base, and fiber genes (Fig. S1). Scanning nucleotide pairwise identity plots revealed that, among the major adenovirus genes, the DNA polymerase and hexon are more conserved, whereas the E1A and fiber are more divergent (Fig. 4). The significant overall sequence divergence of TMAdV from known human and simian adenoviruses is highlighted by the finding that PAdV-A (porcine adenovirus A), a non- primate mammalian adenovirus, shared only a slightly less similar whole-genome pairwise identity to TMAdV of 47.0% (Fig. 4). In fact, in the DNA polymerase gene, TMAdV shared a pairwise identity with PAdV-A of 67.2%, comparable to its pairwise identities with the other human adenoviruses, 59%–71.7% (Figs. 4 and S1). Although TMAdV was found to be highly divergent from other adenoviruses, different isolates of TMAdV from 3 affected titi monkeys were remarkably conserved, sharing 100% identity across the full-length hexon gene (data not shown).

The high level of sequence divergence in TMAdV held true at the amino acid level as well, with amino acid identities relative to other mastadenoviruses ranging from 20.8% to 27.5% for the fiber, the most divergent protein, to 68.7%–78.2% for the hexon (Table 2). Although bearing low sequence similarity to other adenoviruses, the penton base of TMAdV contained an RGD motif that presumably binds α_v integrins. By both nucleotide and amino acid comparisons, the closest phylogenetic relative to

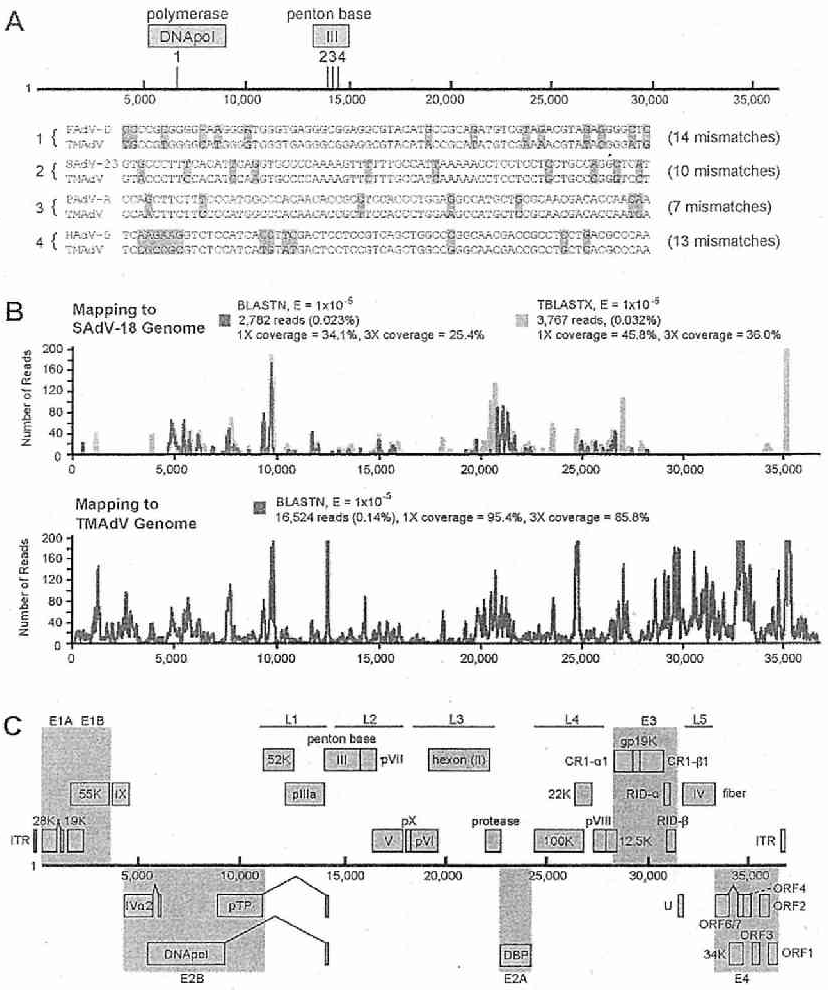

Figure 2. Discovery and whole-genome characterization of the novel adenovirus TMAdV. **(A)** The locations of the 4 Virochip probes derived from adenovirus sequences and used to detect TMAdV are mapped onto the ~37 kB genome. The 4 Virochip probe sequences are also aligned with the corresponding sequence in the TMAdV genome, with mismatches highlighted in pink. **(B)** Coverage map of deep sequencing reads corresponding to TMAdV using BLASTN (blue) and TBLASTX (transparent blue) alignments to SAdV-18. The actual coverage achieved by deep sequencing as determined by alignments to the fully sequenced genome of TMAdV is much higher (red). **(C)** Genome organization of TMAdV. Predicted protein coding regions are shown as boxes. Boxes above the central black line represent open reading frames (ORFs) that are encoded on the forward strand, while boxes underneath the black line represent reverse-strand encoded ORFs. Early region ORFs are shaded in gray. The x-axis refers to the nucleotide position along the ~37k genome of TMAdV. Abbreviations: FAdV, fowl adenovirus; SAdV, simian adenovirus; PAdV, porcine adenovirus; HAdV, human adenovirus, TMAdV, titi monkey adenovirus.
doi:10.1371/journal.ppat.1002155.g002

Table 1. PCR screening for TMAdV.

Sample	Sample Type	PCR Result	Date Presenting with Clinical Signs	Date of Necropsy
Affected, at-risk titi monkeys (died)				
T1	serum[1]	–	5/14/2009	5/19/2009
T26	serum[1]	+	7/23/2009	7/30/2009
T28	conjunctival swab[1]	+	7/16/2009	7/25/2009
	nasal swab[1]	+		
	liver swab[1]	+		
	lung swab[1]	+		
T29	serum[2]	+	7/26/2009	7/31/2009
T30	serum[2]	–	7/25/2009	7/30/3009
T33	lung swab[1]	+	6/23/2009	6/29/2009
	nasal swab[1]	+		
T36	lung swab[1]	+	7/7/2009	7/14/2009
	lung swab[1]	+		
T60	serum[2]	–	7/15/2009	7/22/2009
T63	serum[1]	–	6/20/2009	8/1/2009
T67	nasal swab[#]	–	7/7/2009	8/13/2009
	nasal swab[1]	+		
Affected, at-risk titi monkeys (survived)				
T31	serum[*]	–	7/10/2009	N/A
T32	serum[*]	–	7/12/2009	N/A
T34	serum[*]	–	6/23/2009	N/A
T62	serum[*]	–	7/8/2009	N/A
Asymptomatic at-risk and minimal-risk titi monkeys				
T27	throat swab (n = 1)[§]	–	N/A	N/A
at-risk titi	stool from cages (n = 14)[*]	–	N/A	N/A
at-risk titi	serum (n = 29)[*]	–	N/A	N/A
minimal-risk titi	oral swab (n = 3)[§]	–	N/A	N/A
minimal-risk titi	stool from cages (n = 5)[*]	–	N/A	N/A
minimal-risk titi	serum (n = 8)[*]	–	N/A	N/A
minimal-risk titi	stool from cages (n = 8)[*]	–	N/A	N/A
minimal-risk titi	serum (n = 8)[*]	–	N/A	N/A
Other				
rhesus	rectal swabs (n = 20)[*]	–	N/A	N/A
human	serum (n = 15)[**]	–	N/A	N/A
rodent	droppings (n = 2)[*]	–	N/A	N/A

For titi monkey cage designations (TXX), please refer to Fig. 1.
[1]initial case.
[#]collected prior to outbreak.
[§]collected during outbreak.
[*]collected 2 months after outbreak.
[**]collected 4 months after outbreak.
doi:10.1371/journal.ppat.1002155.t001

TMAdV in GenBank overall was SAdV-3 (Fig. 4; Table 2). Bootscanning analysis revealed no evidence for recombination of TMAdV with other adenoviruses at either the whole-genome or individual gene level (Fig. S2).

The main neutralization determinant for adenoviruses, the epsilon determinant (ε), is formed by loops 1 and 2 in the hexon protein [29]. The epsilon determinant of TMAdV was significantly divergent from that of other mastadenoviruses, with amino acid identities in loop 1 varying from 30.6% to 44.8% and in loop 2 varying from 54.4% to 67.0% (Table 2). This observation suggested that cross-neutralization of TMAdV with sera reactive against other human/simian adenoviruses is unlikely.

Cultivation of TMAdV in human and monkey cells

We next attempted to culture TMAdV in an A549 (human lung adenocarcinoma) cell line, a BSC-1 (African green monkey kidney epithelial) cell line, and PMK (primary rhesus monkey kidney) cells (Fig. 5). Direct inoculation of cell cultures with a lung swab sample from an affected titi monkey produced a weak initial cytopathic

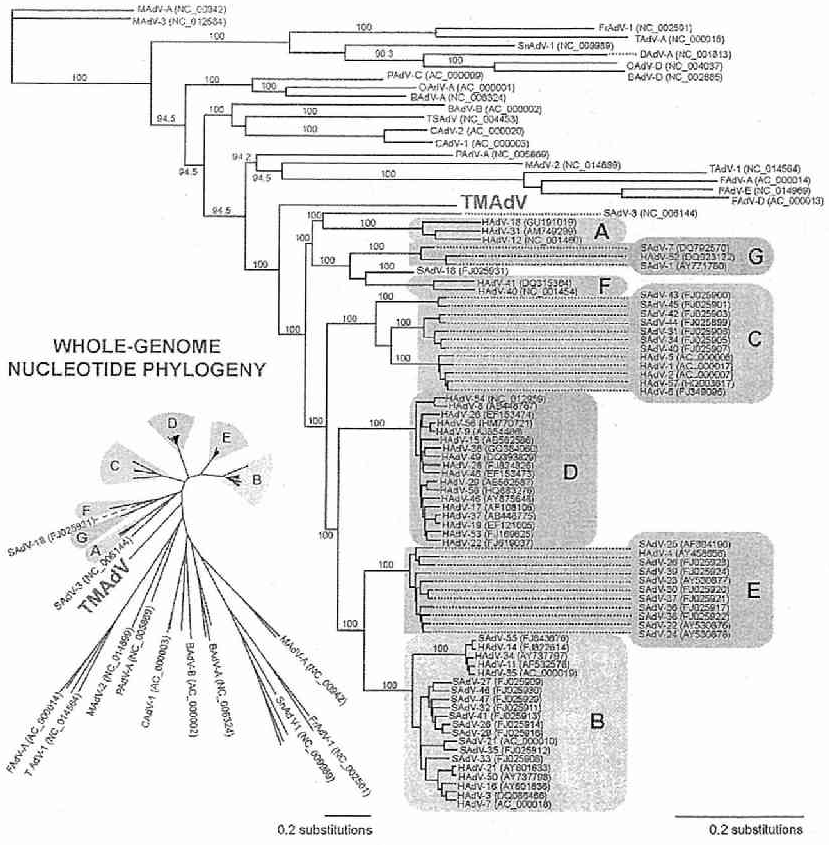

Figure 3. Whole-genome phylogenetic analysis of TMAdV. The whole-genome nucleotide phylogenetic tree is reconstructed from a multiple sequence alignment of all 95 unique, fully-sequenced adenovirus genomes in GenBank and TMAdV. Both rectangular cladogram and radial tree layouts are displayed. The branch corresponding to TMAdV is highlighted in boldface red. Abbreviations: HAdV, human adenovirus; SAdV, simian adenovirus; MAdV, mouse adenovirus, FrAdV, frog adenovirus; TAdV, turkey adenovirus; SnAdV, snake adenovirus; DAdV, duck adenovirus; OAdV, ovine adenovirus; BAdV, bovine adenovirus; PAdV, porcine adenovirus; TSAdV, tree shrew adenovirus; CAdV, canine adenovirus.
doi:10.1371/journal.ppat.1002155.g003

effect in macaque BSC-1 and human A549 cells at day 7. However, despite multiple serial passages, we were unable to propagate the infected cell culture supernatant in either BSC-1 or PMK cells. In contrast, propagation in human A549 cells resulted in viral adaptation by passage 6 and generation of a fully adapted strain of TMAdV by passage 10 that was able to productively infect all 3 cell lines. Thus, culturing and propagation of TMAdV were successful in a human A549 cell line, but not in established or primary monkey kidney cell lines.

An influenza-like illness in a researcher and family members during the titi monkey outbreak

In hindsight, only one individual at the CNPRC reported becoming ill during the titi monkey outbreak, the researcher in closest, daily contact with the animals. Symptoms began near the onset of the outbreak, although whether they began prior to or after identification of the index case is unclear. The researcher, with a past medical history of multiple sclerosis, initially developed symptoms of a viral upper respiratory infection (URI), including

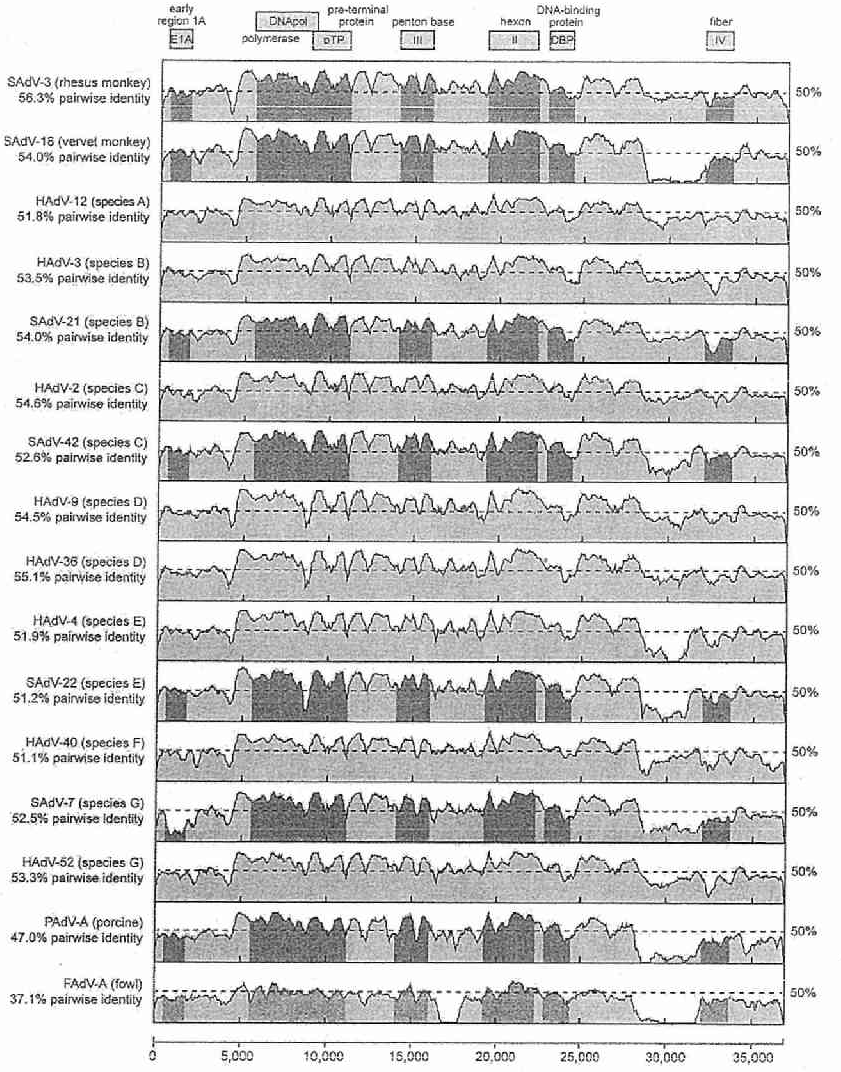

Figure 4. Scanning pairwise alignment of representative adenoviruses with TMAdV. The scanning nucleotide pairwise identities of TMAdV relative to representative human (yellow) or simian (brown) adenoviruses in species A–G, porcine adenovirus (red), and fowl adenovirus (green) are shown. The window size is 400 bp with a step size of 40 bp. The x-axis refers to the nucleotide position along the ~37 k genome of TMAdV. Abbreviations: HAdV, human adenovirus; SAdV, simian adenovirus; PAdV, porcine adenovirus; FAdV, fowl adenovirus.
doi:10.1371/journal.ppat.1002155.g004

Table 2. Amino acid identity of TMAdV relative to other adenoviruses.

	fiber (IV)	E1A	DBP	DNApol	penton base (III)	pTP	hexon (II) whole	hexon (II) ε:L1	hexon (II) ε:L2
SAdV-3 (rhesus monkey)	26.6%	29.9%	37.9%	61.8%	68.2%*	70.6%*	78.2%*	44.8%*	67.0%*
SAdV-18 (vervet monkey)	26.0%	30.7%	39.1%	63.2%	66.5%	68.3%	76.7%	39.1%	63.9%
HAdV-12 (species A)	26.4%	31.0%	38.2%	60.4%	64.3%	67.6%	76.2%	40.1%	64.9%
HAdV-3 (species B)	22.3%	31.4%	36.5%	61.7%	65.8%	68.8%	73.7%	38.3%	63.9%
SAdV-21 (species B)	22.2%	30.6%	36.0%	62.1%	66.1%	68.8%	72.2%	34.1%	61.2%
HAdV-2 (species C)	25.0%	32.6%*	39.5%	62.5%	67.0%	68.0%	71.4%	39.1%	61.4%
SAdV-42 (species C)	26.4%	30.5%	38.8%	62.5%	66.5%	68.1%	72.4%	37.4%	63.3%
HAdV-9 (species D)	21.9%	28.4%	38.3%	63.4%	66.1%	68.3%	74.0%	33.3%	60.0%
HAdV-36 (species D)	20.8%	30.0%	38.1%	63.3%	65.9%	68.3%	73.6%	33.1%	61.9%
HAdV-4 (species E)	26.3%	32.6%*	37.1%	62.8%	67.9%	69.8%	72.7%	30.6%	63.3%
SAdV-22 (species E)	27.5%*	31.2%	36.8%	62.9%	67.6%	70.4%	74.2%	38.6%	63.9%
HAdV-40 (species F)	26.9%	31.0%	40.1%*	62.1%	64.1%	65.9%	77.1%	42.6%	66.3%
SAdV-7 (species G)	25.5%	32.6%*	34.9%	63.3%	67.0%	68.0%	76.4%	43.8%	59.6%
HAdV-52 (species G)	24.1%	30.3%	35.6%	63.8%*	67.8%	67.9%	77.0%	46.2%*	62.6%
PAdV-A (porcine)	26.4%	23.6%	37.4%	55.4%	61.7%	57.7%	68.7%	36.4%	54.4%
FAdV-A (fowl)	1.6%	N/A	25.8%	36.5%	41.6%	31.7%	47.9%	22.3%	39.0%

The amino acid sequences of selected TMAdV proteins and the epsilon determinant of the hexon (ε: L1, loop 1, and ε: L2, loop 2) are compared to the corresponding proteins from representative human, simian, porcine, and fowl adenoviruses.
*For each protein, the entry corresponding to the adenoviral species with the highest percentage identity relative to TMAdV.
doi:10.1371/journal.ppat.1002155.t002

fever, chills, headache, and sore throat, followed by a dry cough and "burning sensation in the lungs" that was exacerbated by a deep breath or coughing. The researcher endorsed a history of recurrent upper respiratory infections, and did not regard the illness as related to the titi monkey outbreak. Although symptoms persisted for 4 weeks, at no time did the researcher seek medical care, and no antibiotics were taken during the illness.

We carried out contact tracing to identify family members and other individuals in close contact with the researcher. Interestingly, two family members in the household also developed flu-like symptoms about 1–2 weeks after the researcher initially became sick. Their symptoms – fever, cough and muscle aches – appeared milder than those of the researcher and completely resolved within 2 weeks. Neither individual sought medical care for these symptoms, and notably, neither had ever visited the CNPRC.

Seroprevalence of TMAdV in monkeys and humans

To explore a potential link between the outbreak and associated illness in humans, we blindly tested available sera from titi monkeys (n = 59), rhesus macaques housed in the same building (n = 36), CNPRC personnel and close contacts (n = 20), and random human blood donors (n = 81) for evidence of recent or prior infection by TMAdV by virus neutralization (Fig. 6). Nineteen serum samples from 15 at-risk affected (symptomatic) titi monkeys were tested. Among 3 affected titi monkeys surviving the outbreak, 2 monkeys mounted a vigorous neutralizing Ab response to TMAdV, with negative pre-outbreak Ab titers (<1:8) but antibody titers 2 months after the outbreak of >1:512, while 1 monkey exhibited a positive but much weaker response. Affected titi monkeys who died during the outbreak exhibited a wide range of neutralizing Ab titers, from <1:8 to >1:512 (those without Ab likely died before mounting a response).

To investigate the possibility of subclinical infection by TMAdV, we also examined serum samples from asymptomatic titi monkeys (n = 40) and nearby rhesus macaques (n = 36), collected 2 months after the outbreak. Fourteen of 40 asymptomatic titi monkeys tested (35%) had antibody to TMAdV, indicating that the incidence of subclinical infection was significant (Fig. 1A; Fig 6). In fact, one of the 14 asymptomatic titi monkeys with positive Ab titers was located in the minimal-risk building. In contrast, only 1 of 36 rhesus macaque samples was positive, with an Ab titer of 1:16. The 1 antibody-positive rhesus serum sample was negative by specific PCR for TMAdV (data not shown), as was stool from the cage in which the rhesus monkey was housed (Table 1).

Approximately 4 months after the outbreak, serum samples were collected from CNPRC personnel in direct contact with the titi monkeys. Serum samples were also collected from the two family members of the clinically ill CNPRC researcher 1 year after the outbreak. Only two samples were found positive for neutralizing Abs to TMAdV: (1) Ab titers for the clinically ill researcher were 1:32, and (2) Ab titers for one of the family members of the clinically ill researcher were 1:8.

Among 81 random blood donors from the Western United States, 2 individuals (2/81, 2.5%) had positive Ab titers of 1:16 and 1:8. Pooled rabbit sera containing antibodies to human adenovirus serotypes 1 through 35, representing species A–E, were unable to neutralize TMAdV (data not shown). Thus, the results of our serological survey appear unlikely to be due to nonspecific cross-reactivity from prior exposure to known human adenoviruses.

Discussion

In this study, we employed a pan-viral microarray assay, the Virochip, to identify a novel adenovirus associated with a fulminant pneumonia outbreak in a colony of New World titi monkeys. Despite the absence of an animal model, which precludes a strict fulfillment of Koch's postulates, there are several lines of evidence implicating this novel adenovirus, TMAdV, as

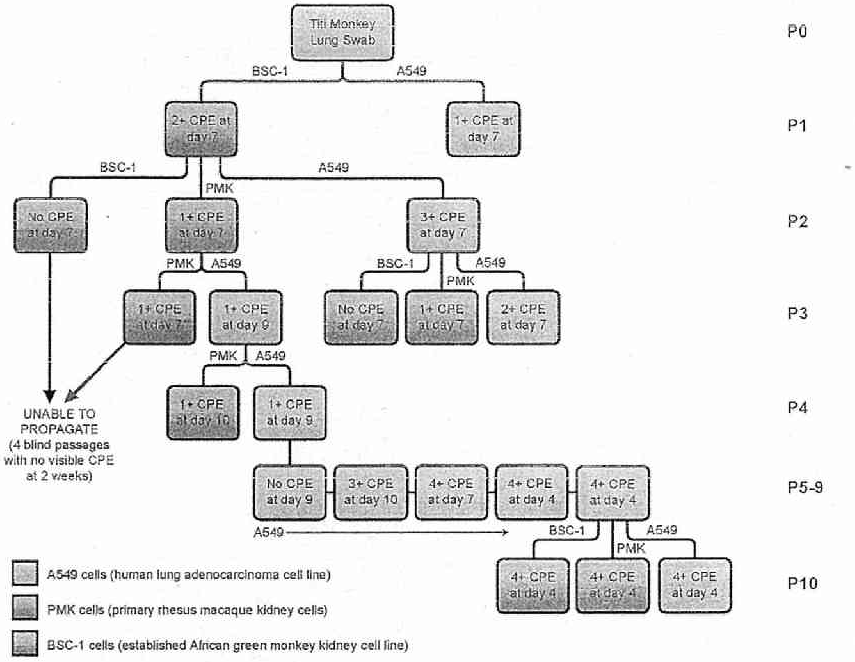

Figure 5. Growth and propagation of TMAdV in cell culture. The flow chart displays 10 passages (P1–P10) of TMAdV cultured in human lung adenocarcinoma (A549, orange), primary rhesus macaque kidney (PMK, brown), or established African green monkey kidney (BSC-1, green) cells.
doi:10.1371/journal.ppat.1002155.g005

the cause of the outbreak. First, conventional testing for other pathogens, including other viruses by Virochip, was negative, and affected monkeys did not respond to empiric therapy with antibiotics or antivirals (ribavirin and oseltamivir in anecdotal use are not effective against adenoviral infections) [30]. Second, the clinical presentation of pneumonia and hepatitis is consistent with the known spectrum of disease associated with adenoviral infections. Third, TMAdV sequence was recovered by PCR in various body fluids and tissues from affected monkeys, including blood, respiratory secretions, and lung/liver tissue (Table 1). Fourth, the finding of intranuclear inclusions in diseased tissues, as well as direct visualization of adenoviral-like particles (TMAdV) in lung alveoli by electron microscopy (Figs. 1D-2 to 1D-4), support a primary role for TMAdV in the pathogenesis of tissue injury in affected monkeys. Finally, there was a significant neutralizing Ab response in surviving animals, with 2 monkeys having titers undetectable prior to the outbreak but rising to >1:512 at convalescence (Fig. 6).

Although TMAdV retains the core genomic features common to all adenoviruses (Fig. 2C), phylogenetic analysis clearly places TMAdV within a separate branch, with no closely related neighbors (Figs. 3 and S1). A phylogenetic distance of >10% combined with the lack of cross-neutralization defines TMAdV as a new species [31]. Since emerging adenovirus strains such as HAdV-14 and HAdV-D22/H8 (otherwise known as HAdV-D53) are known to arise from recombination events among related ancestral strains [32,33], we performed bootscanning analysis to look for such events in TMAdV. The bootscanning analysis, however, failed to show evidence of recombination, likely because closely related and/or ancestral strains to TMAdV have not yet been identified.

Entry of adenoviruses into cells involves an initial attachment of the fiber knob to the cell receptor, followed by internalization via a secondary interaction of the penton base with α_v integrins [34,35]. The presence of an RGD motif in the TMAdV penton base implies that the virus uses α_v integrins for internalization [35]. However, the high sequence divergence in the fiber protein (Table 2), as well as the absence of fiber motifs conserved among adenoviruses that bind CAR [36,37] (coxsackievirus-adenovirus receptor) or CD46 [38,39,40] (data not shown), suggest that neither of these two human adenoviral receptors may be the attachment receptor for TMAdV. Further studies will be necessary to identify the preferred cellular attachment and internalization receptors for TMAdV.

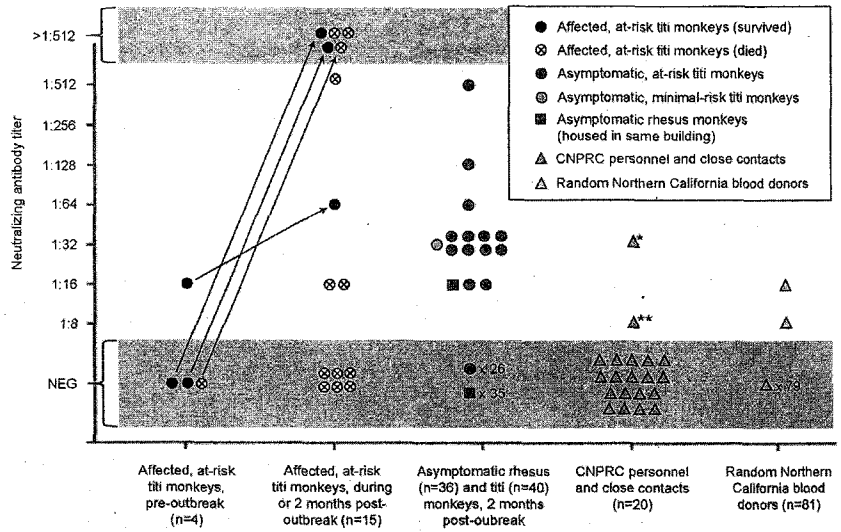

Figure 6. Seroprevalence of TMAdV in humans and monkeys. Sera from titi monkeys (circles), rhesus macaques (squares), and humans (triangles) were tested for antibodies to TMAdV by virus neutralization. Arrows designate pre-outbreak and post-outbreak serum samples from the same individual monkey. Pre-outbreak serum samples were previously banked in 2007. Sera from CNPRC personnel and close contacts (orange triangles) were collected 4 months post-outbreak, except for the two family members of the clinically ill researcher, whose sera were collected 1 year post-outbreak. *, clinically ill researcher; **, family member of the researcher, who was also sick. Abbreviations: CNPRC, California National Primate Research Center; NEG, negative.
doi:10.1371/journal.ppat.1002155.g006

Despite its isolation from affected titi monkeys, we were unable to propagate TMAdV in both established (BSC-1) and primary (PMK) monkey kidney cells (Fig. 4). The virus, however, grew efficiently in a human A549 lung adenocarcinoma cell line. One explanation for this finding is that TMAdV may be unable to productively infect cells derived from Old World monkeys (e.g. rhesus and African green monkeys). An alternative possibility is that successful propagation of TMAdV may depend on infection of a specific host cell type, such as A549 lung, and not BSC-1 or PMK kidney cells. Nevertheless, after 10 passages in human A549 cells, the fully adapted strain of TMAdV exhibits an extended host range with the ability to productively infect both monkey and human cells. This observation implies that TMAdV possesses an inherent capacity to cross the species barrier and infect both humans and nonhuman primates. Efforts to identify host range and cell tropism of TMAdV, as well as the specific sequence changes responsible for adaptation to growth in cell culture, are currently underway.

