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Title: Unknown illness identified as Arenavirus

By Luyanda Makapela

Johannesburg - The virus which has caused the death of three people has been provisionally identified as the rodent-borne Arenavirus.

The Arenavirus, related to the Lassa Fever Virus of West Africa, causes chronic infections in multimammate mice. Infected mice's excretion contains the virus which can contaminate human food or house dust.

A joint statement by the National Institute for Communicable Disease (NICD) and the Department of Health explained that the Arenavirus is a disease spread from human to human through the contact of body fluids.

"Special precautions are required in nursing patients," a statement said.

The finding follows blood samples being sent to Atlanta, in the United States to determine the cause of the deaths of three people who had been suspected of contracting Viral Haemorrhagic Fever.

The virus is similar to Lassa Fever, the department said. It has previously been found in rodents elsewhere in Africa, but has not been found to cause disease in humans other than in West Africa.

Further tests are needed to confirm the diagnosis by growing the virus in culture.

"It needs to be determined whether it is a previously unrecognised member of the Areaviruses, and what its distribution is. There is no indication as yet that Arenaviruses which cause disease in humans are present in South African rodents," the NICD said.

The first victim, who had to be flown in from Zambia in a critical condition, was admitted to the Morningside Medi-Clinic in mid September. She died two days later.

About two weeks later, the paramedic who had flown in with the first victim, was admitted at the same clinic presenting the same symptoms.

A nurse, Gladys Mthembu died shortly afterwards. According to certain reports Ms Mthembu's family has been given a go-ahead to continue with the funeral arrangements as her bedroom had been cordoned off by health officials

Maria Mokubung, a cleaner at the Morningside Medi-Clinic, who also died last weekend has since been ruled out as a possible victim of the virus

Meanwhile the Gauteng Health Department has confirmed that the three other patients, including nurse's female supervisor, who had been under observation for showing symptoms of the virus have been discharged.

They had been in contact with the nurse who died.

However, departmental spokesperson Phumelele Kaunda said there were two contacts that were still under active surveillance after being admitted for observation.

The one patient is a paramedic who had contact with the first patient and developed fever and flu-like symptoms. He was admitted initially in Flora Clinic and then transferred to Morningside Medi-Clinic with a diagnosis of kidney stones.

The other patient is a nurse who attended to the second patient and developed signs and symptoms similar to the first three patients. She is being treated in isolation and received the anti-viral medication, ribavirin. The patient is presently stable.

Gauteng Health MEC Brian Hlongwa meanwhile has sent condolences to the families of those that were killed by the viral infection, particularly families of health professionals who died in the line of duty.

"This illustrates the dedication of our health professionals and the need to society to respect and honour the work that they do," said MEC Hlongwa.

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2008年10月20日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	ProMED-mail, 20081028.3409	公表国 ザンビア・南アフリカ	
販売名（企業名）	別紙のとおり					

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
問題点：南アフリカにおいて、アレナウイルス科の新たなウイルスによる見られる感染により5人の患者が報告された。 初発患者(症例1)の発症は9/2日で、これに続いて3人の二次感染症例と1人の三次感染患者が報告された。初発患者と二次感染の3人は死亡し、三次感染症例は現在入院中である。患者の年齢層は33～47才、女性4人と男性1人。初発患者の感染源は判っていない。他の4人の患者は全員が医療施設内で、初発患者もしくは二次感染患者の血液・体液と接触があった可能性があった。初発患者はザンビア在住で、治療のための南アフリカへの移送後に死亡した。症例2は、症例1の移送に付き添った救急隊員の1人で、症例3は集中治療室にいた症例1の看護を担当していた。症例4は症例1が入院していた部屋の清掃を行った。症例5は症例2の看護を担当した。二次および三次感染患者の潜伏期間は7～13日と考えられている。死亡した4人の患者の発病から死亡までの期間は9～12日であった。患者全員が初発症状として発熱・筋肉痛・頭痛を伴うインフルエンザ様症状を示した。7日間で重症度が増し、いずれも下痢と咽頭痛が見られた。第6～8病日に顔面と躯幹の麻疹様発疹が報告されている。3人に顔面の浮腫があった。死亡した患者では、末期症状として呼吸困難・神経学的症状・循環不全を伴う突然で急速な状態の悪化が見られた。出血症状は著明な特徴ではないが、1人に皮下出血、もう1人は穿刺部位からの持続出血が見られた。暫定的な検査により、今回の感染はアレナウイルス科における新たな異なるウイルスと見られている。 現在(10/28日)まで新たな感染疑い症例は発生していない。感染流行は封じ込められたようであり、医療施設内環境下で濃厚接触者だけに感染が限定されている。病原体の詳細な特徴については、現在調査中であり、初発患者の感染源についての調査も必要である。症候性感染発生の可能性の検討も、感染流行の範囲や臨床像をより理解するために重要である。	記載なし

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

MedDRA/J ver.11.1

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免疫グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗HBs人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免疫グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名（企業名）	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用
報告企業の意見	アレナウイルス属は、エンベロープをもつ1本鎖RNA(－)ウイルスである。齧歯類に寄生し、慢性腎臓感染をおこす。齧歯類の尿中は高ウイルス価であり、ヒトの食品やハウスダストを汚染する。曝露したヒトは偶発的宿主となる。このウイルスの原型はリンパ球性脈絡膜髄膜炎ウイルス（LCMV）であり、ヒトに感染するとインフルエンザ様症状、無菌性髄膜炎もしくは重症髄膜脳炎を発症する。出血熱症候群の原因となるArenavirusesは南米(New World arenaviruses)から数多く報告されている。いわゆるOld World arenavirusesは世界中に分布するLCMVと、西アフリカのナイジェリア、シエラレオネ、リベリア、ギニアを中心に1年間に最大50万人が感染し、実際にはさらに広い地域に分布すると見られているラッサ熱ウイルスである。ラッサ熱ウイルス感染の臨床症状としては、不顕性、軽症発熱性疾患から劇症出血性疾患まで様々であり、致死率は一般的な社会環境における1～2%から、入院患者では20%、院内感染では40%以上に及ぶこともある。西アフリカ一帯に生息する野ネズミの一種であるマストミス（*Mastomys natalensis*）は、ラッサ熱ウイルスの最重要宿主であり、その分布は、西アフリカから東アフリカ一帯と、南アフリカ北東端まで南に広がっている。他のMastomys種とも分布域が重複し、アレナウイルスは過去にはアフリカ南部の齧歯類でも確認されている。 (http://www.forth.go.jp/cgi-bin/promed/search.cgi?title_link=20081029-0050&button_detail=on