The virulence of TMAdV in healthy and apparently immunocompetent titi monkeys (83% case fatality rate) is highly unusual for infections by adenovirus. In humans, deaths due to adenovirus infections or outbreaks are generally low (up to 18% for pneumonia associated with HAdV-14 [4]). Furthermore, severe infections from human adenoviruses are more commonly associated with older age, immunosuppression, and chronic underlying conditions such as kidney failure [4,41]. Young, healthy individuals are in general much less likely to succumb to adenoviral-related illness. The severity of TMAdV-related illness in affected titi monkeys suggests that this species of monkey may not be the natural host for the virus. The failure to detect fecal shedding of TMAdV in convalescent or asymptomatic animals also suggests that the virus does not normally infect titi monkeys (Table 1).

Although the exact origin of TMAdV remains unclear, we can speculate on several possibilities. One possibility is that a cross-species "jump" from captive macaques to a susceptible colony of titi monkeys precipitated the outbreak. As there have been no new introductions of monkeys into the closed colony for the past 2 years, this conjecture relies on asymptomatic infection and transmission of TMAdV in the captive rhesus/cynomolgus macaque population at the CNPRC. CNPRC personnel who visited macaque areas would occasionally enter titi rooms with no change in personal protective equipment, thus providing a potential route of transmission for the virus. In addition, specific antibodies were detected in 1 of 36 (2.8%) asymptomatic rhesus macaques housed in the same building (Fig. 6), indicating that TMAdV has the capacity to infect this species of Old World monkey. Notably, the closest identified phylogenetic relative to TMAdV among the complete genomic sequences available in GenBank is a rhesus monkey adenovirus, SAdV-3 (Fig. 4; Table 2). Furthermore, serological evidence for cross-species adenoviral transmission events between different nonhuman primate species has been previously reported in the literature [42].

Although we failed to detect TMAdV in rodent droppings found near titi monkey cages (Table 2), it is still possible that the virus arose from an unknown animal reservoir. In this regard, the high sequence divergence of TMAdV relative to the known human/simian adenoviruses (Fig. 3), and comparable sequence similarity in the polymerase gene to a porcine adenovirus (Figs. 3 and S1) are striking. The four-week interval between the index case and the second case appears overly long given a typical incubation period for adenovirus infections of no more than 1 week [43]. This may be explained by our finding of a high rate of subclinical infection by TMAdV in asymptomatic titi monkeys (35%), but may also be due to separate introductions of TMAdV into the colony from an as-yet unidentified reservoir.

Our study data also support the potential for cross-species transmission of TMAdV between monkeys and humans. The researcher's fever, cough, and pleuritic symptoms ("burning sensation in the lungs") are consistent with the development of a prolonged viral respiratory illness. Interestingly, pleurisy has been specifically reported in association with certain human adenovirus infections [44]. The clinical presentation, time of illness concurrent with the onset of the outbreak, and presence of neutralizing Abs in convalescent serum all strongly point to primary infection of the researcher by TMAdV. The detection of weakly neutralizing Abs (1:8) in a serum sample from a sick family member of the researcher also suggests that TMAdV may be capable of human-to-human transmission. The decreased levels of neutralizing Abs to TMAdV in the researcher (1:32) and a family member (1:8) relative to those in infected titi monkeys (up to >1:512) are consistent with a recent study showing much higher levels of neutralizing antibodies in chimpanzees than in humans with adenovirus infections, possibly due to more robust adenovirus-specific T-cell responses in humans than in monkeys [45].

Several lines of evidence support the contention that the direction of TMAdV transmission was zoonotic (monkeys to humans) rather than anthroponotic (humans to monkeys). First, the closest known relative to TMAdV in GenBank is SAdV-3, an Old World monkey adenovirus (Fig. 3; Table 2). Second, our results show that PCR assays for human adenoviruses in common use are capable of detecting TMAdV. Although sequencing of PCR amplicons for human adenoviruses is not performed routinely in diagnostic virology, TMAdV would presumably have been detected previously in large-scale studies of hexon sequencing of Ad field isolates if it were circulating in the community [46,47]. Finally, the available sequence data in GenBank is heavily biased towards human adenoviruses, and much less is known about the potential diversity of the simian adenoviruses. We also cannot formally exclude the possibility that the outbreak arose from anthroponotic transmission. In our study, 2 of 81, or 2.5% of random adult blood donors exhibited borderline titers of neutralizing antibody to TMAdV, indicating either a low prevalence of TMAdV in the human population or cross-reactivity to a related virus (although no evidence of cross-reactivity was found with HAdV serotypes 1 through 35). Future large-scale studies of TMAdV seroepidemiology will be needed to better understand transmission of TMAdV between monkeys and humans. Nevertheless, our discovery of TMAdV, a novel adenovirus with the capacity to cross species barriers, highlights the need to monitor adenoviruses closely for outbreak or even pandemic potential.

Materials and Methods

Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The use and care of all animals followed policies and guidelines established by the University of California, Davis Institutional Animal Care and Use Committee (IACUC) and CNPRC (Animal Welfare Assurance #A3433-01). The protocol for the maintenance and breeding of the titi monkey colony was approved by the University of California, Davis IACUC (Protocol #15730). No specific animal research protocol was drafted for this study, as only excess clinical samples were analyzed for diagnostic purposes. Animals in extreme respiratory distress were humanely euthanized by veterinarians. Extensive veterinary care was provided to all animals affected by the outbreak in order to minimize pain and distress.

Serum samples from staff at the CNPRC, close contacts, and random adult blood donors were collected under protocols approved by institutional review boards of the University of California, Davis (Protocol #200917650-1) and University of California, San Francisco (Protocol #H49187-35245-01). Specifically, written informed consent was obtained from staff at the CNPRC and close contacts for analysis of their samples. Any potentially identifying information has been provided with the explicit permission of the individuals involved.

Sera from random blood donors were obtained from the Blood Systems Research Institute (San Francisco, CA); sera were derived from affiliated donor banks in California (Blood Centers of the Pacific, San Francisco, CA), Nevada (United Blood Service, Reno, NV), and Wyoming (United Blood Services, Cheyenne, Wyoming) and de-identified prior to analysis.

The California National Primate Research Center (CNPRC)

The California National Primate Research Center (CNPRC), which houses over 5,000 nonhuman primates, is a part of the National Primate Research Centers Program and is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). At the beginning of 2009, the CNPRC maintained a colony of 74 titi monkeys (*Callicebus cupreus*) and a colony of over 4,500 rhesus macaques (*Macaca mulatta*). No new animals have been introduced into either colony for over 2 years. All titi monkeys are maintained in small social groups, while rhesus macaques are maintained in small or large social groups. All animal facilities are maintained in compliance with United States Department of Agriculture specifications.

Eighty-eight percent of the titi monkey population (n = 65) were housed in 1 quadrant of an indoor animal building, and all titi monkeys demonstrating clinical signs originated from this building (i.e. "at-risk" room) (Fig. 1A). Rhesus macaques (n = 133) were housed in the other 3 quadrants of this same building, and surrounding the building were outdoor housing units with rhesus macaques and cynomolgus macaques (*Macaca fascicularis*). Three additional titi monkeys were moved into the at-risk room less than 2 weeks after presentation of the index case, reflecting a total at-risk population of 68 animals. The remaining 6 titi monkeys were housed in an indoor animal building greater than 500 yards from the at-risk population (i.e. "minimal-risk" room).

Outbreak investigation and microbiological testing

The outbreak lasted approximately 3 months from May to August of 2009. Affected titi monkeys died from 3–24 days after appearance of clinical signs, with an average time to death or humane euthanasia of 8 days. Clinical and epidemiological data, including daily census reports, were tracked and recorded by veterinary and management staff. All personnel entering the titi monkey rooms (both at-risk rooms and minimal-risk rooms)

needed to pass within approximately 20 feet of macaque enclosures prior to entry. CNPRC personal protective equipment (PPE) policy requires a change of PPE between entrance/exit of animal rooms housing different species. Staff compliance of this policy may have been compromised. Measures have since been taken by CNPRC management to ensure compliance with existing policies.

Bacterial, mycoplasma, and fungal cultures were performed at the CNPRC. Clinical samples were also sent to an outside laboratory (Focus Diagnostics, Cypress, CA) for respiratory viral testing by centrifugation-enhanced shell vial culture followed by direct fluorescent antibody staining for 8 viruses (respiratory syncytial virus, adenovirus, influenza virus A and B, parainfluenza virus types 1, 2, and 3, and human metapneumovirus).

Gross, histopathological, and ultrastructural analyses

Gross and histopathological analyses of post-mortem tissues were performed by a board-certified veterinary pathologist specializing in nonhuman primate/laboratory animal medicine, a branch of Primate Services at the CNPRC. At necropsy, tissue samples from the trachea, lung, and liver were collected and fixed in 10% formalin. Tissues were routinely processed and embedded in paraffin. 3-µm sections were stained with hematoxylin and eosin (HE) and examined by light microscopy. For transmission electron microscopy, tissue fragments (2×2 mm) were excised from paraffin blocks of lung, deparaffinized, and processed as previously described [48].

Nucleic acid extraction and cDNA library preparation

Total nucleic acid was extracted from body fluid or swab samples using commercially available kits (Qiagen, Valencia, CA). 200 µL of sample were passed through a 0.22 µm filter (Millipore, Temecula, CA) to remove bacteria and cellular debris and then treated with Turbo DNase (Ambion, Culver City, CA) to degrade host genomic DNA prior to extraction. For tissue samples, lung or liver tissue was homogenized in a 15 mL Eppendorf tube using a disposable microtube pestle (Eppendorf, San Diego, CA) and scalpel, and RNA extraction was then performed using TRIzol LS (Invitrogen, Carlsbad, CA), followed by isopropanol precipitation and two washes in 70% ethanol. Extracted nucleic acid was amplified using a random PCR method to generate cDNA libraries for Virochip and deep sequencing analyses as previously described [18,21].

Virochip analysis

The current 8×60 k Virochip microarrays used in this study contain 19,058 70mer probes representing all viral species in GenBank, and combine probes from all previous Virochip designs [17,18,21,23]. Four probes derived from 2 different *Adenoviridae* genera (SAdV-23, PAdV-A, HAdV-5, and FAdV-D) yielded an adenovirus signature on the Virochip that was found to be TMAdV. With the exception of SAdV-23, these highly conserved probes are part of the core Virochip design and were derived from all available adenoviral sequences in GenBank as of 2002 [21]. One explanation why more high-intensity probes to simian adenoviruses were not seen by Virochip analysis is that the genomes of many simian Ads, including SAdV-3 and SAdV-18 (the two closest phylogenetic relatives to TMAdV in GenBank), were not sequenced until after 2004 [7,49], and thus their genomes are not as well-represented on the Virochip microarray.

Virochip analysis was performed as previously described [21,23]. Briefly, samples were labeled with Cy3 or Cy5 fluorescent dye, normalized to 10 pmol of incorporated dye, and hybridized overnight using the Agilent Gene Expression Hybridization kit (Agilent Technologies, Santa Clara, California). Slides were scanned at 3 µm resolution using an Agilent DNA Microarray Scanner. Virochip microarrays were analyzed with Z-score analysis [18], hierarchical cluster analysis [50], and E-Predict, an automated computational algorithm for viral species prediction from microarrays [51]. Only Z-score analysis, a method for assessing the statistical significance of individual Virochip probes, yielded a credible viral signature on the microarray.

PCR screening

We initially used consensus primers derived from a highly conserved region of the hexon gene to confirm the Virochip finding of an adenovirus by PCR [25]. After recovering the full genome sequence, we then designed a set of specific PCR primers for detection of TMAdV, TMAdV-F (5′-GTGACGTCA-TAGTTGTGGTC) and TMAdV-R (5′-CTTCGAAGGCAAC-TACGC). The TMAdV-specific quantitative real-time PCR was performed on a Stratagene MX3005P real-time PCR system using a 25 µL master mix consisting of 18 µL of water, 2.5 µL of 10X *Taq* buffer, 1 µL of $MgCl_2$ (50 mM), 0.5 µL of deoxynucleoside triophosphates (dNTPs; 12.5 mM), 0.5 µL of each primer (10 µM), 0.5 µL SYBR green, 0.5 µL of *Taq* polymerase (Invitrogen, Carlsbad, CA), and 1 µL of extracted nucleic acid. Conditions for the PCR reaction were 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. Amplicons were purified on a 2% agarose gel, cloned into plasmid vectors using TOPO TA (Invitrogen, Carlsbad, CA), and sent to an outside company (Elim Biopharmaceuticals, Hayward, CA) for Sanger sequencing in both directions using vector primers M13F and M13R.

To assess linearity and limits of sensitivity for the TMAdV PCR assay, 12 serial log dilutions were made of a standard plasmid constructed by cloning the 157-bp TMAdV amplicon into a TOPO plasmid vector. Purified plasmid clones at each serial dilution were quantified using a Nanodrop spectrophotometer and then spiked into negative serum, stool, or oral swab sample matrix, each matrix consisting of a pool of 10 sera, 10 stool samples, or 3 oral swabs, respectively. For each sample type, a standard curve for the TMAdV PCR assay was calculated from 3 PCR replicates at each dilution of nucleic acid extracted from the spiked matrix (data not shown). To determine limits of sensitivity for the assay, probit analysis of results from 6 PCR replicates of 7 serial log dilutions (from a starting concentration of $\sim 1.2\times10^5$ copies/mL) was performed using SPSS 16.0 (SPSS Inc., Chicago, IL). By probit analysis, the 95% limit of detection for TMAdV was 781, 377, or 35 viral genome equivalents/mL for serum, stool, or oral swab samples, respectively (data not shown).

Whole-genome sequencing

To facilitate whole-genome sequencing of TMAdV, we prepared amplified cDNA/DNA libraries for deep sequencing from lung tissue and a lung swab sample from 2 different monkeys using previously published protocols [23,52]. Briefly, randomly amplified libraries were cleaved with a Type IIs restriction endonuclease (*GsuI*) and truncated adapters were ligated on the resulting strand ends. Full-length adapters containing strict 6-nt barcodes were added via an additional 15 cycles of PCR. Amplified libraries were size-selected on a 2% agarose gel at approximately 350 bp average length and then sent to an outside company (Elim Biopharmaceuticals, Hayward, CA) for deep sequencing on an Illumina Genome Analyzer IIx (Illumina, San Diego, CA). Paired-end reads were sequenced for 73 cycles in each direction. Paired-end reads were subsequently filtered to eliminate low-complexity sequences with a Lempel-Ziv-Welch (LZW) compression ratio below 0.4 [53], split into individual reads,

classified by barcode, and stripped of any remaining primer sequences using BLASTN alignments (word size = 11, E-value = 1×10^{-5}). After low-complexity filtering and barcode/primer trimming, 11,950,557 sequence reads remained, with each read consisting of 67 nucleotides, for a total of ~800 megabases of sequence. Reads were then aligned using BLASTN (word size = 11, E-value = 1×10^{-5}) and TBLASTX (word size = 11, E-value = 1×10^{-5}) to the genome sequence of SAdV-18 (Fig. 2B). Overlapping reads aligning to SAdV-18 were used to assemble portions of the TMAdV genome with Geneious software (version 3.6.5) [54], employing the SAdV-18 genome as a reference sequence and requiring a 20-bp minimum overlap and 95% overlap identity. Aligning reads were also used to design PCR primers to close remaining gaps in the TMAdV genome. Amplicons derived from specific TMAdV PCR primers were gel-purified, cloned, and sequenced as described above. The 5′ end corresponding to the inverted terminal repeat (ITR) of TMAdV was obtained by PCR using a forward degenerate consensus primer and a reverse TMAdV-specific primer. The 3′ end was recovered using a forward primer near the 3′ end of the genome and a reverse primer derived from 5′-ITR sequence.

Structural features and phylogenetic analysis

To identify predicted coding regions in the TMAdV genome, we used the fully annotated genome sequence of SAdV-21 in GenBank as a reference. First, we aligned the two genomes and identified all ORFs that were present with Geneious [54]. We then selected the candidate ORF that best matched the corresponding ORF in the annotated reference genome. For adenovirus genes that are spliced (e.g. E1A), the identification of a GT-AG intron start-stop signal was used to pinpoint the correct ORF. To confirm the accuracy of the coding sequence, the sequence of each identified ORF was aligned to a database containing all adenoviral proteins in GenBank by BLASTX.

To generate whole-genome and individual gene nucleotide phylogeny trees, all 95 fully sequenced unique adenovirus genomes were first downloaded from GenBank. Multiple sequence alignments were then performed on a 48-core Linux system using ClustalW-MPI [55]. Trees were constructed after bootstrapping to 1000 replicates by the neighbor-joining method (based on Jukes-Cantor distances) in Geneious [54,56]. Pairwise alignments were calculated using Shuffle-LAGAN (window size, 400 bp; step size 40 bp; translated anchoring), a glocal alignment algorithm that is able to calculate optimal alignments by using both local alignments and global maps of sequence rearrangements (e.g. duplications of the fiber gene in adenovirus genomes with 2 fibers) [57]. Sliding window analysis of the Shuffle-LAGAN pairwise alignments was performed using the online mVISTA platform [58]. More accurate alignments were obtained with Shuffle-LAGAN than with either ClustalW-MPI or Geneious (data not shown). Bootscanning analysis was performed according to the Kimura 2-parameter method using 1000 replicates with Simplot (version 3.5.1) [59]. Pairwise amino acid amino acid alignments between predicted TMAdV proteins and corresponding proteins in other adenoviruses (Table 2) were performed using Geneious [54].

Virus cultivation

A549 (human lung adenocarcinoma) and BSC-1 (African green monkey kidney epithelial) cell lines as well as PMK (primary rhesus monkey kidney) cells are routinely maintained at the Viral and Rickettsial Disease Laboratory (VRDL) branch of the California Department of Public Health. Media consisting of Hank's medium (for A549 cells) or Dulbecco's modified Eagle's medium (DMEM) (for BSC-1 cells) were supplemented with 1×nonessential amino acids (Invitrogen, Carlsbad, CA), 10% fetal bovine serum, 100 U of penicillin/mL and 100 µg of streptomycin/mL. PMK cells were maintained in tubes containing growth media and antibodies to SV-40 and SV-5 polyomaviruses (Viromed, Pasadena, CA). Clinical samples were clarified by centrifugation for 10 min×4000 *g* and passaged through a 0.2-µm filter. Cell culture passages were subjected to 3 freeze-thaw cycles and clarified as above. After achieving 80–90% confluency, cell culture media were changed to maintenance media with 2% FBS and were inoculated with 200 µL of clinical sample or 100 µL of passaged viral supernatant. Viral replication was monitored over 14 days by visual inspection under light microscopy for cytopathic effect (CPE). To confirm the generation of infectious virus, viral supernatants were quantitated by an end-point dilution assay.

Virus neutralization assay (human and monkey sera)

A virus stock of TMAdV (passage 7) was produced on human A549 cells, aliquoted, and quantitated by end-point dilution. To perform the virus neutralization assay, 55 µL of viral supernatant at a concentration of 100 $TCID_{50}$ and 55 µL of serum (starting at a 1:8 dilution) were mixed and incubated for 1 hour at 37°C. As a control for each serum sample, 55 µL of culture media and 55 µL of serum were mixed and treated in an identical fashion. While mixtures were incubating, A549 cells grown in T-25 plates were trypsinized and 4,000 cells in 100 µL of media were added to each well of a 96-well plate. After incubation, 100 µL of mixture were inoculated into appropriate wells containing 4,000 cells per well and the entire plate was placed in a 37°C 5% CO_2 incubator. Cells in the plate wells were observed for evidence of CPE every other day for 1 week. For wells that showed inhibition of viral CPE, the corresponding serum samples were diluted in six 2-fold steps and then retested. The reciprocal of the highest dilution that completely inhibited viral CPE was taken as the neutralizing antibody titer.

Virus neutralization assay (rabbit typing sera)

To assess cross-neutralization of TMAdV by known human adenoviruses, 7 pools of in-house reference sera at the VRDL (rabbit hyperimmune sera) and collectively containing antibodies to human adenovirus serotypes 1 through 35 were available for testing. For each pool, 55 µL of rabbit sera and 55 µL of viral supernatant at a concentration of 100 $TCID_{50}$ were mixed, incubated for 1 hour at 37°C, and inoculated onto A549 cells in wells of a 96-well plate as described above. Cells in the plate wells were observed for evidence of CPE every other day for 1 week.

Microarray and nucleotide sequence accession numbers

All Virochip microarrays used in this study have been submitted to the NCBI GEO database (study accession number GSE26898; microarray accession numbers GSM662370-GSM662391; microarray design accession number GPL11662). The annotated, whole-genome sequence of TMAdV has been submitted to GenBank (accession number HQ913600). Deep sequencing reads have been submitted to the NCBI Sequence Read Archive (accession number SRA031285).

Supporting Information

Figure S1 Phylogenetic analysis of the hexon, polymerase, penton base, and fiber genes of TMAdV. A multiple sequence alignment of selected genes from all 95 unique, fully-sequenced adenovirus genomes in GenBank and TMAdV is performed and the results displayed as a radial phylogenetic tree. The branch corresponding to TMAdV is highlighted in boldface

red. Abbreviations: HAdV, human adenovirus, SAdV, simian adenovirus; PAdV, porcine adenovirus; FAdV, fowl adenovirus.
(TIF)

Figure S2 Bootscanning recombination analysis of TMAdV. Bootscanning analysis was initially performed with all 95 unique, fully-sequenced adenovirus genomes in GenBank (data not shown). After removal of similar viral genomes, bootscan plots of the whole genome and individual genes from a subset representing human/simian adenoviruses in species A–G and all non-primate vertebrate adenoviruses were generated. The window size is 400 bp with a step size of 40 bp for the whole genome, and 200 bp with a step size of 20 bp for the individual genes. The x-axis refers to the nucleotide position. For definition of abbreviations, please refer to Fig. 3.
(TIF)

Acknowledgments

We thank Kathy West for providing a titi monkey photograph, Dr. Don Canfield for the gross pathology and histopathology images as well as expert interpretation, and Grete Adamson for the transmission electron microscopy. We thank Kristine Ren, R.N., for phlebotomy assistance. We are grateful to Alexander Greninger and Guixia Yu for technical support, and to Drs. Eric Delwart and Michael Busch at the Blood Systems Research Institute for providing serum samples from random adult blood donors for screening. We also thank Dr. Sarah Arron at UCSF, Dr. Jean Patterson at the Southwest Foundation for Biomedical Research, and Dr. Sharon Messenger at the California Department of Public Health for helpful advice and feedback. Finally, we would like to recognize all of the CNPRC husbandry, management, pathology, research, and veterinary staff who worked diligently to provide excellent care for the titi monkey colony devastated by the outbreak.

Author Contributions

Conceived and designed the experiments: ECC SY KLB DPS NWL CYC. Performed the experiments: ECC SY SPM NM AR AS CYC. Analyzed the data: ECC SY KRK NM KLB DPS NWL CYC. Contributed reagents/materials/analysis tools: ECC SY SPM NM AR AS KLB DPS NWL CYC. Wrote the paper: ECC SY KRK KLB DPS NWL CYC.

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研究報告の概要

2009 年後半、カリフォルニアデービスにある米国立霊長類研究センターの titi monkey に病原体不明の感染症が流行し、飼育されていた 65 匹中 23 匹のサルが感染して重症肺炎になり 19 匹が死亡した（死亡率 83%）。死亡したサルの肺組織から新種のアデノウイルスが見つかり TMAdV（titi monkey adenovirus）と名付けられた。
この感染事故発生初期にこれらのサルと濃厚接触していた研究者が、発熱、悪寒、頭痛、咳、肺の灼熱感といった、アデノウイルス感染が疑われる症状を呈した。また、研究者の家族 2 人も軽度だが似た症状が認められた。この 3 人は治癒し、ウイルスそのものを検出することはできなかったが、血中に TMAdV に対する抗体が検出されたため、このウイルスに感染していたと考えられた。アデノウイルスは種特異性があり、サルのアデノウイルス、ヒトのアデノウイルス等その動物種のみで感染することが示されており、サルからヒトへの感染（あるいはその逆）はこれまで知られていなかったが、今回、異種間で同一のアデノウイルス感染が認められた。また、軽度の症状を発現した研究者の家族は感染サルへの接触がないことから、このウイルスがヒトからヒトに感染した可能性が示唆された。一方、このウイルスがヒトからサルに伝染した可能性も、完全には、否定できないと報告されている。

使用上の注意記載状況・その他参考事項等

BYL-2011-0407

PLoS Pathogenens
Vol.7 No.7 ; e1002155;
1-16, 2011

報告企業の意見	今後の対応
今回、アデノウイルス科の新たなウイルス種が同定され、アデノウイルスでは初めて動物種を越えた感染（人畜共通感染）が認められた。また、この新型アデノウイルスである TMAdV はヒトーヒト感染も起こすことが確認された。 TMAdV の本来の宿主は不明であるが、本報告にあるような霊長類施設以外で人が感染する確率は低いと考えられる。また、TMAdV に感染したヒトの症状は、人アデノウイルスによるそれと類似していると報告されている。このことから、現時点で、TMAdV がヒトに重大な脅威となるとは考えにくい。本剤の精製工程は、エンベロープを有さないウイルスに対し十分な不活性化なし除去能を有することが示されている。TMAdV に類するウイルスの混入の可能性はきわめて低いと考えられる。 しかしながら、アデノウイルスは種特異性を有するという定説が覆されたことから、新たな人畜共通感染について注視していく必要がある。	現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も，新規人畜共通感染症や新たなウイルス感染症に関する情報収集に努める。

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Canada Communicable Disease Report CCDR WEEKLY

Infectious Diseases News Brief - July 22, 2011

[Current Issue - Table of contents]

Measles And Water-Borne Disease Outbreaks In Horn Of Africa And Kenya Worries World Health Organization

The numbers of people becoming infected with measles and water-borne diseases is growing at an alarming rate in the Horn of Africa and some neighboring countries, says WHO (World Health Organization). Cases of severe diarrhea in Kenya and Ethiopia are a serious concern, the organization adds. Severe drought in the Horn of Africa and Kenya is making millions of people move to other areas, an important factor in the spread of communicable diseases. WHO predicts the problem will get worse. The Horn of Africa, also known as Northeast Africa or the Somali Peninsula is in northern east Africa, it is a peninsula that sticks out hundreds of miles into the Arabian sea. The Horn of Africa has approximately 100 million people, and includes Ethiopia, Somalia, Eritrea and Djibouti. The affected countries have low immunization rates, a shortage of clean water, poor santation and extremely poor health care systems.

Source: Medical News Today 17 July 2011
http://www.medicalnewstoday.com/articles/231242.php

First Adenovirus to Jump Between Monkeys and Humans Confirmed

A novel virus that spread through a California monkey colony in late 2009 also infected a human researcher and a family member, UCSF researchers have found, the first known example of an adenovirus "jumping" from one species to another and remaining contagious after the jump. In a study by the UCSF Viral Diagnostics and Discovery Center, which identified the new virus at the time of the outbreak, researchers confirmed it was the same virus in the New World titi monkeys and the two humans. They also confirmed that the virus is highly unusual in both populations, suggesting that it may have originated from a third, unidentified species. The direction in which the virus spread, however -- from monkeys to humans or vice versa -- remains a mystery. Findings appear in the July 14 issue of *PLoS Pathogens*, a weekly journal of the Public Library of Science. The virus, which researchers have named titi monkey adenovirus (TMAdV), infected more than a third of the titi monkeys in the California National Primate Research Center (CNPRC) in late 2009. In the monkeys, the virus was devastating, causing an upper respiratory illness that progressed to pneumonia and eventually killed 19 of the 23 monkeys (83 percent) that became sick, including healthy young adult monkeys. Around the time of the

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outbreak, a researcher who was taking care of the sick monkeys also developed an upper respiratory infection, with fever, chills and a cough that lasted four weeks, as did two members of the researchers' family who had no contact with the monkey colony. All three recovered fully without medical treatment. The primate center called Chiu when the illness spread through the colony to help identify the pathogen and prevent its spread to other animals. The UCSF Viral Diagnostics and Discovery Center specializes in using a microarray Virochip technology developed at UCSF to identify viruses affecting humans, animals, insects or plants.

Because the researcher's illness was not reported for several months, the virus could no longer be detected directly, so Chiu worked with the California Department of Public Health to conduct antibody testing on the monkeys, the researcher and two of the researcher's family members who also reported having been sick. Antibodies are a product of the body's immune response to pathogens and generally remain in the bloodstream for several months after infection. As a result, they serve as a marker of whether a person was exposed to a specific virus. Both the monkeys and researcher tested positive for antibodies to the TMaDV virus, as did one of the two family members. No other humans at the center were found to have been infected. The UCSF team found that the new virus clearly belonged to the adenovirus family, yet was unlike any adenovirus ever reported to infect humans or monkeys, including from large-scale studies by public health agencies such as the U.S. Centers for Disease Control and Prevention. The new virus was so unusual, in fact, that it shares only 56 percent of its DNA to its closest viral relative.

Source: Science Daily News July 15 2011
http://www.sciencedaily.com/releases/2011/07/110714191427.htm

Date Modified: 2011-07-22

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別紙様式第2-1

No. 13

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 9. 15	該当なし	
一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	EID Vol.17 No.8; Available from: http://wwwnc.cdc.gov/eid/article/17/8/11-0285_article.htm	公表国 米国	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)					

研究報告の概要 -138-

○米国でのヒトにおける新種のアレナウイルス感染
アレナウイルスに属する、ホワイトウォーターアロヨウイルス(WWAV)と他7種類から成るノースアメリカンタカリベセロコンプレックスウイルス(NATSV)及びリンパ球性脈絡髄膜炎ウイルス(LCMV)は、北米で発生することが知られている。5種類のサウスアメリカンタカリベセロコンプレックスウイルス(SATSV)、LCMV、ラッサ熱ウイルスはヒトにおける深刻な発熱性疾患の病原体であるが、NATSVのヒトへの影響は正確に調査されていない。
米国で急性中枢神経疾患や原因不明の熱性疾患の患者1,185人中41人(3.5%)から抗WWAV/抗LCMV-IgG抗体が検出された。ペア血清サンプルの抗体価の分析結果から、NATSVが2人、LCMVが3人の疾患原因であると示唆された。この研究結果は、NATSVもLCMVと同様に米国内でヒトの疾患原因となることを示している。

報告企業の意見	今後の対応
今までヒトへの影響が正確に調査されていなかったノースアメリカンタカリベセロコンプレックスウイルスに対するIgG抗体が米国の急性中枢神経疾患や原因不明の熱性疾患の患者から検出され、ヒト疾患の原因となることが示唆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渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。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。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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Novel Arenavirus Infection in Humans, United States

Mary Louise Milazzo, Grant L. Campbell, and Charles F. Fulhorst

Immunoglobulin G against Whitewater Arroyo virus or lymphocytic choriomeningitis virus was found in 41 (3.5%) of 1,185 persons in the United States who had acute central nervous system disease or undifferentiated febrile illnesses. The results of analyses of antibody titers in paired serum samples suggest that a North American Tacaribe serocomplex virus was the causative agent of the illnesses in 2 persons and that lymphocytic choriomeningitis virus was the causative agent of the illnesses in 3 other antibody-positive persons in this study. The results of this study suggest that Tacaribe serocomplex viruses native to North America, as well as lymphocytic choriomeningitis virus, are causative agents of human disease in the United States.

The arenaviruses (family *Arenaviridae*, genus *Arenavirus*) known to occur in North America include Whitewater Arroyo virus (WWAV), 7 other members of the Tacaribe serocomplex (Table 1), and lymphocytic choriomeningitis virus (LCMV, the prototypic member of the lymphocytic choriomeningitis–Lassa serocomplex). Specific members of the order Rodentia are the principal hosts of the arenaviruses, for which natural host relationships have been well characterized. For example, the hispid cotton rat (*Sigmodon hispidus*) in Florida is the principal host of Tamiami virus (*6*,*7*), and the ubiquitous house mouse (*Mus musculus*) is the principal host of LCMV (*9*).

Five South American members of the Tacaribe serocomplex, LCMV, and Lassa virus are etiologic agents of severe febrile illnesses in humans (*10*,*11*). The human health significance of the North American Tacaribe serocomplex viruses has not been rigorously investigated (*12*).

Studies since the mid-1990s have shown that Tacaribe serocomplex viruses are widely distributed in the United States and Mexico and that woodrats (*Neotoma* spp.) and other members of the family Cricetidae are natural hosts of these viruses (*1*–*5*,*8*,*13*,*14*). The purpose of this study was to investigate whether humans have been infected with North American Tacaribe serocomplex viruses.

Materials and Methods

Samples of serum (n = 1,305), plasma (n = 2), and cerebrospinal fluid (n = 70) from 1,185 persons in the United States with acute central nervous system disease or undifferentiated febrile illnesses were tested for immunoglobulin (Ig) G against the WWAV prototype strain AV 9310135 and LCMV strain Armstrong by using an ELISA as described (*15*). The samples were diagnostic specimens submitted to the Arbovirus Diseases Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention (CDC) (Fort Collins, CO, USA) during 1989–2000 by public health laboratories in the United States. The samples had been tested selectively by CDC laboratorians for evidence of infection with St. Louis encephalitis virus, western equine encephalomyelitis virus, and other arthropod-borne agents of human disease. These tests had not yielded a specific diagnosis for any of the cases in this study.

Information about each case was limited to patient age, sex, date of illness onset, and state from which the samples were submitted. Most (634 [53.5%]) of the 1,185 case-patients were male. Ages at illness onset ranged from 0.2 months to 93 years (median 35 years), and 982 (82.0%) of the case-patients were ≥10 years of age at illness onset.

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DOI: 10.3201/eid1708.110285

Table 1. Natural hosts and geographic distribution of the North American Tacaribe serocomplex viruses

Virus	Natural host(s)	Location	Reference
Bear Canyon	Large-eared woodrat (*Neotoma macrotis*), California mouse (*Peromyscus californicus*)	California, USA	(*1*)
Big Brushy Tank	White-throated woodrat (*N. albigula*)	Arizona, USA	(*2*)
Catarina	Southern plains woodrat (*N. micropus*)	Texas, USA	(*3*)
Rio Catorce	White-toothed woodrat (*N. leucodon*)	San Luis Potosí, Mexico	(*4*)
Skinner Tank	Mexican woodrat (*N. mexicana*)	Arizona, USA	(*5*)
Tamiami	Hispid cotton rat (*Sigmodon hispidus*)	Florida, USA	(*6*,*7*)
Tonto Creek	White-throated woodrat (*N. albigula*)	Arizona, USA	(*2*)
Whitewater Arroyo	White-throated woodrat (*N. albigula*)	New Mexico, USA	(*8*)

The period between illness onset and sample collection ranged from 0 days to 10.1 years (median 31 days). At least 1 sample from each of 580 case-patients was collected before the end of week 4 of illness; for 108 case-patients multiple samples, representing different time points, were available. Cases were geographically distributed as follows: New England, 72 cases; Mid-Atlantic, 50; South Atlantic, 141; East North Central, 96; West North Central, 73; East South Central, 78; West South Central, 42; Mountain, 177; Pacific, 96; and unknown, 360.

A 1:80 dilution and 1:320 dilution of each sample was tested against the WWAV antigen, LCMV antigen, and corresponding comparison (negative-control) antigens. The adjusted optical density (AOD) of a sample-antigen reaction was the optical density of the well coated with the test antigen minus the optical density of the well coated with the corresponding control antigen. A sample was considered positive if the AOD at 1:80 was ≥0.250, the AOD at 1:320 was ≥0.250, and the sum of the AOD at 1:80 and AOD at 1:320 was ≥0.750. Endpoint titers against each antigen were measured in the positive samples by using serial 2-fold dilutions from 1:320 through 1:40,960. The antibody titer of a positive sample was the reciprocal of the highest dilution for which the AOD was ≥0.250. Titers <320 were 160 in comparisons of titers to WWAV and LCMV in individual samples. The apparent homologous virus in an antibody-positive sample was the virus associated with the highest titer if the absolute value of the difference between the titers to WWAV and LCMV was ≥4-fold.

Results

We detected antibody against an arenavirus in 41 (3.5%) of the 1,185 case-patients. Of the antibody-positive case-patients, most (27 [65.9%]) were male. Ages ranged from 4 years to 85 years (median 39 years). Antibody-positive samples were submitted from Florida, Massachusetts, and Wyoming (3 samples each) and Arizona, Idaho, Kansas, Maryland, Michigan New Mexico, New York, North Carolina, Ohio, Rhode Island, Tennessee, Washington, and Wisconsin (1 sample each). For 19 samples, state of submission was unknown.

Twelve persons had positive test results for WWAV but not LCMV; 28 for LCMV but not WWAV; and 1 for WWAV and LCMV (Table 2). In the positive samples, endpoint titers against WWAV and LCMV ranged from <320 to 10,240 and from <320 to 20,480, respectively. The apparent homologous virus was WWAV in 10, LCMV in 24, and indeterminate in 7 of antibody-positive persons (Table 2).

Ages of the 10 persons in whom WWAV was the apparent homologous virus ranged from 5 to 70 years (median 43 years). Samples from these persons were submitted from Arizona, New Mexico, and North Carolina (1 sample each) and Florida and Wyoming (2 samples each); for 3 samples, state of submission was unknown.

The ELISA included paired samples from 8 antibody-positive persons. Time from onset of illness to the first samples from these persons ranged from 0 to 47 days. In side-by-side tests, the endpoint titer to WWAV in the second sample was ≥4-fold higher than that to WWAV in the first sample in paired samples from 2 persons, and the endpoint titer to LCMV in the second sample was ≥4-fold higher than that to LCMV in the first sample in paired samples from 3 of the 6 other antibody-positive persons (Table 3).

Table 2. Antibody (immunoglobulin G) titers against WWAV and LCMV in 1,185 cases of acute central nervous system disease or undifferentiated febrile illnesses, United States*

No. cases	Antibody titer		Apparent homologous virus
	WWAV	LCMV	
5	640	<320	WWAV
1	1,280	<320	WWAV
3	2,560	<320	WWAV
1	10,240	<320	WWAV
7	<320	640	LCMV
3	<320	1,280	LCMV
5	<320	2,560	LCMV
4	<320	5,120	LCMV
2	<320	10,240	LCMV
3	<320	20,480	LCMV
2	320	<320	Indeterminate
1	640	1,280	Indeterminate
4	<320	320	Indeterminate
1,144	<320	<320	None

*WWAV, Whitewater Arroyo virus; LCMV, lymphocytic choriomeningitis virus.

Table 3. Antibody (immunoglobulin G) against WWAV and LCMV in paired serum samples from humans with acute central nervous system disease or undifferentiated febrile illnesses, United States*

Case-patient no.	Age, y, at illness onset	Days after illness onset		Antibody titer, WWAV		Antibody titer, LCMV		Apparent homologous virus
		S1	S2	S1	S2	S1	S2	
1	32	14	44	<320	640	<320	<320	WWAV
2	65	15	61	<320	2,560	<320	<320	WWAV
3	38	14	33	<320	<320	5,120	5,120	LCMV
4	51	2	68	<320	<320	320	20,480	LCMV
5	59	24	38	<320	<320	320	5,120	LCMV
6	72	0	15	<320	<320	<320	640	LCMV
7	12	25	33	<320	<320	320	320	Indeterminate
8	25	47	123	<320	<320	320	320	Indeterminate

*WWAV, Whitewater Arroyo virus; LCMV, lymphocytic choriomeningitis virus; S1, first sample; S2, second (last) sample in paired samples.

Discussion

Previously, antibody to Tamiami virus was found in 5 (3.8%) of 131 Seminole Indians sampled in southern Florida (*16*), and antibody to a Tacaribe serocomplex virus was found in 2 (0.24%) of 829 persons who had worked with cricetid rodents in North America (*15,17*). The results of our current study strengthen the notion that Tacaribe serocomplex viruses enzootic in North America are infectious in humans. The increase in antibody titer against WWAV in cases 1 and 2 in this study (Table 3) suggests that a North American Tacaribe serocomplex virus caused the illnesses in these persons.

The WWAV strain AV 9310135 was originally isolated from a white-throated woodrat (*N. albigula*) captured in northwestern New Mexico (*8*). A recent study demonstrated a high level of diversity among the amino acid sequences of the structural proteins of the North American Tacaribe serocomplex viruses (*5*). Hypothetically, human IgG against some North American Tacaribe serocomplex viruses is not strongly reactive against WWAV in ELISA. If so, the prevalence of antibody to Tacaribe serocomplex viruses in this study actually might be >3.5%.

The severity of human disease caused by LCMV ranges from mild febrile illness to severe encephalitis and disseminated disease (*18*). The results of this study suggest that the illnesses in case-patients 4–6 (Table 3) were caused by LCMV. Whether samples from these 3 persons were tested for anti-LCMV antibody (IgM or IgG) by clinical laboratories could not be determined from records maintained at CDC.

Specimens from 33 of the antibody-positive persons in this study were limited to single specimens. Perhaps these illnesses were caused by a North American Tacaribe serocomplex or by LCMV. The antibody titer to WWAV in the antibody-positive person from New Mexico was 10,240 in a serum sample collected on day 22 day after illness onset.

Future studies on the relevance to human health of the North American Tacaribe serocomplex viruses should include defining the clinical spectrum and epidemiology of human disease caused by these viruses. Some of these viruses may cause aseptic meningitis, encephalitis, or meningoencephalitis. Thus, human disease caused by North American Tacaribe serocomplex viruses may be confused with severe encephalitis caused by LCMV, especially in persons who report recent exposure to rodents.

Acknowledgments

We thank Amanda Panella, Nick Karabatsos, and Stacey Bartlett for technical support.

Financial support for the work done by M.L.M. and C.F.F. was provided by National Institutes of Health grant AI-41435, "Ecology of emerging arenaviruses in the southwestern United States."

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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
				2011. 4. 23	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況		公表国	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)			News release Alabama depertment of public health. Available from: http://www.adph.org/news/assets/110407.pdf	米国	

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
○Serratia汚染原因の同定:速報 アラバマ州公衆保健局(ADPH)は2つの病院から、完全静脈栄養剤(TPN)を投与した患者に*Serratia marcescens*感染が生じたという通報を受けた。米国疾病管理予防センター(CDC)は、共通の原因として可能性のあるTPN製造業者を特定し、その業者からTPNを納入していた6つの病院を特定した。6つの病院で19症例(38歳～94歳;男性8名、女性11名)が報告された。 ADPHとCDCは、遺伝子解析結果、TPNを製造する際に使用していた容器とスターラー、容器をすすぐ為の水道栓及びTPNから分離された菌と、TPNを受けた入院患者12人から分離された*S.marcescens*が同じであったと確認した。さらにTPNの原料である混合アミノ酸1袋も、*S.marcescens*で汚染されていた。TPNを混合する時の殺菌工程の失敗が、汚染の原因になったと考えられる。この製造業者は通知を受け、汚染の可能性を情報提供し、生産を中止し、製品を回収した。 今のところ他の業者からTPN汚染の報告はなく、アラバマ州以外の病院にも当該製品は納入されていなかった。 ADPHは、CDCや他の機関とも協力し、*S.marcescens*感染発生の調査を続ける。	新鮮凍結血漿「日赤」 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 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
アラバマ州公衆保健局と米国疾病管理予防センターは、6つの病院で発生した*Serratia marcescens*感染は、完全静脈栄養剤の製造工程での汚染が原因であると断定したとの報告である。	日本赤十字社では輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に、初流血除去及び保存前白血球除去を導入している。さらに、輸血情報リーフレット等により、細菌感染について医療機関へ情報提供し注意喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌の検出や不活化する方策について検討している。

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ALABAMA DEPARTMENT OF PUBLIC HEALTH
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NEWS RELEASE

Likely source of Serratia contamination identified

FOR IMMEDIATE RELEASE

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The Alabama Department of Public Health and the Centers for Disease Control and Prevention have determined that the *Serratia marcescens* bacteremia in 12 hospitalized individuals who received TPN (total parenteral nutrition) has the same genetic fingerprint as the organism isolated from a container and stirrer used to mix the powdered amino acids, from the tap water spigot used for rinsing the container, and from the TPN.

A bag of compounded amino acids used in the production of TPN has also grown *Serratia marcescens*. Genetic fingerprint results are pending on the compounded amino acids.

The Alabama Department of Public Health is aware of 19 cases of *Serratia marcescens* in patients in six Alabama hospitals. Of these cases, 12 samples from individuals were matched with the bacterium found at Meds IV Pharmacy in Birmingham. Of the remaining seven cases in question, six have no samples available to test for a genetic match and one case is pending.

A failure in a step of the sterilization process in the compounding of TPN was most likely the cause of contamination. Use of these contaminated products led to a bacterial bloodstream infection in these 19 patients.

On March 16, ADPH was notified that an outbreak had occurred in two hospitals among patients receiving TPN. CDC's initial investigation identified TPN produced by Meds IV as a potential common source and determined that six hospitals received TPN from this pharmacy.

Illness with *Serratia marcescens* bacteremia occurred in approximately 35 percent of patients receiving TPN from Meds IV during March. Seventeen cases were reported in March, and two additional cases were retrospectively identified during the investigation, one in January and one in February.

The individuals affected are in the age range from 38 to 94 years; 8 males and 11 females were infected. The numbers of cases and deaths by hospital are as follows: Baptist Princeton, 7 cases, 4 deaths; Baptist Shelby, 5 cases, 2 deaths; Medical West, 3 cases, 1 death; Cooper Green Mercy, 1 case, no deaths; Baptist Medical Center Prattville, 1 case, 1 death; and Select Specialty Hospital of Birmingham, 2 cases, 1 death.

Meds IV was notified and informed its customers of the possibility of contamination. ADPH has been informed that impacted hospitals immediately stopped using TPN received from this

pharmacy and that the pharmacy discontinued all production. On March 24, Meds IV recalled all of its IV compounded products.

ADPH continues an ongoing investigation of the outbreak of *Serratia marcescens* bacteremia in collaboration with the CDC, the U.S. Food and Drug Administration, the Alabama Hospital Association, and the State Board of Pharmacy. At this time, there have been no reports of contaminated TPN, from any other pharmacy, sent to hospitals in Alabama or any other state.

As more information becomes available, ADPH will provide updates.

4/7/11

別紙様式第2-1

No. 27

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
				2011. 6. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)		Eurosurveillance Vol.16 Is.24	ドイツ

研究報告の概要

○志賀毒素／ベロ毒素産生大腸菌感染による出血性下痢及び溶血性尿毒症症候群の大規模アウトブレイク発生時における強化サーベイランス、ドイツ、2011年5月～6月

ドイツでは感染症のための広範な法定サーベイランスシステムが確立している。しかしこのシステムは、患者情報が地域レベルから州や国レベルに報告されるまでに時間を要する。

2011年5月19日、ドイツのロベルト・コッホ研究所は、志賀毒素／ベロ毒素産生大腸菌(STEC／VTEC)O104:H4感染による溶血性尿毒症症候群(HUS)患者がハンブルクで多数発生しているとの報告を受け、翌日当該地域に調査チームを派遣した。患者の急激な増加を受け、強化サーベイランスが必要であることが確認された。このため、以下のような変更を行った。

疫学情報交換の集約化／国レベルまでの情報伝達の迅速化／病院の救急部における出血性下痢症の症候群サーベイランスシステムの導入／ドイツにおけるHUS治療受け入れ能力の評価／検査機関でのアクティブサーベイランスの開始

これらの追加サーベイランスシステムは自主的に行われたものであり、今回のアウトブレイクにおいて、より迅速なモニタリングを可能にした。サーベイランスの強化により、確定患者の実数把握や国際援助が必要かどうかの判断、新規患者発生の発生動向について把握することができた。しかし、法定サーベイランスシステムにおける情報伝達速度については迅速化する必要がある。また新規患者の発生傾向を迅速に探知するため、この先少なくとも3カ月間は病院の救急部における症候群サーベイランスの継続を推奨する。

報告企業の意見	今後の対応
志賀毒素／ベロ毒素産生大腸菌感染症による出血性下痢及び溶血性尿毒症症候群の大規模アウトブレイクが発生した際に強化サーベイランスを実施し、通常のサーベイランスに比べてより迅速にモニタリングを行うことができたとの報告である。	今後も国内外の感染症アウトブレイク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

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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

Enhanced surveillance during a large outbreak of bloody diarrhoea and haemolytic uraemic syndrome caused by Shiga toxin/verotoxin-producing *Escherichia coli* in Germany, May to June 2011

M Wadl (WaldM@rki.de)[1], T Rieck[1,2], M Nachtnebel[1,3,4], B Greutélaers[1,3,4], M an der Heiden[1], D Altmann[1], W Hellenbrand[1], M Faber[1], C Frank[1], B Schweickert[1], G Krause[1], J Benzler[1], T Eckmanns[1], on behalf of the HUS surveillance and laboratory team[5]

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5. The members of the team are listed at the end of the article

Citation style for this article:
Wadl M, Rieck T, Nachtnebel M, Greutélaers B, an der Heiden M, Altmann D, Hellenbrand W, Faber M, Frank C, Schweickert B, Krause G, Benzler J, Eckmanns T, on behalf of the HUS surveillance and laboratory team. Enhanced surveillance during a large outbreak of bloody diarrhoea and haemolytic uraemic syndrome caused by Shiga toxin/verotoxin-producing Escherichia coli in Germany, May to June 2011.
Euro Surveill. 2011;16(24):pii=19893. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19893

Article published on 16 June 2011

Germany has a well established broad statutory surveillance system for infectious diseases. In the context of the current outbreak of bloody diarrhoea and haemolytic uraemic syndrome caused by Shiga toxin/verotoxin-producing *Escherichia coli* in Germany it became clear that the provisions of the routine surveillance system were not sufficient for an adequate response. This article describes the timeline and concepts of the enhanced surveillance implemented during this public health emergency.

On Thursday, 19 May 2011, the Robert Koch Institute (RKI) was informed about a cluster of cases of haemolytic uremic syndrome (HUS) due to Shiga toxin/verotoxin-producing *Escherichia coli* (STEC/VTEC) O104:H4 in the area of Hamburg, Germany. An RKI investigation team visited the affected area the following day. In the face of rapidly rising case numbers, a need for enhanced surveillance was identified on 23 May. We describe here the timeline and concepts of the enhanced surveillance implemented during this massive outbreak of bloody diarrhoea and HUS in May and June 2011 in Germany.

Routine surveillance system

In Germany, STEC/VTEC and HUS have been statutorily notifiable since 2001 according to the Protection against Infection Act (Infektionsschutzgesetz, IfSG [1]). While STEC/VTEC surveillance is based on laboratory analyses, HUS surveillance relies on physicians. Heads of laboratories and physicians must report cases to the local health authorities within 24 hours. The incoming data is validated by the local health authorities and documented electronically. Cases fulfilling the surveillance case definition as issued by RKI [2] are transmitted in anonymous form to the state health authorities by the third working day of the following week. The state health authorities again validate incoming cases and transmit the data to the RKI within the following week. Hence, transferring information on a case from the local to the national health authority may take from a few days up to 16 days.

Epidemiological information is fed back from RKI at least weekly to the stakeholders, e.g. responsible authorities, physicians and laboratories. Information exchange includes teleconferences, reports in the RKI's weekly *Epidemiological Bulletin* and the internet database SurvStat [3].

Enhanced surveillance system

In the context of the outbreak it became immediately clear that the provisions of the routine surveillance system were not sufficient for an adequate response. Hence, the following amendments were implemented:

- Centralising the epidemiological information exchange,
- Accelerating the data flow to the national level,
- Implementing a syndromic surveillance system for bloody diarrhoea in emergency departments,
- Assessing the capacities for HUS-treatment in Germany,
- Initiating active laboratory surveillance.

An overview of routine and newly implemented surveillance systems is given in Figure 1.

Centralising the epidemiological information exchange

On 23 May 2011, the 'Lagezentrum' at the RKI was activated as a central emergency operations centre. A large number of RKI staff was involved in coordinating the collection of epidemiologic information and organising the public health response. From 23 May onwards, teleconferences were conducted almost daily with the responsible state, national and international authorities. Starting on 24 May, epidemiological reports were distributed daily to the responsible authorities, physicians and laboratories to feed back relevant information. Several outbreak-related articles were published in *Eurosurveillance* [4,5] and the German *Epidemiological Bulletin*. The public was regularly informed about the outbreak situation via the RKI website starting on 23 May, press releases were issued on 3 and 10 June. The Federal Centre for Health Education (Bundeszentrale für Gesundheitliche Aufklärung, BZGA), has provided outbreak-related public health advice to the public since 24 May.

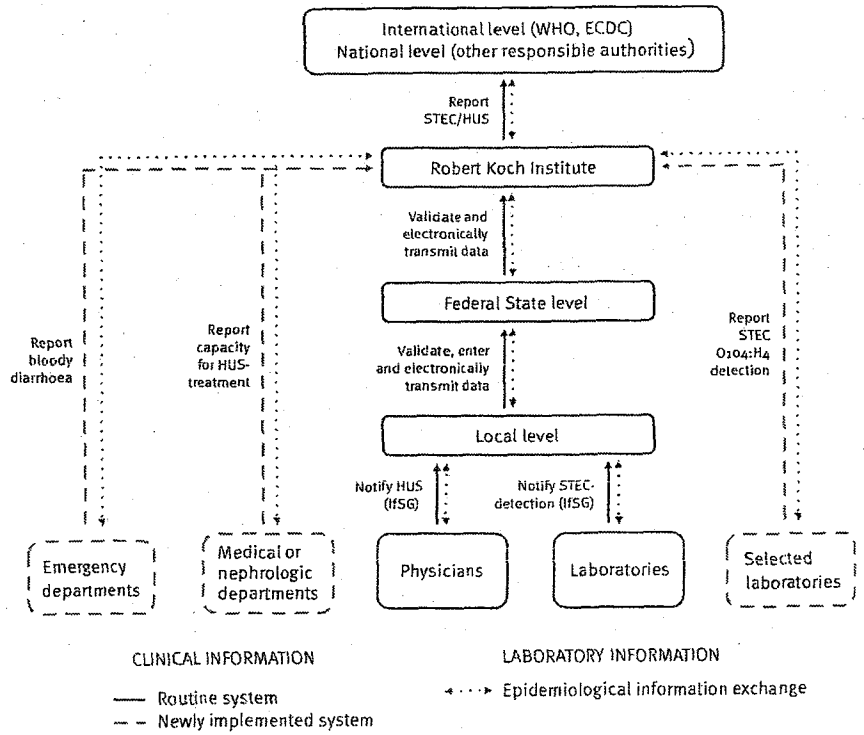
Accelerating the data flow to the national level

From 23 to 27 May 2011, state health authorities were asked to transmit aggregated data via email on a daily basis to the RKI. Concurrently, health authorities were urged to enter and transmit the IfSG data via the electronic surveillance system daily, so that case by case reporting could overtake the aggregated reporting on 27 May. A specific reporting form was published on 26 May to facilitate notification of HUS cases by physicians.

In addition, the existing RKI surveillance case definition was adapted to the outbreak situation to ensure systematic data collection. Modifications included

FIGURE 1

Data and information flow to and from the Robert Koch Institute during the period of enhanced surveillance, STEC/HUS outbreak, Germany, spring 2011



ECDC: European Centre for Disease Prevention and Control; HUS: haemolytic uraemic syndrome; IfSG: German Protection against Infection Act; STEC: Shiga toxin-producing *Escherichia coli*; WHO: World Health Organization.

limitations of time (onset of disease from 1 May 2011), place (epidemiological link to Germany) and person (e.g. consumption of a food item that was acquired in Germany) concerning exposure as well as inclusion of suspected cases [6].

One challenge was counting outbreak-related cases of STEC/VTEC O104:H4 separately from other STEC/VTEC cases, of which a mean of 992 cases annually had been reported to the RKI between 2001 and 2010. In the absence of comprehensive laboratory data for a majority of reported cases, the case definition was revised in a way that listed as exclusion criteria all specific laboratory test results that were not consistent with the characteristics of the outbreak strain.

As of June 12, a total of 3,228 STEC/VTEC and HUS cases in Germany have been associated with the outbreak (Figure 2). The majority of cases (51%) fell ill between 18 and 25 May. The place of exposure was suspected to lie in north-western parts of Germany for most cases (Figure 3). Of the 781 reported HUS cases, 69% were female and 88% were 20 years of age or older. Overall, 22 notified HUS cases have died. Among all 2,447 STEC/VTEC cases, 59% were female and 87% were 20 years of age or older. Thirteen notified STEC/VTEC cases have died.

Figure 4 shows the transmission delay in days from the local to the national level during the STEC/HUS outbreak period among HUS cases. Among the 740 HUS cases (96%) with known date of notification to the local health authorities, the median transmission delay was two days (25th–75th percentile: 1–4 days, minimum–maximum: 0–18 days). The first HUS-case was reported to the RKI through the electronic surveillance system on 18 May. Another three HUS cases were reported on 23 May. Thereafter, the accelerated data flow became evident, for instance, 47 HUS cases were reported to the RKI on 24 May, 50 HUS cases on 25 May, 100 HUS cases on 26 May and 116 HUS cases on 27 May.

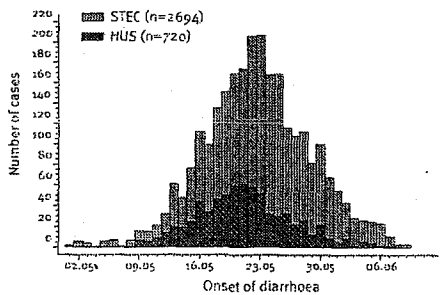
Implementing a syndromic surveillance system for bloody diarrhoea in emergency departments

Since STEC patients often present with bloody diarrhoea, emergency departments (ED) constitute appropriate facilities for the assessment of the temporal trend of an STEC-outbreak. We implemented the surveillance of patients with and without bloody diarrhoea in ED on 27 May.

Participating ED were located in all federal states of Germany, both in areas affected and not affected by the STEC/HUS outbreak (see Figure 4). Data collection covered the total number of new patients in participating ED and the number of patients presenting with bloody diarrhoea by sex and age group (<20 years, ≥20 years). The data were transferred to the RKI by email or fax every day.

FIGURE 2

Reported STEC/VTEC and HUS cases, by date of onset of diarrhoea*, Germany, May–June 2011 (n=2,694)

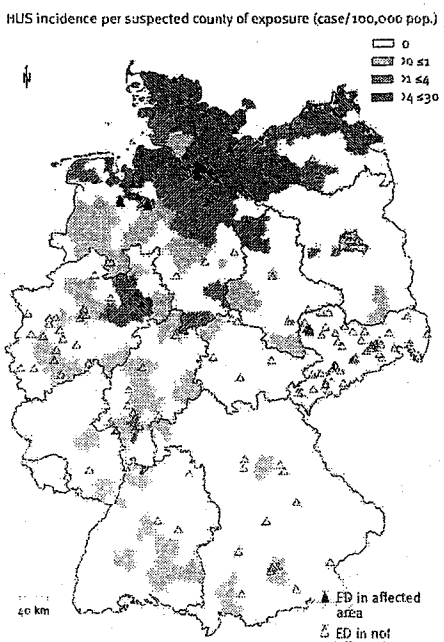


ED: Emergency department; HUS: haemolytic uraemic syndrome; STEC: Shiga toxin-producing *Escherichia coli*.

* Only cases with a notified date of onset since 1 May 2011.

FIGURE 3

Cumulative incidence of HUS cases per suspected county of exposure and emergency departments actively participating in the syndromic surveillance system, Germany, May–June 2011

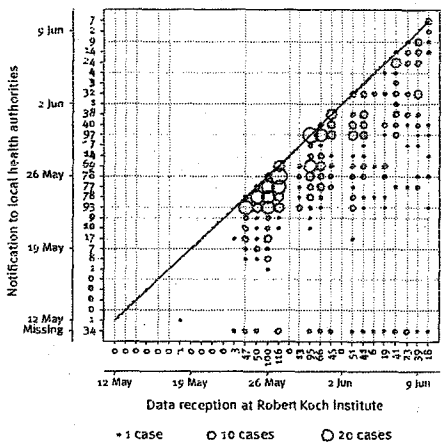


ED: Emergency department; HUS: haemolytic uraemic syndrome; STEC: Shiga toxin-producing *Escherichia coli*.

As of 12 June, a total of 174 ED have participated in the syndromic surveillance system; 27 of which were located within affected areas. The number of ED actively reporting varied from day to day. Thus results may change as further, re¬tro¬spective, reports are received from ED. Between 28 May and 12 June, 4.7% (744/15,884) of all patients presenting to ED in affected regions were reported as having bloody diarrhoea (Figure 5); this proportion was 0.8% (464/55,255) in non-affected regions. Figure 5 shows the sex and age distribution of patients with BD as well as the number of participating ED in affected areas. Women were affected more often than men, with a decreasing proportion of female cases observed after 30 May. Since 6 June, the proportion of all patients with bloody diarrhoea among the patients presenting to emergency departments has remained on an average of 3.6%.

FIGURE 4

Date of notification of HUS cases to local health authority in relation to date of reception at Robert Koch Institute, Germany, May–June 2011



HUS: haemolytic uraemic syndrome; PH: public holiday; STEC: Shiga toxin-producing *Escherichia coli*; WE: weekend.

Weekends and public holidays in bold; the x- and y-axis additionally show the number of reports received.

The size of the circle is equivalent to the number of cases (examples for 1, 10 and 20 cases shown in the legend).

Assessing the capacities for treatment of haemolytic uraemic syndrome in Germany

From 30 May onwards, the German Society for Nephrology collected data on the HUS treatment capacities in Germany and reported these regularly via e mail to the RKI. During the outbreak period, 79 hospitals, located in 15 of the 16 federal states, provided almost daily information: all but two confirmed having sufficient capacities for treating HUS patients.

Initiating active laboratory surveillance

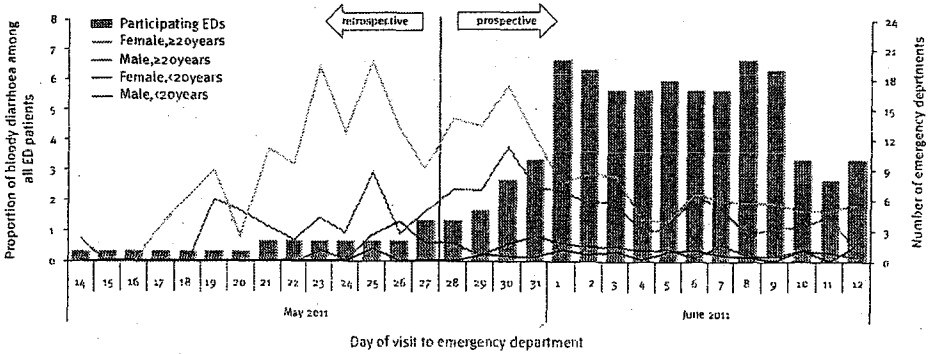
Since 25 May, the RKI has asked four laboratories for daily data transfer per email or telephone. As of 12 June, a total of 195 (6%) of all 3,228 STEC/HUS cases have been confirmed through the routine mandatory system as caused by the outbreak strain STEC/VTEC O104, whereas the active system provided evidence that at least 335 patient samples were related to the outbreak strain.

Reports to the European Union and the World Health Organization

Following international law, Germany informed the European Union (EU) of the STEC/HUS outbreak via

FIGURE 5

Proportions of patients with bloody diarrhoea among all patients presenting to emergency departments, by age and sex, in areas affected by the STEC/HUS outbreak, Germany, May–June 2011 (n=744)



ED: emergency department; HUS: haemolytic uraemic syndrome; STEC: Shiga toxin-producing *Escherichia coli*.

the Early Warning and Response System (EWRS) on 22 May 2011, and notified the event as a potential public health emergency of international concern within the framework of the International Health Regulations (IHR) 2005 on 24 May. The RKI sent updates on the situation to EWRS, the Epidemic Intelligence Information System (EPIS) and the World Health Organization (WHO) on a daily basis.

Both the European Centre for Disease Prevention and Control (ECDC) and the WHO immediately supported the outbreak investigations by staying in close contact with Germany and other countries and reporting imported STEC/HUS cases (in travellers) associated with the outbreak.

Conclusions

Germany has a well established broad statutory surveillance system for infectious diseases. However, the rather long time limits permitted for communicating information on cases from the local to the state/national level led to delayed recognition of this outbreak: The first report at the national level was received on 18 May 2011, while the first outbreak-associated cases fell ill on 1 May, with a sharp increase in case numbers on 9 May. This is a limitation requiring further evaluation. In this specific outbreak situation, the mandatory surveillance system required enhancement that was rapidly and effectively implemented. Physicians, laboratories, local and state health authorities supported the acceleration and extension of the system extraordinarily well. Feedback to the public, the responsible authorities, physicians and laboratories was ensured daily, e.g. by updates on websites, teleconferences and reports.

The additional surveillance instruments were voluntary and allowed for more timely monitoring of this public health emergency. Laboratory surveillance permitted assessment of the actual number of laboratory-confirmed outbreak cases particularly in the early stages. Monitoring capacity for treating HUS patients in German hospitals allowed us to evaluate whether or not international help would be needed. Syndromic surveillance in ED permitted us to follow the temporal trend of bloody diarrhoea patients as a proxy for potentially new STEC/VTEC cases.

We conclude that infectious disease surveillance in Germany can rapidly be adapted to specific outbreak situations. Nevertheless, data flow within the statutory surveillance system should be accelerated, e.g. by use of an electronic notification system by physicians and laboratories, and a common central data base. We recommend continuing syndromic surveillance in ED for at least the next three months to ensure timely detection of possible new trends.

Acknowledgements

We gratefully acknowledge the contribution of all physicians and laboratories as well as the local and state health departments, whose investigations and notifications were the data basis of this report. We especially thank the various emergency departments participating in the syndromic surveillance system, the laboratories involved in the laboratory surveillance system and the German Society for Nephrology for their support.

HUS surveillance and laboratory team

Muna Abu Sin, Susanne Behnke, Bonita Brodhun, Hermann Claus, Yvonne Delere, Lena Fiebig, Barbara Gunsenheimer-Bartmeyer, Andreas Gilsdorf, Karin Haar, Barbara Hauer, Margareta Ung-Zu Kang, Monika Luchtenberg, Inge Mücke, Janina Neifer, Ines Noll, Susanne Schink, Irene Schöneberg, Ed Velasco, Bettina Weiß.

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別紙様式第２-１
番号８

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日		第一報入手日 2011年8月19日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②乾燥抗 HBs 人免疫グロブリン ③ポリエチレングリコール処理抗 HBs 人免疫グロブリン						
販売名 （企業名）	①ヘブスブリン筋注用 200 単位　（ベネシス） ②ヘブスブリン筋注用 1000 単位　（ベネシス） ③ヘブスブリン IH 静注 1000 単位　（ベネシス）		研究報告の公表状況	CDC／MMWR 60 (32) 1083-1086/2011/08/19		公表国 アメリカ	
研究報告の概要	重篤な、時に致命的な肺炎になるレジオネラ病（LD）と、インフルエンザ様の定型的疾患のポンティアック熱（PF）は、レジオネラ菌によって引き起こされるレジオネラ症の二つの最も一般的な症状である。レジオネラ症例は、国立届出疾病監視システム（NNDSS）、及び旅行関連症例の監視データを管理、集団発生の検出を強化するために設計したレジオネラ疾病監視システム（SLDSS）を通じて CDC に報告された。この報告書は、米国 50 州とコロンビア特別区（DC）から 2000 年～2009 年の間 NNDSS へ報告された症例を評価し、10 万人当たりの年齢補正した発生率を計算した。米国のレジオネラ症の年間報告数は 2000 年の 1,110 から 2009 年の 3,522（2000 年から 2009 年の間、NNDSS へ 22,418 例のレジオネラ症が報告された）へ 217%増加し、粗い国内発生率は 2000 年の 0.39/10 万人から 2009 年の 1.15/10 万人へ 192%増加した。						
	報告企業の意見		今後の対応				使用上の注意記載状況・その他参考事項等
	レジオネラは、レジオネラ属に属する真正細菌の総称であり、2～5μm位の好気性グラム陰性の桿菌で、一本以上の鞭毛を持っている。万一、原料血漿にレジオネラ菌が混入したとしても、除菌ろ過等の製造工程にて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。				代表としてヘブスブリン IH 静注 1000 単位の記載を示す。 2. 重要な基本的注意 (1) 本剤の原材料となる血漿については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

18

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People. Saving Money through Prevention.

Morbidity and Mortality Weekly Report (*MMWR*)

Legionellosis --- United States, 2000--2009

Weekly
August 19, 2011 / 60(32);1083-1086

Legionnaires disease (LD), a serious, sometimes lethal pneumonia, and Pontiac fever (PF), an influenza-like, self-limited illness, are the two most common forms of legionellosis, which is caused by *Legionella* bacteria. Legionellosis cases are reported to CDC through the National Notifiable Disease Surveillance System (NNDSS) and a Supplemental Legionnaires Disease Surveillance System (SLDSS) designed to manage surveillance data on travel-related cases and enhance outbreak detection. For this report, cases reported to NNDSS during 2000--2009 from the 50 states and the District of Columbia (DC) were assessed, and crude and age-adjusted incidence rates per 100,000 persons were calculated. U.S. legionellosis cases reported annually increased 217%, from 1,110 in 2000 to 3,522 in 2009, and the crude national incidence rate increased 192%, from 0.39 per 100,000 persons in 2000 to 1.15 in 2009. Because NNDSS is a passive surveillance system dependent on health-care providers and laboratories reporting cases, the actual incidence of legionellosis in the United States likely is higher. Although NNDSS does not record legionellosis cases by type, 99.5% of the legionellosis cases reported to SLDSS during 2005--2009 were classified as LD and 0.5% as PF. Legionellosis surveillance was added to the population-based Active Bacterial Core surveillance (ABCs) system in January 2011 to assess reasons for these increases in numbers of reported cases. The rise in reported cases reinforces the need for health-care providers in all parts of the United States to test and treat adults with severe community-acquired pneumonia for LD, to be vigilant for health-care--associated LD, and to report legionellosis cases to public health authorities.

NNDSS receives reports of cases of nationally notifiable diseases from state health departments, including data on case demographics, the earliest date associated with the patient's illness in public health records (i.e., the date of symptom onset, date of diagnosis, date of confirmatory laboratory test, or the date of the report of the case to the county or state, whichever is earliest), the date of report to CDC, the case status (i.e., confirmed, probable, or suspected), and whether or not the case is part of an outbreak. NNDSS data for 2000--2009 were used to describe legionellosis case demographics, assess seasonal patterns of legionellosis infection, and, using denominators from the 2000 U.S. standard population (*1*) and U.S. Census Bureau estimates, calculate crude and age-standardized incidence rates for the entire United States (excluding U.S. territories) and for each of the nine U.S. Census divisions.* Only cases considered confirmed under the 2005 Council of State and Territorial Epidemiologists' (CSTE) legionellosis case definitions are described in this report.† To be classified as confirmed, cases must be clinically compatible with legionellosis (i.e., fever, myalgia, cough, and/or clinical or radiographic evidence of pneumonia) and meet at least one of the confirmatory laboratory criteria (i.e., recovery of *Legionella* sp. in culture, detection of *Legionella pneumophila* serogroup 1 antigen in urine, or fourfold or greater rise in *L. pneumophila* serogroup 1-specific serum antibodies).

States also are encouraged to report cases to SLDSS to enhance detection of travel-related outbreaks and to provide information on additional legionellosis case variables not captured by NNDSS.§ Legionellosis cases ideally should be reported to both NNDSS and SLDSS. SLDSS collects information related to case demographics, diagnosis, diagnostic testing, hospitalization, outcome, outbreak involvement, nosocomial classification, and recent travel history. In addition to the reports of cases among U.S. residents received from state health departments, SLDSS reports occasionally come from cruise lines, health-care providers, and private citizens. Those additional reports are verified with the relevant state health departments before inclusion in the SLDSS database. Foreign public health authorities also report cases to SLDSS, usually among travelers to the United States. In 2005, CSTE issued a position statement (*2*) requesting that all legionellosis cases be reported to SLDSS, but such reporting is not mandatory, and case follow-up varies by state and county based on staffing availability and perceived public health importance. For this report, SLDSS data were used to characterize diagnoses, diagnostic testing, outcomes, outbreak involvement, and recent travel.¶ Because of potential differences in data received by SLDSS before and after the 2005 CSTE position statement, separate analyses were conducted using cases with onset during 2000--2009 (NNDSS data) and 2005--2009 (SLDSS data).

During 2000--2009, the 50 states and DC reported 22,418 cases of legionellosis to NNDSS. The crude national incidence rate increased 192%, from 0.39 per 100,000 persons in 2000 to 1.15 in 2009, and the age-adjusted incidence of legionellosis in the United States increased 170%, from 0.40 to 1.08 cases per 100,000 persons. In 2000, the age-adjusted incidence varied substantially by U.S. Census division, from 0.09 cases per 100,000 persons in the West South Central division to 0.73 cases in the Middle Atlantic division. This disparity increased absolutely over the decade (Middle Atlantic division: 2.60 cases per 100,000 persons and West South Central division: 0.44 cases in 2009) (Table 1). All reporting divisions had an increase in age-adjusted legionellosis incidence from 2000--2001 to 2008--2009, ranging from a 101% increase in the West North Central division to 294% in the West South Central division. Nationally, 16,595 cases (74%) were in persons aged ≥50 years, and 14,255 (64%) persons were male (Table 2). Legionellosis incidence increased for all age groups from 2000 to 2009, ranging from 8% for persons aged ≤9 years to 287% for persons aged ≥80 years.

Among the 18,392 cases (82%) reported to NNDSS with available information on race, 78% were white, 19% were black, and 3% were American Indian/Alaska Native, Asian, or other (Table 2).** Cases tended to occur in the summer and early fall, with the June--October period accounting for 62% of the cases reported each year (Figure).

During 2005--2009, a total of 5,080 confirmed legionellosis cases among U.S. residents were reported to SLDSS by 47 states,†† accounting for 35% of the 14,554 confirmed cases reported to NNDSS during the same period by all 50 states and DC. An additional 82 confirmed legionellosis cases were reported among foreign visitors to the United States. A total of 1,220 (24%) cases involving U.S. residents were travel-associated; 81% of these involved domestic travel only, and 5% involved cruise ship travel. Travel-associated cases accounted for at least two thirds of the cases reported to SLDSS from 21 states, 11 of which reported only travel-associated cases, suggesting a bias against reporting nontravel-associated cases to SLDSS from some states. Of 3,872 (76%) U.S. resident cases with data available, 4% were associated with a known legionellosis outbreak or possible cluster. Information on clinical outcomes was available for 4,478 (88%) U.S. resident cases, 8% of which resulted in deaths. Urine antigen tests were used to confirm 97% of U.S. resident cases reported during 2005--2009. Only 5% of cases were confirmed by culture during this period, and <1% were confirmed by either serologic or direct fluorescent antigen testing.

Reported by

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Editorial Note

Reported legionellosis incidence rates increased nearly threefold during 2000--2009. In 2009, NNDSS received 3,522 case reports, the most since legionellosis became a reportable disease in 1976 (*3,4*). Increased rates were observed across all age groups and geographic regions. The reported case totals likely underestimate the actual disease burden; the most recent completed U.S. population--based pneumonia etiology study estimated that 8,000--18,000 persons are hospitalized each year with LD (*5*).

An increasing population of older persons contributed to the increase in reported legionellosis cases. Other factors that might have contributed include an increasing population of persons at high risk for infection; improved diagnosis and reporting, possibly stimulated by the 2005 CSTE endorsement of more timely and sensitive legionellosis surveillance; and increased use of urine *Legionella* antigen testing. However, because increases in urine antigen testing began in the 1980s, its use is unlikely to account for the entire increase in legionellosis cases since 2000 (*3,4*).

Urine antigen tests are easy to perform and provide timely, accurate results (sensitivity: 60%--80%; specificity: >99%) for detecting *L. pneumophila* serogroup 1, the causative agent in 70%--80% of LD cases (*6*). In contrast, culture of respiratory samples from possible LD cases (sensitivity: 20%--80%; specificity: >99%) can detect all forms of *Legionella* but has a lengthy turnaround time, and its sensitivity is highly dependent on the skill of laboratory personnel. Similarly, identifying legionellosis through paired serology (sensitivity: 70%--80%; specificity: >95%) involves substantial logistical challenges, whereas direct fluorescent antigen testing for LD (sensitivity: 25%--75%; specificity: >95%) can be technically demanding and can result in false positives resulting from cross-reactions with other bacteria. Only urine antigen and serology are useful for detecting PF, but the sensitivity of these tests for confirmation of PF is substantially lower than for LD (*7*).

Similar to the findings of previous studies, males accounted for >60% of cases, and increasing age was a major risk factor for legionellosis (*3,4*). However, the finding that blacks accounted for a disproportionately high number of cases relative to their 12% share of the population was unexpected. Insufficient information is available to confirm whether these patterns might be the result of differences in underlying risk factors or exposures to *Legionella*, and the high proportion of cases in persons of unknown race limits the interpretation of the racial differences observed.

Legionellosis demonstrates seasonal and geographic variability. During 2000--2009, nearly all regions reported their highest proportion of cases during the summer and early fall. The reported 2009 age-adjusted legionellosis rate in the Middle Atlantic division was nearly six times higher than the rate in the West South Central division. Whether these differences are related to the frequency of testing or reporting is unclear; nonetheless, clinicians should be particularly vigilant for possible LD during the summer and early fall and in geographic areas of relatively high legionellosis incidence. Although use of a urine antigen test for *Legionella* is recommended for cases of severe community-acquired pneumonia (*8*), collection of respiratory specimens for *Legionella*-specific culture also is encouraged as a means to detect all species and subgroups of *Legionella* and enable strain identification in the event of an outbreak. Urine antigen tests and *Legionella*-specific culture also are recommended for suspected cases of health-care--associated LD (*9*).

The findings in this report are subject to at least four limitations. First, current passive surveillance systems cannot determine whether the observed increase in legionellosis cases is actual or an artifact of improved detection or reporting. Second, surveillance likely is biased toward capture of more severe LD cases that are more likely to be tested for *Legionella*, missing those that have been empirically treated with antibiotics active against *Legionella* spp. and those not requiring hospitalization. Third, the nonspecific symptoms of and lack of good diagnostic tests for PF likely result in substantial underdiagnosis of this form of legionellosis. Finally, the proportion of cases that are potentially travel-associated likely is an overestimate resulting from a bias in many states toward primarily reporting travel-associated cases to SLDSS.

A better understanding of the disease burden and the epidemiology of legionellosis is important, but current passive surveillance systems cannot provide all the information required. In January 2011, active laboratory-based and population-based surveillance was launched in 10 ABCs sites around the country.§§ Data from this surveillance will be used to obtain population-based estimates of disease incidence; further describe demographic, seasonal, and geographic variability; and evaluate and improve legionellosis prevention efforts, such as the guidance provided by the American Society of Heating, Refrigerating, and Air Conditioning Engineers on preventing legionellosis associated with building water systems (*10*).

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

† The previous case definition, in use during 1996--2004, also included detection of *Legionella pneumophila* serogroup 1 through direct fluorescent antibody testing as a confirmatory laboratory test. The 2005 case definition is available at http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/legionellosis_current.htm. The 1996 case definition is available at http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/legionellosis1996.htm.

§ A legionellosis case report form is available to state and local health departments at http://www.cdc.gov/legionella/files/legionella_case_report.pdf.

¶ A case of legionellosis was considered to be potentially travel-associated if the patient reported spending at least one night away from home during the 2 weeks before illness onset.

** Data on the ethnicity of cases reported to NNDSS were not included because 39% of cases were in persons of unknown ethnicity.

†† Nebraska, North Dakota, Wyoming, and DC did not report any confirmed legionellosis cases to SLDSS during 2005--2009. During this period, 29--40 states reported cases to SLDSS each year.

§§ Additional information is available at http://www.cdc.gov/abcs/index.html.

What is already known on this topic?

Thousands of cases of legionellosis occur each year in the United States as either Legionnaires disease, an often severe form of pneumonia, or Pontiac fever, an influenza-like, self-limited illness.

What is added by this report?

The incidence of reported legionellosis in the United States nearly tripled during 2000--2009, from 0.39 per 100,000 persons to 1.15. The reasons for this increase are unknown, but increases in the number of older persons and persons at high risk for infection and increased case detection or reporting might have played a role. Incidence increased with age and was highest in the Northeast.

What are the implications for public health practice?

Active, population-based legionellosis surveillance is needed to better assess the epidemiology and apparently increasing incidence of legionellosis in the United States. The rise in reported cases reinforces the need for health-care providers to test adults with severe community-acquired pneumonia or health-care--associated pneumonia for Legionnaires disease and report legionellosis cases to public health authorities.

TABLE 1. Age-adjusted incidence of reported legionellosis cases, by U.S. Census division* and year, 2000--2009

U.S. Census division	Annual incidence per 100,000 population									
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
New England	0.38	0.48	0.81	0.79	0.71	1.00	1.20	1.04	1.43	1.21
Middle Atlantic	0.73	0.67	0.88	1.41	1.25	1.74	2.21	1.86	2.33	2.60
East North Central	0.64	0.68	0.64	0.97	1.03	0.96	1.26	1.24	1.34	1.44
West North Central	0.35	0.27	0.33	0.37	0.38	0.49	0.40	0.54	0.66	0.60
South Atlantic	0.40	0.42	0.42	0.97	0.72	0.73	0.81	0.74	0.79	0.93
East South Central	0.25	0.31	0.26	0.57	0.53	0.47	0.59	0.53	0.61	0.73
West South Central	0.09	0.11	0.12	0.27	0.55	0.24	0.29	0.46	0.34	0.44
Mountain	0.24	0.31	0.31	0.49	0.49	0.49	0.62	0.52	0.46	0.68
Pacific	0.18	0.16	0.17	0.24	0.19	0.26	0.28	0.32	0.48	0.43
Total	0.40	0.41	0.45	0.74	0.70	0.75	0.91	0.86	0.99	1.08

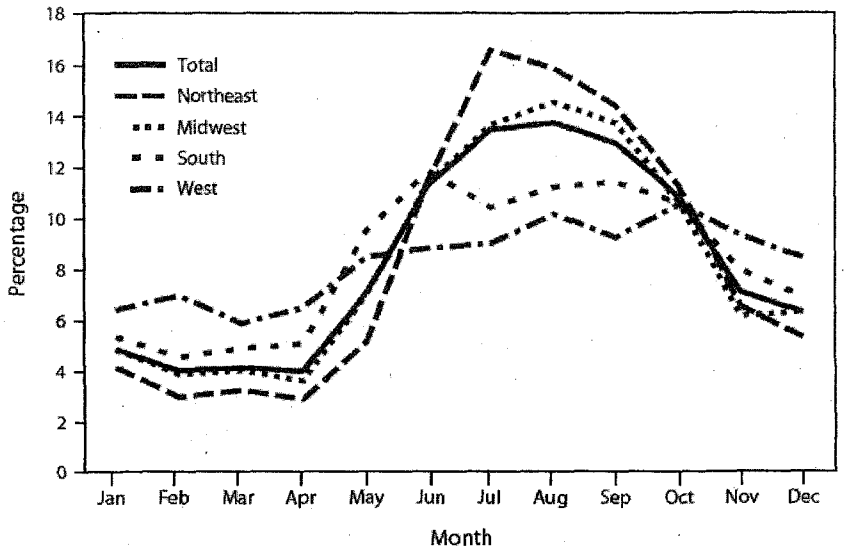
* *New England:* Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont; *Middle Atlantic:* New Jersey, New York, and Pennsylvania; *East North Central:* Illinois, Indiana, Michigan, Wisconsin, and Ohio; *West North Central:* Iowa, Kansas, Missouri, Minnesota, Nebraska, North Dakota,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

TABLE 2. Demographic characteristics of legionellosis cases --- National Notifiable Disease Surveillance System, United States, 2000--2009

Characteristic	No.	(%)	Average per 100,000 population*
Age group (yrs)			
≤9	79	(0)	0.02
10--19	125	(1)	0.03
20--29	516	(2)	0.13
30--39	1,473	(7)	0.36
40--49	3,622	(16)	0.81
50--59	5,401	(24)	1.44
60--69	4,658	(21)	1.94
70--79	3,672	(16)	2.29
≥80	2,864	(13)	2.66
Sex			
Male	14,255	(63)	0.97
Female	8,018	(36)	0.53
Unknown	145	(1)	---
Race			
American Indian/Alaska Native	66	(0)	0.21
Asian	206	(1)	0.14
Black	3,422	(15)	0.87
White	14,287	(64)	0.59
Other	411	(2)	---
Unknown	4,026	(18)	---
Total	**22,418**	**(100)**	**0.75**

* Crude incidence rates, not age-adjusted.

FIGURE. Annual average percentage of legionellosis cases occurring annually, by month and U.S. Census region* --- United States, 2000--2009



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

Alternate Text: The figure above shows the average percentage of legionellosis cases occurring in the United States annually, by month and U.S. Census region during 2000-2009. Cases tended to occur in the summer and early fall, with the June-October period accounting for 62% of the cases reported each year.

Page last reviewed: August 19, 2011

Page last updated: August 19, 2011

Content source: Centers for Disease Control and Prevention

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医薬品
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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 2011年8月31日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②乾燥抗 HBs 人免疫グロブリン ③ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の公表状況	New England Journal of Medicine 2011; 365(6): 422-429	公表国 アメリカ	
販売名（企業名）	①ヘプスブリン筋注用 200 単位 （ベネシス） ②ヘプスブリン筋注用 1000 単位 （ベネシス） ③ヘプスブリン IH 静注 1000 単位 （ベネシス）					使用上の注意記載状況・その他参考事項等
研究報告の概要	エーリキア症（Ehrlichiosis）は、臨床的に重要な新興人獣共通感染症である。Ehrlichia chaffeensis と Ehrlichia ewingii だけが、米国でヒトにエーリキア症を引き起こすと考えられてきた。エーリキア症を疑われる患者は適切な診断を確保し、原因を確かめるために定型的に検査を受ける。我々はエーリキア症の症例の原因を診断し、確かめるために、分子学的方法、培養、及び血清学的検査を用いた。検査では、ミネソタ州とウィスコンシン州のエーリキア症の 4 症例は、Ehrlichia chaffeensis、或いは Ehrlichia ewingii からではないが、その替わり新しく発見されたエーリキア種によって引き起こされたであろうことが分かった。全ての患者は発熱、倦怠感、頭痛、及びリンパ球減少症が見られ、3 人は血小板減少症、2 人は肝酵素濃度上昇を有していた。全員ドキシサイクリン治療を受けた後に回復した。 ミネソタ州とウィスコンシン州で採取された 697 匹のクロアシマダニの少なくとも 17 匹は、ポリメラーゼ連鎖反応検査で同じエーリキア種が陽性であった。遺伝子解析は、この新しいエーリキア種が Ehrlichia muris に密接に関連していることを明らかにした。我々はミネソタ州とウィスコンシン州での新しいエーリキア種を報告し、支援となる臨床的、疫学的、培養、DNA 配列、及び感染源データを提供する。医師は適切な検査、治療、及び地域の監視を確実にするために、この新しく発見された E. muris の近親を知っておく必要がある。					代表としてヘプスブリン IH 静注 1000 単位の記載を示す。 2. 重要な基本的注意 (1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜による ろ過処理を施しているが、投与に際しては、次の点に十分注意すること。
	報告企業の意見		今後の対応			
アナプラズマ属病原体は、直径0.2～2μmの大きさの球状もしくは楕円状のグラム陰性細菌で、反芻動物、ウマ、ヒトの顆粒球で増殖する。万一、原料血漿にアナプラズマ属病原体が混入したとしても、除菌ろ過等の製造工程にて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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ORIGINAL ARTICLE

Emergence of a New Pathogenic Ehrlichia Species, Wisconsin and Minnesota, 2009

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ABSTRACT

BACKGROUND

Ehrlichiosis is a clinically important, emerging zoonosis. Only *Ehrlichia chaffeensis* and *E. ewingii* have been thought to cause ehrlichiosis in humans in the United States. Patients with suspected ehrlichiosis routinely undergo testing to ensure proper diagnosis and to ascertain the cause.

METHODS

We used molecular methods, culturing, and serologic testing to diagnose and ascertain the cause of cases of ehrlichiosis.

RESULTS

On testing, four cases of ehrlichiosis in Minnesota or Wisconsin were found not to be from *E. chaffeensis* or *E. ewingii* and instead to be caused by a newly discovered ehrlichia species. All patients had fever, malaise, headache, and lymphopenia; three had thrombocytopenia; and two had elevated liver-enzyme levels. All recovered after receiving doxycycline treatment. At least 17 of 697 *Ixodes scapularis* ticks collected in Minnesota or Wisconsin were positive for the same ehrlichia species on polymerase-chain-reaction testing. Genetic analyses revealed that this new ehrlichia species is closely related to *E. muris*.

CONCLUSIONS

We report a new ehrlichia species in Minnesota and Wisconsin and provide supportive clinical, epidemiologic, culture, DNA-sequence, and vector data. Physicians need to be aware of this newly discovered close relative of *E. muris* to ensure appropriate testing, treatment, and regional surveillance. (Funded by the National Institutes of Health and the Centers for Disease Control and Prevention.)

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N Engl J Med 2011;365:422-9.

EHRLICHIOSIS AND ANAPLASMOSIS ARE tickborne zoonoses caused by obligate intracellular gram-negative bacteria in the family Anaplasmataceae.[1] Symptoms typically include fever, myalgia, and headache, with rash in rare instances. Severe disease may be associated with gastrointestinal, renal, respiratory, and central nervous system involvement and, in rare cases, death.

In the United States, ehrlichiosis in humans is caused primarily by infection with *Ehrlichia chaffeensis*, which infects monocytes, and less commonly by *E. ewingii*, which infects granulocytes. *Anaplasma phagocytophilum* is closely related to the ehrlichiae and causes human granulocytic anaplasmosis.[1,2] *E. ewingii* and *E. chaffeensis* are transmitted to humans by the bite of an infected tick, *Amblyomma americanum*, whereas *A. phagocytophilum* is transmitted in the United States by the ticks *Ixodes scapularis* and *I. pacificus*.[3]

Ehrlichiosis is a clinically important, emerging zoonosis. *E. chaffeensis*, *A. phagocytophilum*, and *E. ewingii* were first recognized as human pathogens in 1991,[4] 1994,[5] and 1999,[6] respectively. Since then, *E. canis* and *E. muris* have been implicated as causes of human illness in Venezuela and Russia, respectively.[7,8] However, only *E. chaffeensis* and *E. ewingii* have been thought to cause ehrlichiosis in humans in the United States.

METHODS

PATIENTS

EDTA-anticoagulated samples of whole blood obtained from patients throughout the United States with suspected ehrlichiosis or anaplasmosis were submitted for polymerase-chain-reaction (PCR) diagnostic testing for ehrlichia and anaplasma at the Mayo Clinic in Minnesota. Patients with confirmed ehrlichiosis in Minnesota and Wisconsin were interviewed by staff members of local and state health departments according to a standardized questionnaire to obtain demographic, clinical, and epidemiologic information, and medical records were reviewed.

All participants provided written informed consent for collection and testing of additional blood specimens. Research protocols were approved and monitored by the institutional review boards at the Mayo Clinic and the Centers for Disease Control and Prevention (CDC).

REAL-TIME PCR ASSAY

DNA was extracted from the blood specimens (MagNA Pure Instrument, Roche Applied Science) and tested for *E. ewingii*, *E. chaffeensis*, and *A. phagocytophilum* DNA with the use of a real-time PCR assay[9] with primers and fluorescence resonance energy transfer–labeled probes targeting a conserved region of the GroEL heat-shock protein operon. Polymorphisms in the sequence targeted by the probes allowed for differentiation of the three species by means of analysis of melting temperature. Specimens with an atypical result (melting temperature outside the predefined ranges) were tested with the use of a SYBR Green PCR assay targeting the 16S ribosomal RNA gene (*rrs*) of Anaplasmataceae,[10] a nested PCR assay of the GroEL gene (*groEL*),[11] or broad-range *rrs* assays[12] (see Table 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).

DNA SEQUENCING

Amplified DNA fragments were sequenced (3730 DNA sequencer, Applied Biosystems) and analyzed (Sequencher software, version 4.2; Gene Codes). New sequences were submitted to GenBank (accession numbers HM543745 for *rrs* and HM543746 for *groEL*). New, homologous sequences of infective bacteria and related bacteria were aligned with the use of ClustalW software, and phylogenetic analysis was conducted with the use of Molecular Evolutionary Genetics Analysis software, version 4.0.[13]

CULTURE ISOLATION

Buffy-coat and erythrocyte fractions of the whole-blood specimens were processed and inoculated into a tick cell line (ISE6, derived from *I. scapularis*) and a mammalian cell line (RF/6A, derived from rhesus monkey choroid retina; American Type Culture Collection number CRL-1780), according to published protocols.[14] Mammalian cell cultures were incubated in RPMI 1640 medium with 10% fetal bovine serum at 37°C in 5% carbon dioxide, whereas ISE6 cultures were incubated at 34°C in sealed flasks.[15] Cells were examined microscopically for intracellular morulae (bacterial clusters)

of ehrlichia and anaplasma with the use of phase-contrast or bright-field microscopy.

SEROLOGIC TESTING

Serum and plasma specimens from patients with an atypical *gro*EL PCR product were tested for IgG-class antibodies reacting to *E. chaffeensis* or *A. phagocytophilum* with the use of a commercial indirect immunofluorescence assay (Focus Diagnostics).[16] Serum and plasma samples were also tested by means of noncommercial indirect immunofluorescence assays developed and used at the CDC for IgM- and IgG-class antibodies against *E. chaffeensis*, *A. phagocytophilum*, and an ehrlichia species isolated in this study[17]; antigens were derived from canine monocytic DH82 cultures infected with ehrlichia and human promyelocytic HL-60 cultures infected with *A. phagocytophilum*. A reciprocal titer of 64 or higher was considered positive for both assays.

MORPHOLOGIC EXAMINATION OF PERIPHERAL-BLOOD SMEARS

Wright-stained peripheral-blood smears from each patient with an atypical *gro*EL PCR product were screened for the presence of intracellular morulae characteristic of ehrlichia species.

TICK COLLECTION AND DNA EXTRACTION

Ticks were collected in June and July 2009 by the Wisconsin Division of Public Health, Medical Entomology Laboratory, University of Wisconsin–Madison and the Minnesota Department of Health. Tick collection was conducted by dragging a fabric flag (1 m by 1 m) across vegetation at or near residences of patients in northwestern Wisconsin and northeastern, central, and northwestern Minnesota. DNA extraction from ticks was performed with the use of a modified version of a published protocol,[18] with three to five nymphs from Wisconsin processed at a time. DNA was tested with the use of the *gro*EL fluorescence resonance energy transfer assay and the *rrs* SYBR Green PCR assay.

RESULTS

REAL-TIME PCR ASSAY AND SEQUENCING

From June 1 through December 31, 2009, a total of 4247 blood specimens from residents in 45 states were tested by means of *gro*EL PCR assay. Of the 1518 specimens obtained from Wisconsin and Minnesota residents, 163 (10.7%) were positive for *A. phagocytophilum* (35 from Wisconsin and 128 from Minnesota), whereas none were positive for *E. chaffeensis* or *E. ewingii*. Three additional Wisconsin residents and one Minnesota resident had positive PCR tests with a melting temperature that was outside the melting temperature range for *E. chaffeensis*, *E. ewingii*, and *A. phagocytophilum* (Fig. 1 in the Supplementary Appendix). This atypical result was not found for the 2729 specimens collected from the 43 other states.

The four specimens with an atypical *gro*EL PCR melting temperature also tested positive for Anaplasmataceae *rrs* with the use of the SYBR Green PCR assay. The nucleotide sequences of the amplified *rrs* and *gro*EL fragments were identical among the four specimens and shared 98% sequence similarity with the homologous *rrs* and *gro*EL genes of *E. muris* (Fig. 1).

CULTURE ISOLATION

Two ehrlichia species isolates (designated Wisconsin 1 and 2) were cultured from blood specimens obtained from one of the four patients 3 and 4 days before culturing in ISE6 and RF/6A cell lines. Sequence analysis of the PCR-amplified portions of *rrs* showed that they were identical to each other and to the sequences obtained from the clinical specimens with the atypical melting-temperature results.

Morulae were detected with the use of phase-contrast microscopy of live RF/6A cultures 5 weeks after inoculation. Fixed and stained ISE6 cells contained one to three large morulae per cell, whereas RF/6A cells contained numerous, small morulae (Fig. 2).

SEROLOGIC TESTING

Of the four patients with atypical PCR results, two (Patients 2 and 4) (Table 1) were tested by means of the commercial indirect immunofluorescence assay. Serum samples collected from Patient 2 were negative for IgG antibodies to *E. chaffeensis* (i.e., titer <64) on days 2 and 15 after the onset of fever, whereas serum specimens from Patient 4 were positive (i.e., titer ≥64) for IgG antibodies on day 5 (titer of 256) and day 54 (titer of 1024) after the onset of fever.

In addition, serum and plasma specimens from three of these four patients were tested by means of the CDC indirect immunofluorescence assays. At least one specimen from each patient tested was positive for IgM or IgG antibodies reacting to *E. chaffeensis*, and the titers were even higher in response to the new ehrlichia species. A specimen obtained 15 days after the onset of illness from Patient 2 had high titers of IgM and IgG antibodies against the new ehrlichia species; in a specimen obtained 188 days after onset, IgM and IgG antibody titers were substantially reduced. Patient 4 had a strong seroconversion with a high IgG antibody titer 76 days after infection. No antibodies reacting to *A. phagocytophilum* antigens were detected (i.e., titer <64) in three patients with the use of the commercial or noncommercial assay. Specimens from Patient 3 were not available.

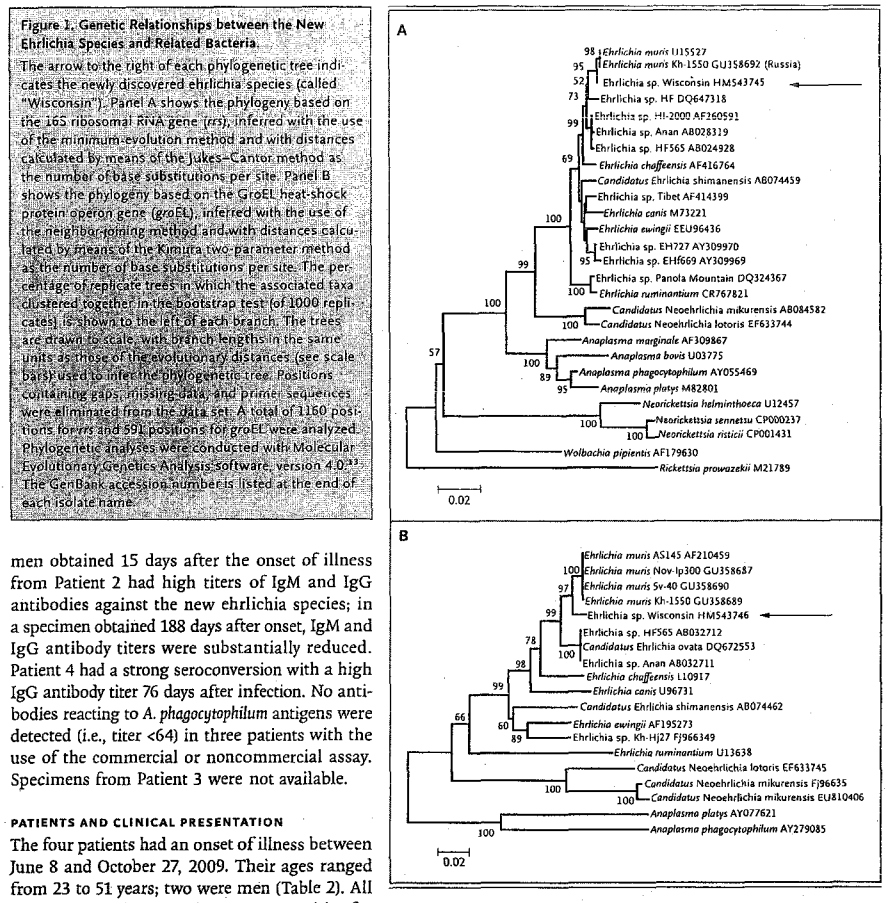

Figure 1. Genetic Relationships between the New Ehrlichia Species and Related Bacteria.

The arrow to the right of each phylogenetic tree indicates the newly discovered ehrlichia species (called "Wisconsin"). Panel A shows the phylogeny based on the 16S ribosomal RNA gene (*rrs*), inferred with the use of the minimum-evolution method and with distances calculated by means of the Jukes–Cantor method as the number of base substitutions per site. Panel B shows the phylogeny based on the GroEL heat-shock protein operon gene (*gro*EL), inferred with the use of the neighbor-joining method and with distances calculated by means of the Kimura two-parameter method as the number of base substitutions per site. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (of 1000 replicates) is shown to the left of each branch. The trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances (see scale bars) used to infer the phylogenetic tree. Positions containing gaps, missing data, and primer sequences were eliminated from the data set. A total of 1160 positions for *rrs* and 591 positions for *gro*EL were analyzed. Phylogenetic analyses were conducted with Molecular Evolutionary Genetics Analysis software, version 4.0.[33] The GenBank accession number is listed at the end of each isolate name.

PATIENTS AND CLINICAL PRESENTATION

The four patients had an onset of illness between June 8 and October 27, 2009. Their ages ranged from 23 to 51 years; two were men (Table 2). All four patients whose specimens were positive for the newly discovered ehrlichia species reported fever, fatigue, and headache. Patient 2 also reported nausea and vomiting. The interval between the onset of illness and the physician visit was 1 to 4 days. Laboratory findings included lymphopenia (in all four patients), thrombocytopenia (in three), elevated hepatic aminotransferase levels (in one of the three patients tested), and mildly elevated alkaline phosphatase levels (in one of the two patients tested). No morulae or other blood parasites were detected in peripheral-blood smears.

Two patients had previously received solid-organ transplants and were taking immunosuppressive drugs at the time of diagnosis. One patient had cystic fibrosis and had undergone bilateral lung transplantation 2 years before the onset of illness; medications received included mycophenolate mofetil, cyclosporine, and prednisone. This patient was hospitalized for 3 days during the acute illness for management of an infiltrate in the left lung and pleural effusion on the left side for which a specific cause was not

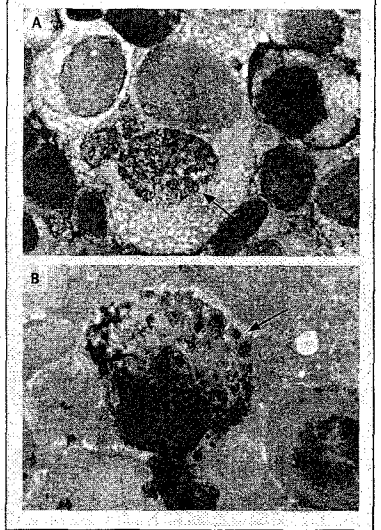

Figure 2. Intracellular Morulae of the New Ehrlichia Species in the ISE6 and RF/6A Cell Cultures.
Panel A shows the ISE6 cell line, and Panel B shows the RF/6A cell line. Morulae are indicated by arrows (Giemsa stain).

determined. The symptoms improved after administration of ceftazidime and doxycycline. The second patient had received a renal allograft 4 years before the onset of symptoms and was receiving mycophenolate mofetil, tacrolimus, and prednisone. This patient had acute kidney injury (serum creatinine level, 2.2 mg per deciliter [194.5 μmol per liter] vs. a baseline of 1.2 mg per deciliter [106.1 μmol per liter]) and was successfully treated with doxycycline. The two immunocompetent patients had relatively mild illnesses and were successfully treated with doxycycline. The patient from Wisconsin received doxycycline at 100 mg twice daily for 21 days, and the patient from Minnesota received the same regimen for 10 days.

EPIDEMIOLOGIC INVESTIGATION

All patients reported peridomestic (e.g., from mowing the lawn) or recreational exposure to ticks or wooded areas (Fig. 2 in the Supplementary Appendix). The three Wisconsin patients resided in Eau Claire or Burnett County, and one reported traveling to Bayfield County in northwest Wisconsin 1 week before the onset of illness. The Minnesota patient resided in Rice County and had traveled to a wooded area in Pine County, Minnesota, within 30 days before the onset of symptoms.

TICK COLLECTION AND PCR ASSAY

A total of 697 ticks were tested. DNA from the newly discovered ehrlichia species was detected in 16 of 534 *I. scapularis* ticks (7 nymphs and 9 adults) from Minnesota, as well as in 1 group of 5 nymphs (of 154 total) from Wisconsin (where the minimum infective rate is 6.5 infected nymphs per 1000 nymphs tested) (Table 2 in the Supplementary Appendix). No DNA from the newly discovered ehrlichia species was detected in 9 *I. scapularis* adults from Wisconsin or 88 *Dermacentor variabilis* adults from Wisconsin.

DISCUSSION

We have identified a new ehrlichia species (subsequently referred to as ehrlichia species Wisconsin) in blood from four patients living in Wisconsin or Minnesota, by using molecular, culture, and serologic methods. The presence of ehrlichia species Wisconsin DNA in blood specimens from these patients collected during the period of acute illness suggests that this organism was the etiologic agent of their infection. This is supported by the results of serologic testing with whole-cell antigens of the Wisconsin isolate: IgM and IgG antibody responses against the species were positive in the three patients tested, with consistently higher titers than those to *E. chaffeensis*. All four patients recovered after administration of doxycycline, which is the antibiotic of choice for the treatment of ehrlichiosis.

The identification of ehrlichia species Wisconsin in humans has important clinical and epidemiologic implications. Ehrlichiosis was not previously thought to be endemic in Minnesota and Wisconsin and would not be routinely tested for among patients from these areas. Also, commercial tests for ehrlichiosis may fail to provide an accurate identification of this organism. The considerable serologic cross-reactivity of the Wisconsin isolate with *E. chaffeensis* could confound diagnostic and epidemiologic studies and may explain the recent increase in the numbers of cases attributed to *E. chaffeensis* infection in Wisconsin and Minnesota on the basis of serologic testing

Table 1. Results of Tests for IgM and IgG Antibodies against the New Ehrlichia Species, *Ehrlichia chaffeensis*, and *Anaplasma phagocytophilum*.*

Patient No.†	Days from Illness Onset to Specimen Collection	Specimen Type	Reciprocal IgM and IgG Titer, CDC Assay						Reciprocal IgG Titer, Mayo Clinic Assay	
			New Ehrlichia Species		*E. chaffeensis*		*A. phagocytophilum*		*E. chaffeensis*	*A. phagocytophilum*
			IgM	IgG	IgM	IgG	IgM	IgG		
1	198	Plasma	256	128	256	<16	<16	<16	NA	NA
2	2	Serum	NA	NA	NA	<32	NA	<32	<64	<64
2	15	Serum	512	256	256	64	<16	<16	<64	<64
2	188	Plasma	16	64	<16	32	<16	<16	NA	NA
4	7	Serum	NA	NA	NA	NA	NA	NA	256	<64
4	46	Serum	NA	NA	NA	NA	NA	NA	1024	<64
4	76	Plasma	32	2048	<16	2048	<16	<16	NA	NA

* Antibody titers were assessed with the use of a noncommercial indirect immunofluorescence assay at the Centers for Disease Control and Prevention and with the use of a commercial indirect immunofluorescence assay (testing for IgG antibody but not IgM antibody) at the Mayo Clinic. NA denotes not available.
† Specimens from Patient 3 were not available for testing.

*only. In addition, PCR assays for E. chaffeensis and E. ewingii may not detect ehrlichia species Wisconsin because of lack of primer and probe homology. The ehrlichia–anaplasma real-time groEL PCR assay used in our investigation has the advantage of providing differential detection of ehrlichia species Wisconsin from *E. chaffeensis*, *E. ewingii*, and *A. phagocytophilum* on the basis of differences in DNA composition of the amplified fragment.[9] Finally, detection of morulae in peripheral-blood samples from infected persons is an unreliable means of diagnosing infection with ehrlichia species Wisconsin. Morulae are detected infrequently in blood from patients infected with ehrlichia species[19] and were not found in blood from our four patients.

Ehrlichia infections in the United States are commonly transmitted by *A. americanum*. However, the northern range for *A. americanum* is not thought to extend into Wisconsin and Minnesota, and public submissions of *A. americanum* ticks to the University of Wisconsin–Madison or the Minnesota Department of Health are uncommon. In contrast, both *D. variabilis* and *I. scapularis* are abundant, human-biting species in northwestern Wisconsin and Minnesota. The presence of ehrlichia species Wisconsin DNA in at least 17 *I. scapularis* nymphs and adults, as well as the absence of ehrlichia DNA in the *D. variabilis* ticks tested, suggests that *I. scapularis* is a vector for ehrlichia species Wisconsin. Extended investigation and tick surveillance are required to understand the distribution of this agent in Wisconsin and Minnesota and to definitively implicate a specific tick vector.

The new ehrlichia species reported in this study is closely related to *E. muris* (with approximately 98% sequence homology), but its exact taxonomic placement cannot yet be determined, because only a few isolates and limited genetic data are available. *E. muris* is considered to be an Old World pathogen found in different ticks of the *I. persulcatus* complex ranging from Eastern Europe to Japan.[10,20,21] *E. muris* DNA has been detected in the blood of small rodents and deer from these areas,[22] suggesting that these animals may be reservoirs of *E. muris* and related organisms. We are also aware of at least 2 PCR-confirmed and 84 serologically diagnosed cases in humans attributed to *E. muris* infection in the Perm region of Russia.[12] Similarly, Japanese investigators reported a 1.1% seroprevalence of antibodies against *E. muris* among 1893 Tokyo residents, with an even higher seroprevalence among rodents (6 to 63%)[23]; however, it is difficult to ascertain whether these antibodies in mice and humans are related to *E. muris* or to other antigenically related organisms, because multiple ehrlichia agents have been reported from the same region.[10,21,24]

In summary, we have characterized a newly discovered ehrlichia species with supportive clini-

Table 2. Laboratory Test Results in the Four Patients Infected with the New Ehrlichia Species, Shortly after Presentation.*

Patient No.	Age	Sex	White-Cell Count	Lymphocyte Count	Platelet Count	AST	ALT	Alkaline Phosphatase
	yr			×10⁻⁹/liter			U/liter	
1	51	M	3.4	0.48	87	76	75	NA
2	23	M	3.6	0.41	104	42	NA	134
3	50	F	5.0	0.84	132	NA	NA	NA
4	50	F	3.6	0.54	212	16	NA	88
Normal range			3.5–10.5	0.9–2.9	150–450	8–48	7–55	45–115

* ALT denotes alanine aminotransferase, AST aspartate aminotransferase, and NA not available (not performed or not reported).

cal, epidemiologic, culture, DNA-sequence, and vector data. Further assessment of the ecologic, epidemiologic, and clinical features of the infection caused by this species is required to facilitate its distinction from other known tickborne infections in this region. To guide diagnostic testing and treatment, physicians should be aware that a novel pathogenic ehrlichia agent is present in Minnesota and Wisconsin and that organism-specific PCR and serologic testing can be used to identify the cause of suspected infections.

Note added in proof: After this article was submitted, Telford et al. reported findings of an *E. muris*–like bacterium in Wisconsin *I. scapularis* ticks collected during the 1990s.[25]

The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Presented in part at the annual meeting of the American Society of Tropical Medicine and Hygiene, Washington, DC, November 18–22, 2009; the annual conference of the Epidemic Intelligence Service, Atlanta, April 19–23, 2010; the International Conference on Emerging Infectious Diseases, Atlanta, July 11–14, 2010; the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, September 12–15, 2010; the annual meeting of the American Society of Tropical Medicine and Hygiene, Atlanta, November 3–7, 2010; and the 6th International Meeting on Rickettsiae and Rickettsial Diseases, Heraklion, Greece, June 5–7, 2011. The findings reported here were also described in a Health Alert Network public health announcement by the Minnesota Department of Health and the Wisconsin Division of Public Health.

Supported in part by a grant from the National Institutes of Health (R01 AI042792, to Dr. Munderloh) and a cooperative agreement with the CDC (5U50C1000483-03, to Mr. Neitzel).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Richard Thoune, Denise Wirth, and Paulette Magur from the Eau Claire City County Health Department; Carol Larson from the Burnett County Department of Health and Human Services; Sue Shea from the Mayo Clinic Health System–Eau Claire; Richard Heffernan, Tom Haupt, and Kristin Hardy from the Wisconsin Division of Public Health; and Gregory A. Dasch, Aubree Roche, and Arianna Salazar from the Rickettsial Zoonoses Branch, CDC.

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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
			2011. 6. 23	該当なし	
一般的名称	新鮮凍結人血漿	研究報告の公表状況	Levicnik-Stezinar S. 21st Regional Congress of the International society of Blood Transfusion, June 18-22, 2011, Lisbon, Portugal.	公表国 スロベニア	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)				

研究報告の概要

○ヒト顆粒球エーリキア症の輸血感染

ヒト顆粒球アナプラズマ症(HGA;以前はヒト顆粒球エーリキア症として知られていた)は、アナプラズマ・ファゴサイトフィルムによるダニ媒介性の人畜共通感染症である。赤血球輸血によるアナプラズマ感染の可能性について報告する。

36歳女性が帝王切開術を受け、その際に6単位の赤血球と2単位の新鮮凍結血漿が輸血された。9日後に発熱を生じ、後に急性呼吸窮迫症候群(ARDS)に転帰した。PCRによってアナプラズマ・ファゴサイトフィルムが検出され、HGAであることが確認された。感染原因として可能性のあるものは輸血のみであった。輸血された8単位についてPCR及び間接蛍光抗体法により検査を行った結果、1検体が陽性を示した。

今回、ARDSを伴う輸血感染HGAの重篤症例を確定した。感染は白血球除去赤血球に起因したため、白血球除去の効果は小さいと思われる。この感染症例は、ダニ咬傷歴のある供血者の一時的な供血停止を支持する。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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
急性呼吸窮迫症候群を伴う重篤な輸血感染ヒト顆粒球エーリキア症が発生したとの報告である。	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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2009 (none WNV positive) and 14.786 in 2010 (2, 1.3% WNV positive). For both the considered methods the need of test repetition was low (under 3%).

Discussion: WNV is a relatively new health threat in Italy and its impact on the National Blood systems is not predictable. Thus, the most effective strategies: surveillance, donors deferral, NAT screening will have to be re-evaluated over time. The rapid introduction of the WNV NAT assay in response to the notification, in 2009, of human neuro-invasive NWV infections is an example of the ability of regulatory agencies, blood testing organizations, and test manufacturers to respond quickly to emerging blood-borne pathogens.

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TRANSFUSION TRANSMITTED HUMAN GRANULOCYTIC EHRLICHIOSIS

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Background: Human granulocytic anaplasmosis (HGA; previously known as "human granulocytic ehrlichiosis") is a tick-born zoonosis that is caused by an obligate intracellular bacterium, Anaplasma phagocytophilum. Human infection is usually tick borne.

Aims: A possible transmission of anaplasma acquired through a transfusion of red blood cells is reported.

Case report: An elective caesarean section and consecutive surgical revision was performed on a 36-year-old woman. 6 units of RBC and 2 units of FFP were transfused. 9 days later the patient developed a fever, which in few days turned into acute respiratory distress syndrome (ARDS). An infection with HGA was confirmed. The only possible cause of infection could have been in the transfusion of blood components.

Methods: The diagnosis of the patient was confirmed when Anaplasma phagocytophilum was detected using PCR. The PCR and IFA serology testing for IgG Anaplasma phagocytophilum was performed on the 8 transfused donations.

Results: One of the blood donor samples was retrospectively identified as PCR positive and IgG reactive (>1:1024)

Table 1: Results of testing donor and recipient for anaplasma phagocytophilum

	RECIPIENT			DONOR		
	PCR	IFA		PC R	IFA	
			-11B days	-	-	
Day of transfusion	-	-	Day of donation (9 days before transfusion)	+	>1:1.024	
+ 15 days	+	-	+ 43 days	-	>1:2.048	
+ 19 days	+	+				
+ 25 days	-	+				

Conclusions: We investigated and confirmed the case of severe HGA with ARDS transmitted by blood transfusion. The infection occurred after the transfusion of leucoreduced red blood cells. The efficacy of leucoreduction is probably small. There were no other recipients of the disputable donation. A psoralen inactivated unit of pooled platelets had already expired and was not used for transfusion. The FFP was destroyed. This proven transmission supports the indication for the temporary deferral of blood donors with a history of tick bites.

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SEN VIRUS INFECTION IN IRANIAN THALASSEMIC PATIENTS

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Background and objectives: SEN virus (SEN-V) is a blood-borne single-stranded, non enveloped DNA virus.

Beta-thalassemia is very common in Iran and some Mediterranean countries. Most of these patients need blood transfusion which increases the risk for blood -borne viruses. The aim of this study was to investigate the prevalence of SEN-V-D and -H viremia among thalassemic patients.

Material and methods: Sera of 260 blood donors and 40 thalassemic patients,who were negative for serum Hepatitis B surface antigen (HBs-Ag) and third-generation HCV antibody (anti-HCV) were tested for SEN-D and -H DNA. DNA was extracted from plasma samples and amplified by seminested PCR.

Results: SEN-D viremia was detected in 4 (1.54%) and 2 (5%) of 260 blood donors and 40 thalassemic patients respectively. SEN-H viremia was detected in 47 (18.08%) and 18 (45%) of 260 blood donors and 40 thalassemic patients respectively. SEN-D or SEN-H viremia was identified in 60 (23.08%) and 19 (47.5%) of 260 blood donors and 40 thalassemic patients respectively.

Conclusions: The prevalence of SEN-H is more than SEN-D. SENV infection is higher in thalassemic Hepatitis than in blood donors. The high frequency of SENV infection among thalassemic patients could be associated with blood transfusion.

Key Words: Blood donor, Prevalence, SEN Virus, Thalassemic patient.

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EFFICACY OF TEMPORARY EXCLUSION OF TRAVELLING BLOOD DONORS

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Background: Travelling donors who unknowingly import exotic infections endanger the safety of the blood supply. Outbreaks should be evaluated rapidly after the first signals of an emerging disease appear only a limited time frame is available for analysis, decision making and implementation of safety measures. Using deterministic calculations we estimated in hindsight the threat of five emerging epidemics abroad, that did (n = 3) and did not cause (n = 2) temporary deferral of travelling Dutch blood donors.

Method: Three outbreaks were analysed that caused temporary exclusion of donors returning from the area involved: (1) an outbreak of West Nile Virus (WNV) in Emilia Romano-Italy (78 notified cases with hospitalizations); (2) WNV outbreak in West Macedonia-Greece (108 notified cases); and (3) the Chikungunya outbreak in Thailand (12115 notified cases). Two outbreaks without deferral of returning donors were analysed: (4) the Sandfly Fever Sicilian Virus (SFSV) outbreak in the Ankara region-Turkey (260 notified cases) and (5) the Hepatitis A outbreak in Latvia (2817 notified cases).

Sources of data and assumptions:

(1) Data on Dutch tourists were obtained from the Dutch Bureau of Statistics. We assume that 5% of Dutch tourists are blood donors. Because donors are relatively healthy they are assumed to travel twice as much as the general population. (2) Dutch tourists are assumed to visit the same regions as all tourists; as reported by statistical offices in the countries involved. (3) The incidence among tourists (i.e. per time unit) is assumed to equal the reported incidence in the local populations.

Results: The estimated numbers of returning infected Dutch blood donors are as follows: (1) WNV outbreak in Emilia Romana resp. in Macedonia: 0.2 resp. 0.5 infected returning donors.

(2) Chikungunya outbreak in Thailand: 1.6 infected returning donors.

(3) Sandfly virus fever in Ankara region: 0.06 infected returning donors.

(4) Hepatitis A in Latvia: 0.6 infected returning donors.

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
				2011. 9. 15	該当なし	
一般的名称	解凍人赤血球濃厚液		研究報告の公表状況		公表国	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			IASR Vol.32 No.8 (No.378) August 2011; Available from: http://idsc.nih.go.jp/iasr/32/378/dj3781.html	日本	

研究報告の概要

○北海道のライム病の発生状況と症例－自験113例の検討

ライム病は起因菌であるスピロヘータの一種*Borrelia burgdorferi* sensu lato(ボレリア)を保有するマダニ類(日本ではシュルツェマダニ)の媒介により生じる全身性感染症である。臨床症状は早期(I、II期)、後期(III期)に大別され、早期は慢性遊走性紅斑(ECM)、顔面神経麻痺などの神経症状、関節痛などをきたし、後期には慢性萎縮性肢端皮膚炎(ACA)、関節炎などを呈する。日本におけるライム病は1987年に長野で1例目が報告されて以来、主に北海道、本州中部以北で200例以上の確定例の存在が推定される。ライム病がマダニ刺咬症のうちどのくらいの頻度で発症するかは不明であったが、1995年～2000年の6年間に北海道の道北道東地方の関連病院を中心に集積したマダニ刺咬症700例のうち確定例が56例(8.0%)であることから、ボレリア汚染地域においてライム病が発症する頻度はマダニ刺咬症の10%未満と推定される。また1989年から2004年までに113例の確定例を集積し、そのうち52例は皮疹部からのボレリア分離培養に日本で初めて成功した。北海道のライム病はECMに代表される皮膚症状が主体で、第II期以後の出現頻度も9例(8.0%)と欧米に比べ低い。また発熱、全身倦怠感などの全身症状の出現頻度もそれぞれ29例(26%)、11例(9.7%)と低く、抗菌薬に対する反応も良好で、一般に軽症例が多い。北海道に代表される日本のライム病が概して軽症である原因は、ボレリアそのものの病原性の違いや、人種的遺伝的違い、抗菌薬を早期に使用できる医療状況、vectorであるマダニの違いなど、複数の要因が関与していると推定される。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
北海道を中心とした日本国内のライム病症例を検討することにより、日本におけるライム病の特徴を示した報告である。	今後も引き続き情報の収集に努める。

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MedDRA/J Ver.14.1J

IASR

(Vol. 32 p. 218-219: 2011年8月号)

JRC2011T-035

北海道のライム病の発生状況と症例—自験113例の検討

1. はじめに

ライム病は起因菌であるスピロヘータの一種の*Borrelia burgdorferi* sensu latoを保有するマダニ類(日本ではシュルツェマダニ)の媒介により生じる全身性感染症である。臨床症状は早期(I, II期)、後期(III期)に大別され、早期は慢性遊走性紅斑(erythema chronicum migrans; ECM)、顔面神経麻痺などの神経症状、関節痛などをきたし、後期には慢性萎縮性肢端皮膚炎(acrodermatitis chronica atrophicans; ACA)、関節炎などを呈する。特徴的なECMがみられれば比較的容易に診断可能だが、マダニ刺咬の既往が不明で、関節、神経症状主体の症例は診断が困難で、血清診断や、病変部の培養などの検査を必要とする。筆者は2004年までに旭川医大皮膚科で113例のライム病を経験し1)、これは本邦の確実例の半数以上を占めると推定される。筆者が現在の病院へ赴任後も毎年3～4人のライム病患者が来院しており、北海道に確実例が年間10例は発生すると推定している。したがって2010年までに、北海道のライム病確実例は200例を超えると推定する。ここで自験113例のライム病の臨床的特徴を概説し、ライム病の最近の症例も含めて紹介したい。

2. ライム病の一般的臨床所見と自験ライム病のECM

ライム病の症状は早期(I, II期)、後期(III期)に大別され、以下に概説する。

I期(局在期):ECMはマダニ刺咬部から紅斑性丘疹で始まり、周辺に紅斑が拡大する。易疲労感、発熱、筋肉痛、頸部痛などの症状を伴ったり、関節痛、リンパ節腫脹もみられることがあり、約4週間続く。

II期(播種期):ECMが多発性にみられたり(二次性遊走性紅斑)、皮膚リンパ球腫、循環器症状としてA-V blockや心臓炎などが稀にみられる。また、顔面神経麻痺、神経根炎、髄膜炎などもみられ、数週間～数カ月続く。

III期(慢性期):数カ月～数年にわたり、ACA、慢性の髄膜炎、末梢神経委縮、大関節の腫脹と疼痛を伴った慢性関節炎がみられる。

これらI～III期の症状が順番に出現せずに、いきなりII期の症状(顔面神経麻痺)が発症することもあるが、後に詳細な病歴をとるとECMの存在していたことが発覚することもある。欧米でもECMの出現頻度は当初は50～70%といわれていたが、患者教育とライム病の診断を厳格化することで、その頻度は90%に達するといわれている。また、自験例では後に述べるようにECM主体の皮膚症状(I期)でとどまる症例が多い。以下にECMの臨床像を述べる。

ECMはマダニ刺咬後、数日～1カ月後に刺咬部を中心に丘疹状紅斑で始まり、急速に拡大して環状になり、径10cm以上になる。典型的なものでは「bull's eye」と表現されるring-shaped erythema(環状紅斑)が多く、homogeneous erythema(均一紅斑)も頻度が高い。稀に紅斑紫斑状になり、hemorrhagic erythema(出血性紅斑)になることもある。稀に浮腫性紅斑で、小水疱、膿疱を伴い蜂窩織炎様2)の臨床像も呈する。さらに径1～2cm程度のatypical stationary erythemaも稀に存在する。ふつう自覚症状は強くないが、掻痒感、灼熱感も伴う。自験113例では環状紅斑が72例(64%)、均一紅斑が36例(32%)であった。ダニ刺咬部は多くは硬結、時に壊死、痂皮を伴う。皮膚外症状としてはECMに伴って発熱、全身倦怠感、頸部痛、筋肉痛、ダニ刺咬部の近くの関節痛、リンパ節腫脹がみられる。本邦では5%以下に顔面神経麻痺などの神経症状がみられると推定される。

3. 自験ライム病113例の特徴

本邦では1987年に長野で1例目が報告3)されて以来、主に北海道、本州中部以北で200例以上の確実例の存在が推定される。発症地域が限られる理由は、起因菌ボレリア(*B. burgdorferi* sensu lato)を保有するマダニが現時点ではシュルツェマダニに限られ、このマダニは本州では標高1,000m以上の山岳地帯および、北海道などの寒冷地域に生息するためと推定される。シュルツェマダニのすべてが*B. burgdorferi* sensu latoを有しているわけではなく、15～22%の保有率である。本邦症例はマダニ刺咬の既往を患者が記憶していることが多いが、欧米ではマダニ刺咬の既往歴は1/3程度である。これは欧米ではサイズの小さな若虫による刺咬が多く、患者が気づかないのに対して、わが国のシュルツェマダニの刺咬はほとんどが成虫によるため、吸血によりかなり大きなサイズになり、患者が認識しやすいためと推定される。

ライム病がマダニ刺咬症のうちどのくらいの頻度で発症するかは不明であったが、1995～2000年の6年間に北海道の道北道東地方の関連病院を中心に我々が集積したマダニ刺咬症4)は700例あり、そのうちECMが発症し、ボレリア培養陽性あるいは血清抗体陽性のライム病確実例が56例(8.0%)であることから、ボレリア汚染地域においてもライム病が発症する頻度はマダニ刺咬症の10%未満と推定される。我々は1989年に1例目5)のライム病を報告して以来、前述したごとく2004年までに113例の確実例を集積し、そのうち52例はBSKII

培地を用いて、皮疹部からのボレリア分離培養6,7) に本邦で初めて成功した。

本症の生命予後は良好であり、北海道のライム病はECMに代表される皮膚症状が主体で、第II期以後の出現頻度も9例(8.0%)と、欧米に比べ低い。また、発熱、全身倦怠感などの全身症状の出現頻度もそれぞれ29例(26%)、11例(9.7%)と低く、抗菌薬に対する反応も良好で、一般に軽症例が多い。また、欧米の第III期にみられるような慢性のリウマチ様関節炎を呈した症例はなく、一過性の関節痛が22例(19%)に認められた。これらの関節痛は治療に対する反応もよく、ECMの消褪とともに症状が消失する。ただし、1999年に胸鎖関節炎の合併を整形外科医によって診断されたIII期の確実例も道東で発生した。顔面神経麻痺が3例8-10) (2.7%)にみられ、髄膜炎9) も認められた。また稀ではあるが、治療に伴うJarisch-Herxheimer反応11) が生じることも留意すべきである。また、最近ではかなりの肝障害がみられた症例もあり、この症例では担当の内科医もライム病関連の肝炎を疑っている(本号6ページ参照)。

北海道に代表される本邦のライム病が概して軽症である原因は、ボレリアそのものの病原性の違いや、人種的遺伝的違い、抗菌薬を早期に使用する医療状況、vectorであるマダニの違いなど、複数の要因が関与していると推定される。他方、世界的にみると、慢性期ライム病では、抗菌薬による治療後も年余にわたって、倦怠感、全身の筋肉痛、知覚異常、言語の記憶力低下などの神経症状が継続することが知られ、急性期症状から引き続き生じる鬱症状との鑑別が以前から問題になっていた。これについて最近は症例の集積がなされて、meta-analysis の結果12) 、2006年のISDA(Infectious Disease Society of America)のガイドラインではpost-Lyme disease syndrome (PLDS)が提唱されている。PLDSの治療については抗菌薬の追加投与が有効か否かのcontrolled trialがなされて、プラセボと有意差がない結果となった。したがって、このような症例を経験した場合は、適切な抗菌薬による治療を1コースのみ追加して行い(エビデンスがないことを念頭に入れて)、その後は対症療法(たとえばアミトリプチリン・商品名トリプタノール)などが推奨されている。我々は適切な治療後も軽度の倦怠感、マダニ刺咬部位近くの神経知覚障害、関節痛が持続するPLDSと思われる症例を1例経験したので報告する。

4. 症例報告

症例:41歳、男性
初診:2006年10月20日
主訴:左下肢のしびれ、索状硬結、倦怠感、眩暈、動悸

現病歴:2006年6月8日、上ノ国町の山で左下腿をマダニ刺症。自分で抜去した。9月中旬から同部位に浸潤性紅斑出現し、拡大するため札幌医大皮膚科初診し、血清ウエスタンブロットにて*B. garinii* 抗体がIgG、IgMともに陽性でライム病と診断され、ミノサイクリン投与をうけた。紅斑は消退したが、主訴の訴えが残り、当科を初診した。2週間テトラサイクリンの内服でも軽快せず、集中力低下、倦怠感が強く、入院治療を希望したため、2006年11月9日当科に入院治療となった。

現症:左下肢に淡褐色の約1cmの硬結(マダニ刺咬部)とその上方に静脈に沿って淡い紅斑が認められた。

入院時検査所見:血液生化学所見はWBC 5,700、Hb 13.7、PLT 23.5万、CRP 0.37、RF 3.4、ASO 18、ALP 263 、CH50 53.1、AST 18、ALT 10、LDH 171、BUN 11.9、CRE 0.94、CPK 185と異常なく、ボレリア抗体0.42(EIA)、心電図、心エコー異常なし、HLA検索はHLA A2、A33、B61、B44、DR9、DR13。

臨床経過:PLDSまたは慢性期ライム病を考え、セフトリアキソン2g/日の点滴を4週間継続した。下肢の索状硬結は改善し、動悸もみられなくなった。しかし、倦怠感と下肢の鈍痛が持続した。退院後はEBM がないことを説明の上、ドキシサイクリン内服を開始した。1カ月後から倦怠感、下肢の鈍痛、集中力低下も改善。本人の希望もあり、さらに1カ月内服して治療中止した(2007.3.16)。仕事への意欲も出て、4月から復帰するという。その後、2カ月に1回経過を見せにくるが、元気である(2008.1.18終診)。経過を通じてアミトリプチリンは使用しなかった。

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JA旭川厚生病院皮膚科 橋本喜夫

今月の表紙へ戻る

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Return to the IASR HomePage(English)

IASR *Infectious Agents Surveillance Report*

HOME IDSC
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別紙様式第2-1

No. 18

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
				2011. 9. 15	該当なし	
一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	CDC Media Relations, September 6, 2011; Available from: http://www.cdc.gov/media/releases/2011/p0906_parasitic_infection.html?s_cid=2011_p0906_parasitic_infection.html	公表国 米国	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)					

研究報告の概要

○ダニ媒介の寄生虫感染に対する米国における血液供給の脆弱性 リスク軽減のためには供血者スクリーニングのバベシア検査が必要

米国疾病管理予防センター(CDC)と共同研究者が行った過去30年にわたる研究の結果によると、バベシアは赤血球内のダニ媒介性寄生虫であり、輸血によって感染しうる。輸血関連バベシア症は初めて発生した1979年以来次第に認識されてきた。感染しているにも関わらず自覚のない供血候補者に対してFDA認可のバベシア検査は万能ではない。バベシア症は生命に関わるが治療可能な疾患であるため、スクリーニング検査の向上を含めて予防の戦略が必要である。複数のメーカーが血液事業者とともにバベシア検査の開発に取り組んでいる。

バベシア症は通常ダニによって媒介されるため、ダニに刺咬され無意識のうちに感染した人からの輸血で伝播することもある。従って、ダニ媒介感染の予防が血液供給の安全対策を助けることとなる。

米国のほとんどのダニ媒介バベシア症は7つの州で(コネチカット州、マサチューセッツ州、ミネソタ州、ニュージャージー州、ニューヨーク州、ロードアイランド州、ウィスコンシン州)、特に暖かい時期に発生している。しかし輸血関連バベシア症は19州において認識されており、年間を通して発生している。

バベシア症はマラリアと誤診されることがあり、専門家は、診断が考慮されない限り重症例でも容易に見逃されると指摘している。2011年1月、バベシア症は全国的な届出疾患となり、州保健省はバベシア症例に関してCDCと情報を共有することを奨励している。バベシア症に関する正確な情報を得ることは、血液供給をより安全にするために有益である。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2011年1月、バベシア症が全米において届出疾患となったことを述べた米国疾病管理予防センターからの報告である。	日本赤十字社では問診時にバベシア症の既往を確認し、該当する場合は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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MedDRA/J Ver.14.1J

CDC Media Relations - Press Release: September 6, 2011

JRC2011T-034

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People. Saving Money through Prevention.

Press Release

For Immediate Release: September 6, 2011
Contact: CDC Media Relations
(404) 639-3286

U.S. blood supply vulnerable to parasitic infection spread by ticks

To reduce risk, Babesia test is needed to screen blood donors

Babesia, a tickborne parasite of red blood cells, is being transmitted through blood transfusions, according to results of a collaborative study, led by the Centers for Disease Control and Prevention, of data from the past three decades. Transfusion-associated cases of babesiosis have been increasingly recognized since 1979, the year the first known case occurred.

The article about the study and an accompanying editorial appear today online in the Annals of Internal Medicine.

In the report, CDC and collaborators describe 159 transfusion-related babesiosis cases that occurred during 1979-2009, most (77 percent) from 2000 to 2009. No Babesia test approved by the Food and Drug Administration is available for screening prospective blood donors, who can feel fine despite being infected.

Babesiosis is a potentially fatal but treatable complication of transfusion. Severe consequences, such as multi-organ failure and death, are most often seen in persons without a spleen, the elderly, and those with a weak immune system. The study authors say prevention strategies, including development of a screening test, are needed. Some manufacturers are working with investigators at blood establishments to develop FDA-approved tests for Babesia for donor-screening purposes.

"We want clinicians to become more aware of babesiosis, including the small possibility of transmission via blood transfusion," says Barbara Herwaldt, M.D., M.P.H., CDC medical epidemiologist, and lead author of the article. "If a patient develops unexplained fever or hemolytic anemia after a transfusion, babesiosis should be considered as a possible cause, regardless of the season or U.S. region."

Because babesiosis is spread most commonly by ticks, the risk of this disease is another reason for people prevent tick bites. People who unknowingly become infected through the bite of a tiny tick (about the size of a poppy seed) can transmit the parasite via blood transfusion. Therefore, prevention of tickborne infection can help safeguard the blood supply.

Most U.S. tickborne Babesia cases have occurred in seven states in the Northeast and the upper Midwest (in parts of Connecticut, Massachusetts, Minnesota, New Jersey, New York, Rhode Island, and Wisconsin), particularly during the warm months of the year. However, transfusion-associatedBabesia cases have been identified in 19 states and have occurred year-round.

Dr. Herwaldt points out that even severe Babesia cases, not just cases that are asymptomatic or mild, are easily missed unless the diagnosis is considered. Even then, babesiosis often is mistakenly diagnosed as malaria, which also infects red blood cells.

In January 2011, babesiosis became a nationally notifiable disease, which means state health departments are encouraged to share information about cases of babesiosis with CDC. More accurate information about tickborne and transfusion-transmitted cases of babesiosis will help CDC and its partners, including the Food and Drug Administration, in their continued efforts to make the blood supply even safer.

Annals of Internal Medicine
Article: http://www.annals.org/content/early/2011/09/02/0003-4819-155-8-201110180-00362
Editorial: http://www.annals.org/content/early/2011/09/02/0003-4819-155-8-201110180-00363

links below for two government–sponsored events that focused on improving blood safety from babesiosis risk.

Information on babesiosis: http://www.cdc.gov/parasites/babesiosis/index.html

Information on the *Babesia* parasite: http://www.dpd.cdc.gov/dpdx/HTML/Babesiosis.htm

Information on FDA public workshop

Information on the Blood Products Advisory Committee meeting

Information on ticks: http://www.cdc.gov/ticks/

For information on CDC's roles in monitoring blood safety: http://www.cdc.gov/bloodsafety

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Historical Document: September 6, 2011
Content source: Office of the Associate Director for Communication, Division of News and Electronic Media
Notice: Links to non-governmental sites do not necessarily represent the views of the CDC.

Centers for Disease Control and Prevention 1600 Clifton Rd. Atlanta, GA 30333, USA
800-CDC-INFO (800-232-4636) TTY: (888) 232-6348, New Hours of Operation 8am-8pm ET/Monday-Friday
Closed Holidays - cdcinfo@cdc.gov

別紙様式第2-1

No. 8

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2011. 7. 14	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿		研究報告の公表状況	百瀬 俊也, 三浦左千夫, 佐藤陽子, 大塚裕司, 平力造, 日高敏, 北折健次郎, 濱口元洋, 高松純樹, 日野学. 第59回 日本輸血・細胞治療学会総会; 2011.4.14-16; 東京都	公表国 日本	使用上の注意記載状況・その他参考事項等 新鮮凍結血漿「日赤」 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)					

研究報告の概要

○在日ブラジル人献血者における*Trypanosoma cruzi*抗体検査の研究的試行とシャーガス病に関するアンケート結果について

目的: サシガメを媒介虫とするシャーガス病は中南米諸国で流行し、感染者は*Trypanosoma cruzi*(以下*T.cruzi*)を長期間体内に保有する無症候性のキャリアとなることが知られている。日本でも中南米からの定住者が約40万人いると言われているが、献血者の中にキャリアがどの程度存在するのかは不明である。今回、日本に定住しているブラジル・サッカーチームのブラジル人サポーター(多くは日系人)の献血イベントに際し、同意を得た上で*T.cruzi*抗体検査と出身地域等のアンケート調査を実施したので報告する。

対象と方法: 在日ブラジル人グループから愛知県赤十字血液センターへ献血協力の申し込みが事前にあったので、献血イベントに参加した献血希望者に対し、あらかじめ*T.cruzi*抗体検査の研究的試行の説明を行い、同意した者から検体を採血しアンケート調査に協力していただいた。*T.cruzi*抗体検査は迅速法(イムノクロマト法)及びELISA法を同センターにて実施し、追加検査を慶応大学医学部にて行った。その他の献血にかかる問診、検査は通常の献血と同様に実施した。

結果: 献血希望者は20名であり、全員が*T.cruzi*抗体検査の研究的試行に同意した。ELISA法は20名全員陰性であったが、迅速法は19名陰性、1名判定保留であった。追加検査の結果、判定保留の1名は偽陽性であると判断した。20名の献血希望者は、男性13名(うち1名VVRにより不採血)、女性7名(うち4名Hb濃度不足により不採血)であり、年齢は20代9名、30代11名であった。出身地はサンパウロ州17名、パラナ州2名、ミナスジェイラス州1名であった。多くはシャーガス病やサシガメを知っていたが、全員家族にシャーガス病の者はおらず、また過去に*T.cruzi*抗体検査をした者は1名のみであった。

結語: 在日ブラジル人献血希望者20名に対し同意を得て*T.cruzi*抗体検査を実施した結果、全員陰性であった。20-30代の若い世代であり、家族にもシャーガス病の者はいなかったが、中南米に居住歴を有する献血者へのさらなる検討が必要と考える。

報告企業の意見	今後の対応
同意を得た在日ブラジル人献血者20名に対し*T.cruzi*抗体検査を実施したところ、全員が陰性であったとの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。

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Japanese Journal of Transfusion and Cell Therapy, Vol. 57, No. 2

JRC2011T-030

O-119 輸血後劇症肝炎にみた感染症検査の問題点と今後の課題

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山田千亜希[1]，藤原晴美[1]，百瀬俊也[2]，内田茂治[3]，鞭波宏樹[4]，平松久弥[4]，小川奈緒子[1]，芝田大樹[1]，府内紘憧[1]，金子 誠[1]，遠藤弘子[1]，鈴村妙子[1]，竹下明裕[1]
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【緒言】本邦の輸血医療の進歩は輸血後感染症を激減させたが，改善のために不断の努力が必要な課題である．今回，輸血後劇症肝炎を発症した症例が発生したが，輸血の安全性の更なる向上を図るため，家族の同意を得た上で当大学倫理委員会の指針に従い症例を詳細に検証し，感染症検査の重要性と今後の課題を検討した．【症例】2008年1月，患者は手術に伴いRCC2袋，計3単位を輸血した．輸血67日前に測定したHBs抗原，HCV抗体，HIV抗体は全て陰性であり，輸血直前に遡及調査用検体を採取していた．献血者2名は献血時のHBs抗原，HBc抗体，20プールNAT検査が全て陰性であった．輸血32日後，患者は他施設でHBs抗原を測定し陰性であった．輸血95日後より全身状態の急速な悪化を認め劇症肝炎と診断された．この時の検査でHBs抗原，HBe抗原，HBc抗体，HBV DNAが陽性となった．血漿交換を含む治療が施行されたが，入院後3日で不幸な転帰をとった．患者の輸血前保存検体ではHBs抗体，HBc抗体，個別NAT検査が全て陰性であったが，1名の献血者由来検体の個別NATが陽性となった．献血者及び患者間で調査のウイルスgenotypeは一致した．患者は生物由来製品感染等被害救済制度による救済給付の対象となった．【考察】現行の20プールNATの検出感度は64IU/mlとされ，それ以下のウイルス濃度での肝炎発症が認められたことになる．感度やコスト等にも限界があるが，輸血用血液に対する検査技術の更なる向上が求められる．また現在推奨されている輸血後感染症検査の実施時期（3ヶ月）に肝炎を発症した．感染の早期発見によって抗ウイルス薬による早期治療が可能な場合もあり，症状の軽減化が期待されるため，検査時期を考える上でも貴重な症例である．輸血後感染症検査の重要性を医療従事者に加え患者自身にも理解してもらい，検査の遂行率を上げていく必要がある．

O-120 在日ブラジル人献血者における *Trypanosoma cruzi* 抗体検査の研究的試行とシャーガス病に関するアンケート結果について

日本赤十字社血液事業本部[1]，慶應義塾大学医学部熱帯医学・寄生虫学教室[2]，愛知県赤十字血液センター[3]
百瀬俊也[1]，三浦左千夫[2]，佐藤陽子[2]，大塚裕司[1]，平 力造[1]，日高 敏[2]，北折健次郎[2]，
濱口元洋[3]，高松純樹[3]，日野 学[1]
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【目的】サシガメを媒介虫とするシャーガス病は中南米諸国で流行し，感染者は *Trypanosoma cruzi*（以下，*T.cruzi*）を長期間体内に保有する無症候性のキャリアとなることが知られている．日本でも中南米からの定住者が約40万人いると言われているが，献血者の中にキャリアがどの程度存在するのかは不明である．今回，日本に定住しているブラジルサッカーチームのブラジル人サポーター（多くは日系人）の献血イベントに際し，同意を得た上で *T.cruzi* 抗体検査と出身地域等のアンケート調査を実施したので報告する．
【対象と方法】在日ブラジル人グループから愛知県赤十字血液センターへ献血協力の申込が事前にあったので，献血イベントに参加した献血希望者に対し，予め *T.cruzi* 抗体検査の研究的試行の説明を行い，同意した者から検体を採血しアンケート調査に協力していただいた．*T.cruzi* 抗体検査は迅速法（イムノクロマト法）及びELISA法を同センターにて実施し，追加検査を慶應大学医学部にて行った．その他の献血に係る問診，検査は通常の献血と同様に実施した．
【結果】献血希望者は20名であり，全員が *T.cruzi* 抗体検査の研究的試行に同意した．ELISA法は20名全員陰性であったが，迅速法は19名陰性，1名判定保留であった．追加検査の結果，判定保留の1名は偽陽性であると判断した．20名の献血希望者は，男性13名（うち1名VVRにより不採血），女性7名（うち4名Hb濃度不足により不採血）であり，年齢は20代9名，30代11名であった．出身地はサンパウロ州17名，パラナ州2名，ミナスジェイラス州1名であった．多くはシャーガス病やサシガメを知っていたが，全員家族にシャーガス病の者はおらず，また過去に *T.cruzi* 抗体検査をした者は1名のみであった．
【結語】在日ブラジル人献血希望者20名に対し同意を得て *T.cruzi* 抗体検査を実施した結果，全員陰性であった．20-30代の若い世代であり，家族にもシャーガス病の者はいなかったが，中南米に居住歴を有する献血者へのさらなる検討が必要と考える．

別紙様式第2-1
番号9

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医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2011年8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	①②乾燥抗HBs人免疫グロブリン ③ポリエチレングリコール処理抗HBs人免疫グロブリン		研究報告の公表状況	http://www.hhs.gov//2011/07/27	公表国 アメリカ	
販売名（企業名）	①ヘブスブリン筋注用200単位　（ベネシス） ②ヘブスブリン筋注用1000単位　（ベネシス） ③ヘブスブリンIH静注1000単位　（ベネシス）					

-180- 研究報告の概要

FDAは、サウジアラビアでBSE原因物質に感染したと考えられるヒトでvCJDが3例発生したことに基づき、血液及び血液成分（原料血漿を含む）のドナーならびにヒト細胞、組織、及び細胞と組織をベースとした製剤（HCT/P）のドナーについての現行のvCJD関連の安全性勧告事項を改変すべきか否か、そしてサウジアラビアでの滞在期間について、特定の血液ドナーを不適格とすることの勧告、またはHCT/Pのドナーで特定の不適格なドナーを見出すことに関する勧告について、伝達性海綿状脳症諮問委員会（TSEAC）からの助言を求めている。

2011年3月にHealth Canadaは最近移住してきた1人がvCJDのほぼ確実な例であるとの報告を行った。その患者は若い男性で1986年生まれ、少年期は殆どサウジアラビアで過ごしており（12年間）、その後隣接するドバイ（UAE）で4年間を過ごした。この患者のvCJDの最初の症状は、ドバイからカナダへ移住する前の2011年初期に現れていた。Health Canadaの専門家は、その男性はこれまで手術を受けたことも輸血を受けたこともないので、おそらくはアラビア半島に居住していた間に、さらに言えばドバイよりもこの患者が少年期の殆どを過ごしていたサウジアラビアで、BSE原因物質に食物を介して暴露されて感染したのであろう、と結論づけている。この患者はサウジアラビアで食物を介してBSE原因物質に暴露されたと考えられる第3例目である。以前に報告されたvCJDの1例は、UCSF病院（University of California, San Francisco）で診断され、概略がCDCに報告された例で、ヴァージニア州に住んでいた患者で、サウジアラビア人ではないが、サウジアラビアで生まれ育っていた。更にそれ以前の2003年のvCJD症例は、詳細な報告はないが、33歳のサウジアラビア人で、おそらくはサウジアラビア国内で感染したものであろうと結論づけた。
サウジアラビアから世界動物保健機関（World Organisation of Animal Health）へのBSEの症例報告はこれまで全くない。しかし、サウジアラビアは問題となっていた期間（1980年～1996年）に英国からウシ及び牛肉製品を輸入していたことが確認されており、またサウジアラビアは肉骨粉（MBM）がBSE原因物質によって汚染されていた可能性があるとされている1988～1993年の間に、英国起原のMBMの荷受け先であったことが確認されている。我々は、サウジアラビアでのBSE原因物質へのヒトへの暴露危険性は、懸念されている年度である1980年から1996年までの間にこの地域に輸出された英国起原の牛肉及び生牛が、主要なものであると推測した。

サウジアラビアに居住していたことによるものと考えられるvCJDの3例の報告は、米国の血液安全性についての勧告事項やHCP/Pの安全性に影響を与えるが、それはドナーとなる可能性のある次の4群の人々の適格性に影響を及ぼす：
1980-1996年の間に (1) サウジアラビアに駐留していた米軍関係者
(2) サウジアラビアの米軍活動をサポートしていた契約請負業者の米国人労働者
(3) サウジアラビアで非軍事の請負業者に雇われていた米国人労働者
(4) サウジアラビアに住んでいたが米国に移住した移住者
現在のところサウジアラビアは、FDAがドナーの不適格性を判断することを勧告している国々のリストには含まれていない。FDAは現行の適格性/不適格性の勧告事項を改定してその中に、1980年から1996年末までの間にサウジアラビアに軍関係者として累積で6ヶ月以上滞在した、血液及び血液成分（原料血漿及びHCT/Pを含む）のドナーを含めることを考慮中である。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリンIH静注1000単位の記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・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は排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

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議題： 1. 以下の者について、原血漿を含む血液と血液製剤のドナーの延期を推奨、及びHCT/Pの不適格なドナーとする a) 1980年初め～1996年末までの間、米軍としてサウジアラビアに累積6ヵ月以上滞在した者 b) 1980年初め～1996年末までの間、サウジアラビアに累積5年以上滞在した者 2. 血液、血液製剤、血漿由来品、HCT/Pの供給の影響の可能性、製品の安全性についてのこれら推奨事項の寄与について 3. FDAの案、または更なる安全性基準について TSEACは、サウジアラビアを訪れた一部ドナー（1980年初めから1996年末まで米国軍人としてサウジアラビアに累計6ヵ月以上滞在していた人、或いは同期間に累計5年間以上滞在していた人）は献血延期すべきであることについて合意した。	

報告企業の意見	今後の対応
血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、本剤の製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	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。

ヘブスブリン

FDA Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC)
23rd Meeting, August 1, 2011
Gaithersburg, MD

Issue Summary

Donor Deferral/Ineligibility for Time Spent in Saudi Arabia to Reduce Risk of vCJD Transmitted by Blood and Blood Products and by Human Cells, Tissues and Cellular and Tissue-Based Products (HCT/Ps)

Issue

FDA seeks advice from TSEAC on whether, based on three cases of vCJD in individuals likely to have been infected with the BSE agent in Saudi Arabia, to modify current vCJD-related safety recommendations for donors of blood and blood components, including Source Plasma, and for donors of HCT/Ps, to recommend deferring of certain blood donors or finding ineligible certain donors of HCT/Ps for time spent in Saudi Arabia.

Background

vCJD in recent Saudi immigrant to Canada. In March 2011 Health Canada described a probable case of vCJD in a recent immigrant. The diagnosis of vCJD was supported by results of a tonsil biopsy in Canada showing accumulation of abnormal prion protein [26]. The patient is a young man, born in 1986, who spent most of his early life in Saudi Arabia (12 yr) and, later, in neighboring Dubai, United Arab Emirates (4 yr) [4, 11]. He first showed symptoms of vCJD early in 2011 before emigrating from Dubai to Canada. The probable time of food-borne infection with the BSE agent for individuals with the patient's prion-protein-encoding (*PRNP*) genotype (129MM) falls within the years he lived in the Arabian Peninsula (estimated median incubation period of food-borne vCJD for persons with the *PRNP*-129-MM genotype, estimated to be 12 to 13 yr [1, 18]). Authorities at Health Canada [19] have concluded that the man, who has no history of surgery or blood transfusion, was probably infected by dietary exposure to the BSE agent while in the Arabian Peninsula, more likely in Saudi Arabia, where he spent most of his early years, than in Dubai. It is much less likely, though not impossible, that he might have been exposed to the BSE agent during a visit of two weeks to the UK in 1995 (near the end of the UK BSE dietary risk period). He paid a second brief visit to the UK in 2002, six years after the UK dietary risk period is thought to have ended [25]. This is the third case of vCJD plausibly attributed to a dietary exposure to BSE agent in Saudi Arabia.

vCJD in other persons born in Saudi Arabia. One previous case of vCJD, diagnosed by brain biopsy at the UCSF Hospital, University of California, San Francisco, and briefly described by the CDC, occurred in a person living in Virginia who was a non-Saudi Arabian national born and raised in Saudi Arabia [5]. That person's family recalled no history of travel to UK except for connecting flights. An earlier case of vCJD in 2003, never described in detail, affected a 33-year-old Saudi citizen who underwent brain biopsy at a hospital in Saudi Arabia; vCJD was diagnosed from a sample of the biopsy sent to the Mayo Clinic, Rochester, Minnesota, and confirmed by the National Prion Disease Surveillance Center, Case Western Reserve University, Cleveland, Ohio [1]; CDC noted that the patient "may have visited the UK, if at all, only for several days"

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(although he had visited France) and concluded that he was most likely to have been infected in Saudi Arabia [5].

BSE in Saudi Arabia. Saudi Arabia has not reported any case of BSE to the World Organisation for Animal Health (OIE) [36]. BSE has very rarely been recognized in other countries of the general region: two cases of BSE were reported in cattle imported into Oman in 1989 [37] and one case in a native bovine in Israel was reported in 2002 [38]. However, Saudi Arabia was identified as having imported live cattle and beef products from the UK during the period of concern (1980-1996) [24], and Saudi Arabia was identified as a consignee of meat-and-bone meal (MBM) of UK origin, during the years 1988-1993 when MBM might have been contaminated with the BSE agent [23].

We have assumed, for the purposes of this analysis, that the major risk of human exposure to the BSE agent in Saudi Arabia was from beef and live cattle of UK origin exported to the region during the years of concern: 1980 through 1996. According to Sanchez-Juan and colleagues [24], the UK exported to Saudi Arabia almost 1,000 live bovines (1980-1990) and about 32,000 tons of carcass meat (1980-1996). Earlier estimates of exports reported by a representative of the World Health Organization (WHO) to TSEAC were roughly similar [23]. However, we cannot verify the accuracy of those figures. We have also assumed that exports of live cattle, beef, MBM and other bovine-derived products exports from the UK to Saudi Arabia ceased when the European Commission prohibited such exports both to Member States of the European Community and to "third countries" in March 1996 [16]. Furthermore, the UK implemented an enhanced prohibition of mammalian proteins in ruminant feed in 1996 and other controls to enhance the safety of food for humans and animal feeds by the end of 1996 [25]; therefore, we conclude that the risk of exposure to the BSE agent in any products and live cattle exported from the UK to Saudi Arabia after that time was small.

We acknowledge that other BSE countries (i.e., countries of Europe) might also have exported beef to Saudi Arabia and neighboring countries both during the years 1980 through 1996 and afterwards, however (1) the much lower rates of both diagnosed BSE and vCJD cases in other countries relative to the UK suggest that the risk associated with beef from those countries must be considerably less than for UK beef, and (2) we have not been able to estimate imports of beef from non-UK countries into Saudi Arabia. We are also unable to estimate cross-border sales of cattle or beef products in the region or the possibility that BSE might have been introduced into native ruminants in Saudi Arabia by the use of MBM—either imported or domestically produced—in animal feed supplements. Saudi Arabia is estimated to have had about half a million cattle in 1998 and far larger numbers of camels, goats, and sheep [2]. While acknowledging the theoretical possibility of BSE infections in local ruminants, we concluded that the risk of such infections is probably much less than that of beef products from the UK and too uncertain to consider unless and until reliable information becomes available.

Estimating the possible risk of dietary exposure to the BSE agent in US donors of blood and tissues during residence in Saudi Arabia. Since 1999, FDA's recommendations regarding deferral of blood and ineligibility of donors of HCT/Ps potentially exposed to the BSE agent in various countries—geographic deferrals—have been based on rough comparisons of the estimated risk of oral exposure to the BSE agent

in various groups of people compared to the risk of the UK population from the beginning of 1980 until the end of 1996, when UK food/feed protections were fully implemented. FDA, in 2001, announced a model that estimated the risk in most countries of Western Europe assigned as a relative-risk compared to the UK. The risk of dietary exposure to the BSE agent was assumed to be stochastic and directly (linearly) related to the time spent in a country where the BSE agent contaminated beef products [6]. In principle, the exposure of concern was consumption of beef products, but dietary histories were unavailable and are probably unreliable, so donor days in country were taken as a surrogate. Based on a number of other assumptions, the following relative risks were assigned: UK=1.0, France=0.05 (i.e., 5% of beef in France assumed to have been imported from the UK [3] and other countries of Western Europe=0.015 (extrapolating to the rest of Western Europe the results of intensive surveillance of BSE in Switzerland) [6]. For purposes of deferral policy, and in consideration of the absence of more detailed information, vCJD risk in Western Europe was taken as comparable to that in France as a worst case. A risk relative to UK of 0.35 was assigned to US military bases that obtained beef from the UK in various years using estimates of UK beef sourcing provided to the FDA by the US Department of Defense (DoD) [9]. These estimated relative risk factors are highly uncertain because of uncertain simplifying assumptions that underlie them. In fact, the model appears to have predicted fewer cases of vCJD than have been recognized in France (25 to date or more than 10% of the UK per capita rate) and overestimated cases in US military personnel and dependents (no cases to date among as many as 4.8 million active duty personnel and an unknown number of dependents and employees [32]). At the time, FDA also attempted to predict the possible loss of otherwise suitable blood donors that might result from various vCJD-related geographic donor deferral policies, based on a travel survey of donors in 12 blood centers [6]. Insofar as limited information has been available to us, we attempted a similar assessment of vCJD-related risk in Saudi Arabia, an assessment of reduction in that risk by donor deferral policies, and an estimation of the possible loss of otherwise suitable blood donors that might result.

1) **Estimates of relative prevalence of vCJD in various countries compared with Saudi Arabia.** We attempted to estimate vCJD risk in donors resident in Saudi Arabia by comparing the crude rate of vCJD attributed to residence in Saudi Arabia with rates for seven European countries currently on the FDA deferral list that have had cases attributed to infection within the country, not including three cases attributed to infection during residence in the UK [27]. Information to date, summarized in **Table 1,** suggests that the crude recognized prevalence of vCJD attributed to exposure to the BSE agent in Saudi Arabia to date (three cases in a total population estimated by the US Census Bureau earlier this year to be 26,132,000 [29]) resembles that in a number of European countries (somewhat lower than estimated prevalences in Ireland and France, both of which have lower rates than UK) for which FDA currently recommends geographic deferrals of blood donors [9] and screening of HCT/P donors [8]. It is important to note that the crude prevalence estimates provided in Table 1 have not been adjusted either for ages of the populations (younger persons being more often affected by vCJD than older persons) in the different countries or for probable differences in vCJD case recognition and reporting.

2) **Potential consumption of UK beef products by persons resident in Saudi Arabia 1980-1996.** In trying to estimate exposure to UK beef products, we addressed two groups of residents.

(a) Estimated consumption of UK beef by the general population of Saudi Arabia, including Saudi nationals and foreign residents. We considered two factors affecting the risk of dietary exposure to the BSE agent: **(i)** estimated UK exports of beef to Saudi Arabia during the years 1980 through 1996, and **(ii)** estimated total beef consumption in Saudi Arabia. The latter adjustment was based on published data reporting that residents of Saudi Arabia, on average, consume considerably less beef than do residents of the UK and other Western European countries. Published sources suggested that about 10% of beef imported into Saudi Arabia during the years of concern might have originated in the UK [24] [31] and that average annual per capita beef consumption in Saudi Arabia was about a quarter of that in the UK (lamb and poultry being more popular) [2, 17, 30]. Taken together, these figures, although not validated and admittedly uncertain, suggested that a reasonable average relative risk estimate for dietary exposure to BSE agent in UK beef by persons resident in Saudi Arabia during the years 1980-1996 might be 0.025 (i.e., 0.10 x 0.25) that of persons resident in the UK during the same period and not unlike the risk previously estimated for most countries of Western Europe.

(b) US military personnel on bases in Saudi Arabia. We also considered information provided to FDA by the Armed Services Blood Program Office, DoD, about sources of beef supplied by the US Government to the US military personnel stationed in countries of the Arabian Peninsula during the years of concern (which include the years of the First Persian Gulf War). Information about military beef was taken from a recent DoD review of procurement records. Beef in field rations/"meals ready to eat" [MREs] during those years was all of US origin. However, an uncertain but possibly significant amount of the beef sold to and consumed by US military personnel living on US bases in Europe and Saudi Arabia after 1980 originated in the UK, though such procurement decreased after 1989. We cannot assume with confidence that the origin of beef consumed on US bases in Saudi Arabia differed significantly from that on European bases south of the Alps. Acknowledging the uncertainties, we therefore assumed that the risk of dietary exposure to the BSE agent for US military personnel living on bases in Saudi Arabia from 1980 through the end of 1996 was similar to that FDA previously assigned to US military living on European bases south of the Alps, taken to be about 35% of that for UK residents during the same period. Unlike US military stationed on European bases, no military dependents lived in Saudi Arabia. For the most part, US military contractors were not supplied with food by DoD, purchased their food locally—"on the economy"—and so are assumed to have shared the general dietary risk of exposure to the BSE agent with other residents of Saudi Arabia.

Canadian deferral of blood donors resident in Saudi Arabia. Since November 2007, Héma-Québec [21], a blood establishment operating in the Province of Quebec, has requested deferral of blood donors resident in Saudi Arabia for any period of six months

or more from 1980 through 1996 [20]. Since March 2011, Canadian Blood Services (CBS) has required the same deferral [19]. Canadian blood donor deferral policies for residents of Saudi Arabia do not include donors with history of blood transfusion in that country. **Table 2a** compares current Canadian and US blood donor deferral policies for vCJD risk. The policies, while similar, are not identical. We are not aware that any other country has recommended blood donor deferral for residents of Saudi Arabia.

Canadian assessments for determining suitability of donors of cells and tissues. Health Canada requires that travel information be collected for cell and tissue donors and some other questioning of donors or their proxies about vCJD risk factors. There are, however, no exclusion criteria based on risk factors associated with residence in or travel to specific geographic areas [19]. US donor screening recommendations regarding vCJD for donors of HCT/Ps are summarized in **Table 2b.**

FDA's proposed response to reports of three vCJD cases in individuals likely to have been infected with the BSE agent in Saudi Arabia. The reports of three cases of vCJD attributed to residence in Saudi Arabia has implications for US blood safety recommendations and for the safety of HCT/Ps, affecting the suitability of four groups of potential donors: US military personnel serving in Saudi Arabia, US guest workers who were military contractors supporting US forces in Saudi Arabia, other US guest workers employed as non-military contractors in Saudi Arabia and immigrants to the US who lived in Saudi Arabia, during the years 1980-1996. Saudi Arabia is not currently included on the list of countries for which FDA has recommended deferral/ineligibility of donors [9].

FDA is considering modifications to current suitability/eligibility recommendations to include donors of blood and blood components, including Source Plasma and HCT/Ps who spent any cumulative period of six months or longer as military personnel serving in Saudi Arabia from 1980 through the end of 1996. This recommendation is similar to the current recommendation to defer donors resident on US military bases in Europe during years when they were supplied with UK beef (comprising an estimated 35% of the beef supply through 1996 south of the Alps [9]). FDA is also considering modifications to current suitability/eligibility recommendations to include any other donors of blood and blood components, including Source Plasma and HCT/Ps who spent any cumulative period of five years or longer living in Saudi Arabia from 1980 through the end of 1996. This modification is similar to the current recommendation to defer donors resident in France, except that, because of continuing reports of BSE affecting native cattle in several European countries and a lack of reliable information regarding implementation of food safety measures and cross-border trade in beef products in Europe [7], FDA continues to consider the period of potential dietary exposure to the BSE agent for France and most other European countries (except UK) to extend to the present. We have assumed that the BSE risk for Saudi Arabia was associated with importation of live cattle and beef from the UK and that the risk became negligible at the end of 1996.

We acknowledge that Saudi Arabia might have imported live bovines and beef from other BSE countries after 1996 [2], but we have not included that assumption in developing the proposed recommendations. Saudi Arabian authorities have assured FDA that, since at least 1996, the Kingdom has prohibited the importation of live bovines and

beef products from countries reporting BSE to the OIE, as suggested by public sources [14]. As noted above, Saudi Arabia has reported no case of BSE to the OIE, and we assume that native Saudi cattle have probably not been infected. The likelihood that BSE infection was established in the substantial number of small ruminants (sheep and goats—far outnumbering cattle in Saudi Arabia [2, 17]) seems remote. We do not have information regarding rendering and animal feeding practices in Saudi Arabia (specifically on production of MBM and use of MBM in feeds) that would allow more reliable assumptions.

Potential impact on US blood supply and on HCT/P supply resulting from proposed deferral of certain blood donors or ineligibility of certain cell and tissue donors resident in Saudi Arabia during the years 1980-1996

We considered four potential at-risk groups that would be affected under the proposed recommendation for US donors with a history of residence in Saudi Arabia during the years 1980-1996. The groups include: **(1)** US military personnel; **(2)** US guest workers who were contractors to the US military; **(3)** US guest workers who were contractors but not for the US military; and **(4)** immigrants from Saudi Arabia to the US (both Saudi and non-Saudi nationals, regardless of current citizenship). **Table 3** below summarizes the predicted number of US donors and blood donations lost as a result of the proposed changes to recommendations for determining suitability of blood and plasma donors based on residence in certain countries. Because the more limited available information on donors and donations of HCT/Ps, FDA has not been able to analyze the possible impact of the proposed recommendation on the US supply of HCT/Ps.

US military personnel. Based on information provided to FDA by DoD, approximately 600,000 US troops were deployed to Saudi Arabia for a period ≥ 6 months in the years 1980-1996; that number represents about 90% of total deployments to Saudi Arabia during that period. Those persons would all be deferred from blood donations or ineligible to donate under the proposed geographic risk factor recommendations. However, DoD estimates that approximately 30% of this population are already deferred from donating due to the vCJD European deferral and other reasons. In addition, a large number of this population retired or left the military and may be donating to civilian blood collection facilities.

US military contractor guest workers. Information from DoD indicated that approximately 200,000 personnel including DoD civilians and contractors were employed in Saudi Arabia during the years 1980-1996. We assumed that all had cumulative stays of ≥ 6 months but less than 5 years. Under the proposed recommendation, they would not be deferred from donating blood and would remain eligible to donate HCT/Ps.

US non-military contractor guest workers. We assumed all US non-military guest workers who lived in Saudi Arabia during the years 1980-1996 had cumulative stays ≥ 6 months with an average length of stay of four years [10, 12, 13, 15, 22]. We further assumed that 30% of US guest workers lived in Saudi Arabia for more than 5 yr [10, 12, 13, 15, 22] and thus would be deferred from blood donation and ineligible to donate HCT/Ps under the proposed geographic BSE risk factor recommendations. The *Average Annual Number* of US guest workers in Saudi Arabia was estimated using data from the

US State Department on the number of registered US citizens in Saudi Arabia in 1999 [35]. We used these data for the year 1999, extrapolated and summed each year to derive the total number of US guest workers in Saudi Arabia during 1980 and 1996.

Immigrants. Our estimates assume that all immigrants from Saudi Arabia since 1985 had stayed for ≥ 5 yr in Saudi Arabia during the years 1980-1996, and they would be deferred from blood donations or ineligible to donate under the proposed geographic BSE risk factor recommendations. The *Average Annual Number* of persons emigrating from Saudi Arabia to the US from 1985 to the present was derived from Immigration Statistics 1989-2010 released by the US Department of Homeland Security [34]. Our estimates do not capture non-Saudi nationals immigrating to the US from Saudi Arabia as the last residence of record and thus may somewhat underestimate the number of donors and donations in this category that would actually be lost.

Donor loss calculation. We calculated blood donor loss based on the assumption that individuals who resided in Saudi Arabia during the years 1980-1996 have a 5% rate of donation [28, 33], which is the donation rate for the general US population. Our calculation for the potential loss of blood units assumes that each donor donates approximately 1.7 units of blood each year [33]. The estimated potential impact on US blood supply resulting from the proposed donor deferral recommendation is summarized in Table 3

Questions for TSEAC

Question 1. Do available data support the consideration by FDA to recommend deferring donors of blood and blood components, including Source Plasma, and to determine to be ineligible donors of HCT/Ps, who

a) spent six months or more cumulatively in Saudi Arabia as US military personnel from the beginning of 1980 through the end of 1996 or

b) otherwise spent more than five years cumulatively in Saudi Arabia from the beginning of 1980 through the end of 1996?

Question 2. Please discuss the likely contribution of those recommendations to the safety of the products involved and the possible impact on supplies of blood, blood components, plasma derivatives and HCT/Ps.

Question 3. Please comment on additional information that might better inform FDA's consideration of the proposed or any further safety measures.

Table 1.

Reported vCJD cases per estimated total population 2011

Country	vCJD Cases	Estimated* Population 2011	Crude Rate
UK	175	62,698,362	2.8×10^{-6}
Ireland	2	4,670,849	4.3×10^{-7}
France	24	65,102,719	3.7×10^{-7}
Portugal	2	10,760,305	1.9×10^{-7}
Netherlands	3	16,847,007	1.8×10^{-7}
Spain	5	46,754,657	1.1×10^{-7}
Saudi Arabia	3	26,131,703	1.1×10^{-7}
Italy	2	61,016,804	3.3×10^{-8}
Japan	1	126,475,664	7.9×10^{-9}

vCJD cases are attributed to exposure in a country according to the conclusion of the CJD Surveillance Unit, Edinburgh [27]. Cases resident for ≥ 6 mo in UK are attributed to UK. Rates are not adjusted for differences in population age profiles or for efficiency of case recognition and reporting in various countries.

*Population estimates for various countries were taken from the Web site of the US Census Bureau for mid-year 2011 [28].

Table 2a

Comparison of Geographic vCJD-related *Blood* Donor Deferral Policies Recommended by FDA and Required by Canadian Blood Services

	USA FDA	*Canadian Blood* Services	Hema-Québec
UK	≥ 3 mo 1980-1996[a]	≥ 3 mo 1980-1996[b]	≥ 1 mo 1980-1996
France	≥ 5 yr 1980-present	≥ 3 mo 1980-96	≥ 3 mo 1980-96
Other Western Europe (WE)	≥ 5 yr 1980-present ≥ **28** countries[a]	≥ 5 yr 1980-present **12** countries[b]	≥ 6 mo 1980-present **12** countries[c]
Transfusion history	UK, France 1980-present	UK, France, WE 1980-present	UK, France, WE 1980-present
Saudi Arabia	no deferral	≥ 6 mo 1980-1996	≥ 6 mo 1980-1996
Other countries	no deferral	no deferral	no deferral

a *US* definition of United Kingdom = England, Northern Ireland, Scotland, *Wales*, the *Isle of Man*, the *Channel* Islands, Gibraltar, and the Falkland Islands

US definition of WE (excluding UK, France) = Albania, Austria, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Demark, Finland, Germany, Greece, Hungary, Republic of Ireland, Italy, Liechtenstein, Luxembourg, Macedonia, Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, and the Federal Republic of Yugoslavia ([sic] now Kosovo, Montenegro, and Serbia)

FDA also recommends deferral of US military personnel who spent ≥ 6 mo on certain military bases in Europe 1980-1996 [9]

b Canadian definition of United Kingdom = England, Northern Ireland, Scotland, Wales, the Isle of Man, the Channel Islands (excludes Gibraltar and the Falkland Islands). Canadian definition of WE (excluding UK, France) = Austria, Belgium, Demark, Germany, Republic of Ireland, Italy, Liechtenstein, Luxembourg, Netherlands, Portugal, Spain, Switzerland

c http://www.hema-quebec.qc.ca/donner/don-de-sang/qui-peut-donner-du-sang/creutzfeldt-jakob.en.html

Table 2b.

Geographic vCJD-related Cell and Tissue Donor Eligibility Policies Recommended by FDA

Ineligible Donors with history of:	USA FDA
Residence in UK	≥ 3 mo 1980-1996[a]
Residence in Other Western Europe	≥ 5 yr 1980-present ≥ **28** countries[b]
Blood transfusion	UK, France 1980-present
Residence in Saudi Arabia	no current recommendation
Residence in Other Countries	no current recommendation

a US definition of United Kingdom = England, Northern Ireland, Scotland, Wales, the Isle of Man, the Channel Islands, Gibraltar, the Falkland Islands

b US definition of Europe = Albania, Austria, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Demark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Liechtenstein, Luxembourg, Macedonia, Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, United Kingdom, Federal Republic of Yugoslavia ([sic] now Kosovo, Montenegro, Serbia)

FDA also recommends deferral of US military personnel who spent ≥ 6 mo on certain military bases in Europe 1980-1996 [8]

Note: Health Canada requires that travel information be collected and some donor other screening for vCJD-related risk factors. There are, however, no exclusion criteria for cell and tissue donors based upon risk factors associated with residence or travel history to specific geographic areas.

Table 3.

Estimated loss of blood donors and blood donations resulting from proposed recommendations to defer certain blood donors with history of residence in Saudi Arabia during 1980-1996

	US Military Personnel	US Guest Workers Military Contractors	US Guest Workers Non-Military Contractors	Immigrants to US	Total
Average annual number	n/a	n/a	36,000[a]	920[b]	n/a
Population to be deferred	420,000[c]	0[d]	45,900[e]	24,800[f]	490,000
Blood donors lost[g]	21,000	0	2,300	1,200	24,500
Blood units lost[h]	35,700	0	3,910	2,040	41,700

Notes:

[a]Average annual number of US guest workers in Saudi Arabia: based on data from US State Department for registered US Citizens living in Saudi Arabia in 1999 (http://overseasdigest.com/amcit_nu2.htm).
[b]Average annual number of immigrants from Saudi Arabia: based on data from US Department of Homeland Security, Yearbooks of Immigration Statistics 2004 and 2010 (http://www.dhs.gov/files/statistics/publications/yearbook.shtm).
[c]Number of military personnel to be deferred calculated by:
600,000 (total number of military personnel who stay for ≥ 6 months, DoD 2011) x 70% (percentage individuals having already been deferred, DoD 2011)
[d]Total number of military contractors who stay for ≥ 5 years, and thus to be deferred (DoD, 2011)
[e]Total number of US guest workers non-military contractors to be deferred calculated by:
(Average Annual Number of US guest workers x 17 years (from January 1, 1980 to December 31, 1996) / Average Length of Stay) x 30% (percent stay for ≥ 5years)
[f]Total number of immigrants to be deferred, calculated by:
Average Annual Number of immigrants x 27 years (from 1985 to current)
[g]Blood donors lost, calculated by:
Population to be deferred x 5% (donation rate)
[h]Number of blood units lost, calculated by:
Number of donors to be deferred x 1.7 (average number of donations per donor per year)

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別紙様式第 2-1
番号 14

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2011 年 11 月 11 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	Haemophilia 2011; 17: 931-937	公表国 イギリス		
販売名（企業名）	ハプトグロビン静注 2000 単位「ベネシス」（ベネシス）					

研究報告の概要

英国血友病センター医師機構(UKHCDO)が、787 人の先天性出血性疾患患者の血漿製剤による vCJD 感染リスクを評価した。
患者は、供血後に vCJD を発症したドナー由来血漿を含む 1987-1999 年に製造した 25 バッチの何れかの製剤を投与された。これらのバッチの vCJD 感染性を血漿の画分の感染性とバッチの製造データから推測した。各患者の受けた総 vCJD 感染性は、薬剤の総投与量から推測される累積感染性から推算した。
787 人の内、604 人(77%)は汚染バッチの投与を受けてから 13 年間以上追跡調査した。この 604 人の推定 vCJD リスクは、595 人が 1%以上、164 人が 50%以上、及び 51 人が 100%。これらのリスクが英国人の背景的リスクである食事によるリスクに上乗せされる。604 人の患者の内、94 人(16%)は vCJD を発症した患者由来のバッチを、供血後 6 カ月以内に投与された。151 人(25%)は 10 歳以下で製剤の投与を受けていた。
2009 年 1 月 1 日現在、これらの患者は一人も vCJD を発症していない。血漿画分の感染性を過度に見積もったか、輸血用血液成分の受血者よりも潜伏期間が長いことを示唆する。

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)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・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は排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
血漿分画製剤は理論的な vCJD 伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を 2003 年 5 月から添付文書に記載している。2009 年 2 月 17 日、英国健康保護庁(HPA)は vCJD に感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD 異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内での BSE の発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは 1999 年以前の英国に比べて極めて低いと考える。また、本剤の製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	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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Haemophilia

Haemophilia (2011), 17, 931–937 DOI: 10.1111/j.1365-2516.2011.02508.x

ORIGINAL ARTICLE *Transfusion transmitted disease*

The risk of variant Creutzfeldt-Jakob disease among UK patients with bleeding disorders, known to have received potentially contaminated plasma products

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Summary. The risk of variant Creutzfeldt-Jakob disease (vCJD) from potentially infected plasma products remains unquantified. This risk has been assessed for 787 UK patients with an inherited bleeding disorder prospectively followed-up for 10–20 years through the UK Haemophilia Centre Doctors' Organisation (UKHCDO) Surveillance Study. These patients had been treated with any of 25 'implicated' clotting factor batches from 1987 to 1999, which included in their manufacture, plasma from eight donors who subsequently developed clinical vCJD. Variant CJD infectivity of these batches was estimated using plasma fraction infectivity estimates and batch-manufacturing data. Total potential vCJD infectivity received by each patient has been estimated by cumulating estimated infectivity from all doses received during their lifetime. Of 787 patients, 604 (77%) were followed-up for over 13 years following exposure to an implicated batch. For these 604 patients, the estimated vCJD risk is ≥1% for 595, ≥50% for 164 and 100% for 51. This is additional to background UK population risk due to dietary exposure. Of 604 patients, 94 (16%) received implicated batches linked to donors who developed clinical vCJD within 6 months of their donations. One hundred and fifty-one (25%) had received their first dose when under 10 years of age. By 1st January 2009, none of these patients had developed clinical vCJD. The absence of clinical vCJD cases in this cohort to date suggests that either plasma fraction infectivity estimates are overly precautionary, or the incubation period is longer for this cohort than for implicated cellular blood product recipients. Further follow-up of this cohort is needed.

Keywords: haemophilia, inherited bleeding disorders, risk assessment, UK plasma products, variant Creutzfeldt-Jakob disease

Introduction

The bovine spongiform encephalopathy (BSE) epidemic in UK cattle occurred from 1980 to 1996. Evidence has been presented that a distinct clinicopathological variant of Creutzfeldt-Jakob disease (vCJD), first described in 1996 [1], is the human manifestation of BSE [2–4]. Concerns that vCJD may be transmissible by blood and blood products, and actions taken to reduce the risk to UK patients with an inherited bleeding disorder, have recently been reported [5].

The vCJD risks from plasma products linked to donors who later developed vCJD, remain unquantified. Det Norske Veritas's (DNV) risk assessment informed the introduction of further public health measures for recipients of UK-sourced plasma products in 2004 [6]. These recipients included patients with inherited bleeding disorders who had been treated with UK-sourced plasma products between 1980 and 2001. On the advice of the CJD Incidents Panel (CJDIP), and facilitated by the Health Protection Agency (HPA), these patients were informed of their risk by the UK Haemophilia Centres Doctors' Organisation (UKHCDO) *via* their

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Accepted after revision 17 January 2011

Haemophilia Centres and asked to implement public health measures to reduce the possible risk of vCJD spreading to others [5].

The nature of the blood-associated vCJD agent and the impact of processing technologies on the nature and distribution of vCJD infectivity in human blood components and plasma products were unknown. Therefore, the DNV risk assessment was based on data from published animal studies and a number of assumptions [6]. Three options were developed: (i) the fractionation step with the largest clearance of infectivity represents the entire process, (ii) the reduction in infectivity when separating blood into blood components and plasma fractions is the only step that reduces infectivity when producing plasma products, and (iii) the infectivity level correlates with the protein content of plasma products. Option (iii) was rejected as it was considered scientifically invalid. The CJDIP adopted option (ii) rather than (i) on the basis that it was more precautionary and because there were uncertainties around the clearance values in option (i).

This article presents the application of this risk assessment to 787 bleeding disorder patients who have received implicated clotting factor batches linked to donors who later developed clinical vCJD. The identification of the abnormal prion protein associated with asymptomatic vCJD postmortem in a patient in this cohort has prompted this assessment [7]. The implications to inform further public health responses are discussed.

Materials and methods

Implicated plasma product batches

In the UK, a total of 178 plasma product batches have been linked to 25 plasma donations from 11 donors who subsequently developed clinical vCJD [8]. These include 25 implicated clotting factor[1] batches linked to 18 plasma donations from eight donors that have been used to treat 787 UK patients with inherited bleeding disorders. The batches had expired before the 2004 patient notification.

Calculation of infectivity of plasma products

Plasma from many thousands of donations is pooled prior to fractionation. The DNV risk assessment provided estimates of potential infectivity of different plasma fractions. Infectivity was quantified using the ID_{50}, *where one* ID_{50} *is the dose required to produce* infection in 50% of recipients.

[1]Factor concentrates are made from pooled plasma and include FVIII, FIX, FVII, FXI, FXIII and prothrombin complex concentrates as well as antithrombin.

In 2004, the HPA used a 'Product Risk Calculator' tool to estimate the infectivity of each implicated batch (Appendix 1 Supporting information). The tool combined the DNV infectivity estimates with fractionators' batch-manufacturing data. For each batch, it calculates the dose estimated to contain 0.02 ID_{50}. This represents a 1% risk of infection in addition to the general background population risk from potential dietary exposure. This is the level of risk the CJDIP considered sufficient to warrant patient notification and public health action [9]. The cumulated lifetime infectivity received by each patient was estimated using the data on each batch and the total quantity received.

Identification and management of patients with bleeding disorders

A policy decision was taken that all bleeding disorder patients treated with UK-sourced clotting factors from 1980 to 2001 (rather than just those who had received implicated clotting factors) should be considered 'at risk' of vCJD for public health purposes [5]. This decision was made because: (i) a single dose of implicated clotting factor was thought to contain *sufficient infectivity for a recipient to cross the* 1% additional risk threshold (high risk plasma product), and (ii) it was considered likely that further implicated clotting factors would be identified if future clinical vCJD cases were found to have donated plasma.

Haemophilia clinicians used locally held or National Haemophilia Database (NHD) records to identify all recipients of UK-sourced plasma products from 1980 to 2001 and used product information from two UK fractionators to identify patients who had received implicated clotting factors. Patients notified as being 'at-risk' of vCJD for public health purposes were able to choose whether or not to find out if they had received implicated clotting factors. Haemophilia clinicians were encouraged to report these patients (unless they had withheld consent) to the NHD, for follow-up. This has been in the UKHCDO vCJD Surveillance Study following ethical approval from the London Multicentre Ethics Committee (MREC/01/2/11).

National haemophilia database

Data on product type and batch number of implicated batches, total doses received and start and completion dates of each treatment were collected by haemophilia centres. The NHD is updated annually by individual UK haemophilia centres with treatment data sets and information about new diagnoses and deaths. All deaths and causes of death are verified as patients are flagged with the Office for National Statistics. Person-years at risk of vCJD were calculated by subtracting the date of

the first dose of an implicated batch from either 1st January 2009 or the date of death as appropriate.

Results

Patient population in NHD

A total of 8547 patients with inherited bleeding disorders were registered on the NHD on 1st January 2009 (Table 1). Of these, 3735 have been identified as having received UK-sourced clotting factors between 1980 and 2001 and therefore are defined as 'at risk' of vCJD for public health purposes. Of these, 787 had received implicated clotting factors batches ('implicated batch') linked to donors who later developed clinical vCJD. Auditing notification data for each centre against implicated batches supplied to them by the two UK fractionators show that 11 million IUs (about 50%) of implicated batches remains unaccounted for [5]. As a result of this under-notification, it is estimated that the 787 patients represent approximately 50% of all patients who had received implicated batches. The following results/data concern these 787 implicated batch recipients.

Outcome, deaths and autopsy

No clinical cases of vCJD have been observed in these patients as of 1st January 2009. Fifty-one (6.5%) deaths were reported by 1st January 2009 but none was related to vCJD. Only four autopsies have been performed in this cohort. Abnormal prion protein, indicating vCJD infection, has been detected in a single postmortem spleen sample of a haemophilia patient who died of causes unrelated to vCJD 11 years after receiving 9025 IUs (estimated vCJD infectivity ID_{50} 0.21) from two implicated FVIII batches [7]. These batches were linked to two plasma donations from a donor who developed vCJD within 6 months of the second donation.

Estimated infectivity of implicated batches

Table 2 is the list of implicated batches showing the quantities of each batch used with their estimated infectivity, and the number of patients treated with each batch. Two hundred and sixty three (33%) patients received >1 implicated batches and 229 (29%) patients received implicated batches linked to >1 donors. A total of 12.7 million IUs of implicated FVIII and FIX was used to treat 787 patients from 1987 to 1999. On average each patient received 10 000 IUs (median) (range 240–169960) and estimated vCJD infectivity 0.443 ID_{50} (median) (range 0.010–9.593). A total of 773 (98%) patients received estimated vCJD infectivity ≥0.02 ID_{50} (Fig. 1). Of 604 (77%) patients who have been followed-up for over 13 years, which is the predicted incubation period of primary vCJD [10,11], 595 have ≥1%, 164 have ≥50% and 51 have 100% estimated vCJD risk in addition to the background UK population risks due to potential dietary exposure.

Donors linked to implicated batches

Table 3 is the list of eight donors showing data on implicated batches and the number of recipients linked to each of them. These donors developed vCJD 88 months (median) (range 6–143) following their last donations. One hundred and forty-nine (19%) patients received implicated batches linked to donors who developed vCJD within 6 months of donation and 552 (70%) linked to donors who developed vCJD within 6 years of donation. When estimated infectivity is plotted against interval between donation and onset of vCJD in donors the distribution of patients for these parameters can be clearly seen (Fig. 2). The patient in whom the abnormal prion protein associated with vCJD was found at postmortem received two implicated batches from donor 1 [7]. For one of these batches, the interval between donation and onset of vCJD in the donor is 6 months but with a relatively

Table 1. Patients with inherited bleeding disorders registered in the National Haemophilia Database on 1st January 2009 by diagnosis and subgroups at risk of vCJD for public health purposes.

	Number of patients with bleeding disorders by diagnostic subgroups				
Patient group and subgroups	Haemophilia A	Haemophilia B	von Willebrand	Other	Total
Total registered in the National Haemophilia Database (NHD)	3 281	729	2 996	1 541	8 547
Registered patients who are at risk of vCJD: (treated with UK sourced plasma products between 1980 and 2001)	2 246	562	518	409	3 735
Registered patients at risk of vCJD who are known to have received implicated clotting factor batches	556	168	39	24	787*

*11 million IUs (about 50%) of implicated batches remain unaccounted for [5]. As a result of this under-notification, it is estimated that the 787 patients represent approximately 50% of all patients who had received implicated batches.

Table 2. Description of each implicated clotting factor batch used to treat 787 patients with inherited bleeding disorders.

Batch number	Brand name[†]	Donor IDs linked to implicated clotting factor batches*	Months between donation and onset of vCJD in donors	Estimated infectivity (ID_{50}) per IU for each batch[†]	Total quantities (IUs) of each batch used	Estimated total infectivity (ID_{50}) received from each batch	Total number of patients registered as treated with each batch (n = 787)[‡]
FHB4547[§]	8Y	1	6	0.0000199	873 821	17.424	61
FHB4596	8Y	6	31	0.000043	1 054 410	45.340	93
FHC4237[§]	8Y	1	46	0.0000472	983 977	46.444	117
FHB4189	8Y	8	112	0.0000486	735 725	35.756	71
FHB4419	8Y	3	15	0.0000584	656 600	38.345	55
FHB4116	8Y	2	31	0.0000774	280 710	21.727	34
FHC0369	8Y	8	139	0.000088	199 060	17.517	52
FHC0289	8Y	2	59	0.0000948	266 960	25.308	46
FHC0059	8Y	5	143	0.0001135	58 560	6.645	10
FHM4054	High purity F8	8	127	0.0000662	304 500	20.158	33
FHM3990	High purity F8	8	134	0.0000738	169 055	12.476	11
FHE4548[§]	Replenate	1	6	0.0000246	965 400	23.749	88
FHF4625	Replenate	7	58	0.0000262	1 035 900	27.141	47
FHE4536	Replenate	6	40	0.000029	1 224 270	35.504	97
FHE4437	Replenate	8	82	0.0000388	818 095	31.742	73
0304-70510	Z8	4	134	0.0009526	16 150	15.385	3
FJA4308	9A	8	94	0.0000343	379540	13.031	20
FJA4239B[§]	9A	1	46	0.0000548	141435	7.755	9
FJA0092	9A	2	59	0.0000735	92990	6.835	18
FJA0020	9A	5	143	0.0000948	88025	8.349	10
3502-70210	HT Defix	4	138	0.0001391	216220	30.083	26
FJM4327	Replenine	8	98	0.0000226	1129915	25.536	80
FJM4625	Replenine	7	58	0.0000434	22145	0.961	4
FJM4437	Replenine	8	82	0.0000592	379380	22.459	29
FJM4596	Replenine	6	31	0.0000604	592380	35.780	49

*These numbers have been assigned to anonymize the donors for this study.
[†]Sorted by brand name and estimated infectivity per IU.
[‡]15 patients were treated with the same batch of an implicated clotting factor in more than one treatment episode. 256 patients were treated with different batches of implicated clotting factors in more than one treatment episode.
[§]Four implicated clotting factor batches were linked to the donor whose donations were linked to vCJD infection of a patient with bleeding disorder [7]. This patient received implicated clotting factor batches: FHB4547 and FHC4237.

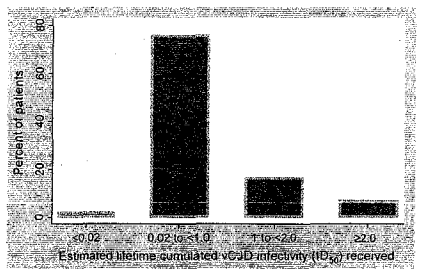

Fig. 1. Distribution of patients with bleeding disorders by estimated lifetime cumulated vCJD infectivity received (n = 787).

low estimated infectivity dose. Others have received higher estimated infectivity from the same donor and the same donation, but none of them has developed clinical vCJD.

Age at exposure and person-years at risk

The median age at which patients received their first dose of an implicated batch was 22 years (range 0.3–87). 174 (22%) patients were under 10 years, 362 (46%) under 20 years, and 628 (80%) were under 40 years of age when they received their first dose. The median age of patients who were alive on 1st-January-2009 (n = 736) was 35 years (range 13–92). The median follow-up time from the date of the first dose of an implicated batch to 1st January 2009 or the date of death was 15 years (range 2 days–22 years) (person-years at risk). Plotting the estimated infectivity against person-years at risk reveals many patients with more event free person-years at risk than the patient with known abnormal prion protein [7] (Fig. 3).

Discussion and conclusion

This article reports the absence of clinical vCJD cases among 787 patients with an inherited bleeding disorder who have been treated with high risk[2] implicated clotting factors. These include 604 (77%) patients who have lived longer after receiving the first doses of implicated clotting factors than the predicted incubation period of 13 years for primary vCJD [10,11]. Of them, one quarter (n = 164) have ≥50% estimated risk

[2]A single dose of implicated clotting factor was thought to contain sufficient infectivity for a recipient to cross the 1% risk threshold.

Table 3. Description of implicated donations, manufactured clotting factors, known quantities used, the number of identified patients treated, average quantities and infectivity received by each patient from individual donors.

Donor IDs	Months between donations and onset of vCJD in a donor*	Number of batches of each product linked to a donor (n = 25)		Total quantities (IUs) of implicated clotting factors linked to a donor	Total number of patients linked to a donor (n = 787)†	Average quantities (IUs) received by each patient (median, range, quartiles)	Average vCJD infectivity (ID_{50}) received by each patient (median, range, quartiles)
1‡	6,§ 46§	8Y	2	2 963 633	257	9000	0.245
		9A	1			260–96000	0.010–4.531
		REPLENATE	1			3000–15700	0.118–0.481
2‡	31, 59	8Y	2	640 660	83	5900	0.483
		9A	1			255–29820	0.024–2.527
						1770–11000	0.145–0.914
3	15	8Y	1	656 600	55	9400	0.549
						470–54990	0.027–3.211
						4700–16000	0.274–0.943
4‡	135, 138	HT DEFIX	1	232 370	29	5520	0.883
		Z8	1			552–48852	0.077–9.593
						2760–8800	0.384–1.690
5	143	8Y	1	146 585	20	4800	0.502
		9A	1			240–35645	0.027–3.381
						1928–9650	0.197–1.089
6‡	31, 40	8Y	1	2 871 060	238	10000	0.373
		REPLENATE	1			500–102960	0.015–3.851
		REPLENINE	1			4755–15300	0.155–0.616
7	58	REPLENATE	1	1 058 045	51	13510	0.472
		REPLENINE	1			965–57900	0.025–1.517
						5150–30880	0.152–0.809
8‡	82, 94, 98, 112, 127, 134, 139	8Y	2	4 112 770	336	9700	0.376
		9A	1			460–100000	0.011–5.511
		HPF VIII	2			3475–15343	0.178–0.660
		REPLATE	1				
		REPLENINE	2				

*Median interval between donation and onset of vCJD in donors was 88 months. 149 (19%) of patients received implicated batches linked to donors who developed vCJD within 6 months of donation and 552 (70%) linked to donors who developed vCJD within 6 years of donation.
†The figures in the column do not add to 787 because of exposure to multiple implicated donors. 557 patients were treated with implicated clotting factor batches linked to one donor, 182 to two donors, 45 to three donors, two to four donors, and one to five donors.
‡Donors 1, 2, 4, 6 and 8 donated more than once.
§The vCJD infected patient was treated with implicated clotting factor batches linked to two donations from this donor [7].

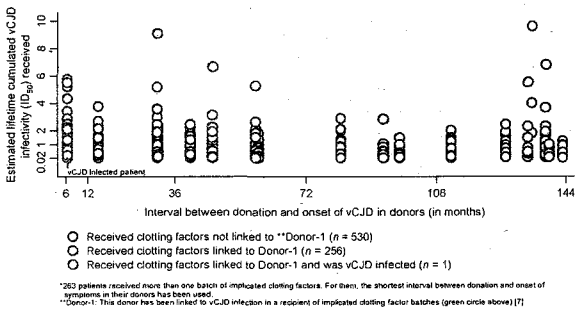

Fig. 2. Scatterplot showing estimated lifetime cumulated vCJD infectivity of implicated clotting factors received by patients with bleeding disorders by interval between donation and onset of symptoms in donors* (n = 787).

(received ≥1ID_{50}) and 8% (n = 51) have 100% risk (received ≥2ID_{50}) of vCJD in addition to background UK population risk due dietary exposure. Forty-nine of the 51 patients who have 100% risk were still alive on 1st January 2009. Thirteen of these 49 patients had received clotting factors linked to donors who developed vCJD within 6 months of their donation. The risk to these patients was calculated using estimates from the DNV risk assessment, and batch-manufacturing data.

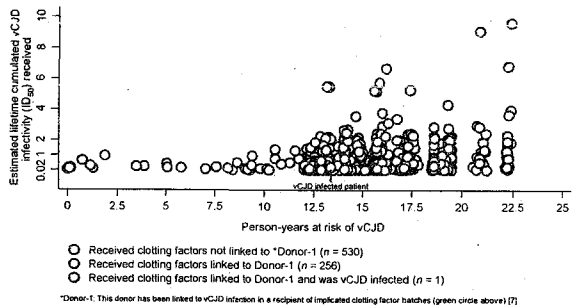

Fig. 3: Scatterplot showing estimated lifetime cumulated vCJD infectivity of implicated clotting factors received by patients with bleeding disorders by their person-years of exposure (n = 787).

The incubation period of vCJD within this at risk group may prove to be longer than the predicted incubation period of primary vCJD and secondary vCJD due to non-leucodepleted packed red cells transfusion. The infective dose in the plasma and red cell components is assumed equal but the implicated plasma is diluted in the plasma pool and then distributed between many vials. A large body of data from different experimental approaches (including endogenous infectivity models) consistently show that conventional bio-separation processes used in plasma product manufacturing are capable of removing prion agents to a significant extent [12,13]. These data question whether the highly precautionary approach as adopted in the UK is still judged as appropriate. It is possible that the infectivity clearance assumptions made in the DNV risk assessment, and the option chosen by the CJDIP are overly precautionary.

Other countries have adopted less precautionary approaches. Authorities in France concluded that the risk posed by implicated batches, even in the most pessimistic scenario, was very low. Consequently, they decided to continue to fractionate plasma sourced from domestic blood supply, introducing nano-filtration as an additional step in the process [14]. Authorities in Canada concluded that the risk of transmission of vCJD for patients who have received FXI linked to UK donors is in the range of 1 in 100 000 to 1 in 1 000 [15]. In their risk assessment, the US FDA included infectivity reductions associated with various processing steps in the production of FVIII and has concluded that the risk of vCJD infection is likely to be extremely low ranging from 1 in 9.4 million to 1 in 15 000 [16].

Age dependent susceptibility is required to fully account for observed age distribution of primary vCJD cases [11]. Age at treatment (8–10 years) with human growth hormone has been found to be a risk factor for secondary CJD in the UK [17]. If age dependent susceptibility is a risk factor for secondary vCJD, then the 174 (22%) patients who received their first dose of implicated clotting factors before 10 years of age may have an increased susceptibility to vCJD infection. The median follow-up time from first exposure in this subgroup is 16 years (range 12–22).

It is of interest that a recent publication links impaired scrapie agent neuroinvasion in aged mice with effects of host age on follicular dendritic cell status [18]. If immune function affects vCJD neuroinvasion in man, then it can be speculated that the immune modulation and deficiency associated with blood borne virus infections in some of this cohort may make subclinical vCJD infection more likely rather than clinical disease.

The dose response relationship has not been established for TSE infections. Experimental estimation of dose response relationship requires a large number of experimental animals, particularly if the level of infectivity is low. Unfortunately, there is very little data on dose response relationship in TSE infections. The DNV risk assessment considered different models on dose response relationship in TSE infections using available data and came up with the assumption that the dose-response function for vCJD infectivity is linear without any threshold [6]. More experimental data are required to validate this assumption to improve the risk assessment.

The DNV risk assessment assumes that risk from regular equal doses of vCJD implicated plasma product over a 1-year-period is additive, and it ignores doses received after the first year. Where the patients have received variable doses from different batches and/or from different donors during several years with wide variations in the estimated levels of infectivity, it is difficult, and somewhat meaningless to calculate an annual dose. Therefore, the CJDIP took a precautionary approach and decided to estimate cumulative lifetime infectivity.

While the under reporting of implicated batch recipients is a concern, it does not invalidate the descriptive data on risk assessment. These may inform any future risk assessment should vCJD develop in a patient who has received implicated batches of clotting factors.

Other factors, such as, prion protein genotype, age at exposure, interval between donation and development of vCJD in the donor, lifetime cumulative infectivity received and the number of donor exposures may also help assess the vCJD risk in this cohort. The continuance of this surveillance study especially with improved recruitment to its postmortem and biopsy arm may provide valuable information that aids our understanding of developing vCJD after exposure to implicated clotting factor batches and allows more informed risk counselling of patients.

Acknowledgements

The article is submitted on behalf of the UK Haemophilia Centre Doctors' Organisation (UKHCDO) by the Transfusion Transmitted Infection Working Party of UKHCDO in collaboration with the Health Protection Agency. We thank the Department of Health for funding the Surveillance Study; the patients who have permitted their data to be recorded on the UKHCDO National Haemophilia Database (NHD); the doctors of UKHCDO who submitted data to the NHD; Ms Lynne Dewhurst, Administrator of the NHD for supplying data; Bio Products Limited (BPL); Scottish National Blood Transfusion Service (SNBTS) and UK National Blood Service (NBS) for their collaboration. Carolyn Millar is the coordinator of the study. The lead investigator was initially Christine Lee, and is currently Frank Hill on behalf of UKHCDO.

Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Users Guide to the Product Risk Calculator.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

B 個別症例報告概要

○ 総括一覧表
○ 報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料3において集積報告を行っているため、添付していない）。

別紙様式第4

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢（歳）	発現時期（年/月/日）	転帰	出典	区分	識別番号	報告日	備考 MedDRA (Ver.)
		器官別大分類	基本語										
第17回	17 -1	感染症および寄生虫症	C型肝炎	米国	女性	不明	1993	不明	自発報告	当該製品	11065985	2011/7/12	14.0
第17回	17 -2	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	死亡	自発報告	当該製品	11000014	2011/8/31	14.0
第17回	17 -3	感染症および寄生虫症	C型肝炎	フランス	男性	不明	不明	不明	自発報告	当該製品	11000015	2011/9/1	14.0
第17回	17 -4	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000016	2011/9/1	14.0
第17回	17 -5	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000017	2011/9/1	14.0
第17回	17 -6	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000018	2011/9/1	14.0
第17回	17 -7	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000019	2011/9/1	14.0
第17回	17 -8	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000020	2011/9/1	14.0
第17回	17 -9	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000021	2011/9/1	14.0
第17回	17 -10	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000022	2011/9/1	14.0
第17回	17 -11	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000023	2011/9/1	14.0
第17回	17 -12	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000024	2011/9/1	14.0
第17回	17 -13	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000025	2011/9/1	14.0
第17回	17 -14	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000026	2011/9/1	14.0
第17回	17 -15	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000027	2011/9/1	14.0
第17回	17 -16	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000028	2011/9/1	14.0
第17回	17 -17	感染症および寄生虫症	C型肝炎	スペイン	不明	不明	不明	不明	自発報告	当該製品	11000029	2011/9/1	14.0
第17回	17 -18	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000030	2011/9/1	14.0
第17回	17 -19	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000031	2011/9/1	14.0
第17回	17 -20	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000032	2011/9/1	14.0
第17回	17 -21	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000033	2011/9/1	14.0
第17回	17 -22	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000034	2011/9/1	14.0
第17回	17 -23	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000035	2011/9/1	14.0
第17回	17 -24	臨床検査	B型肝炎コア抗体陽性	米国	不明	不明	不明	不明	自発報告	外国製品	11103879	2011/9/15, 2011/9/28	14.0
第17回	17 -25	感染症および寄生虫症	C型肝炎	米国	女性	不明	不明	不明	自発報告	外国製品	11000040	2011/11/10	14.1
第17回	17 -26	感染症および寄生虫症	C型肝炎	米国	男性	不明	不明	不明	自発報告	外国製品	11000043	2011/11/17	14.1

血対ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
100473	27-Dec-11	110791	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	無	有	無
100474	27-Dec-11	110792	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	無	有	無

別紙様式第4

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第17回	17-1	感染症および寄生虫症	C型肝炎	コロンビア	男	8歳	不明	不明	症例報告	当該製品	識別番号：11000011 報告日：2011年8月22日 MedDRA: Version(14.0)
第16回	16-1	感染症および寄生虫症	非A非B型肝炎	アルゼンチン	男	46歳	不明	回復	文献報告	当該製品	識別番号：11000001 報告日：2011年4月15日 MedDRA: Version(14.0)

100476	20-Jan-12	110803	バクスター	ルリオクトコグアルファ(遺伝子組換え)	ルリオクトコグアルファ(遺伝子組換え)	遺伝子組換えチャイニーズハムスター卵巣細胞株	該当なし	有効成分	無	有	無
100477	20-Jan-12	110804	バクスター	ルリオクトコグアルファ(遺伝子組換え)	ウシ血清アルブミン	ウシ血液	米国	製造工程	無	有	無
100478	20-Jan-12	110805	バクスター	ルリオクトコグアルファ(遺伝子組換え)	インスリン(抗第Ⅷ因子モノクローナル抗体製造用)	ウシ膵臓	米国	製造工程	無	有	無
100479	20-Jan-12	110806	バクスター	ルリオクトコグアルファ(遺伝子組換え)	アプロチニン	ウシ肺	ニュージーランド	製造工程	無	有	無
100480	20-Jan-12	110807	バクスター	ルリオクトコグアルファ(遺伝子組換え)	ウシ胎児血清(抗第Ⅷ因子モノクローナル抗体製造用)	ウシ血液	オーストラリア	製造工程	無	有	無
100481	20-Jan-12	110808	バクスター	ルリオクトコグアルファ(遺伝子組換え)	培養補助剤(抗第Ⅷ因子モノクローナル抗体製造用-1)	ウシ血液	米国	製造工程	無	有	無
100482	20-Jan-12	110809	バクスター	ルリオクトコグアルファ(遺伝子組換え)	培養補助剤(抗第Ⅷ因子モノクローナル抗体製造用-2)	ウシ肝臓	米国又はカナダ	製造工程	無	有	無
100483	20-Jan-12	110810	バクスター	ルリオクトコグアルファ(遺伝子組換え)	人血清アルブミン	人血漿	米国	添加物	無	有	無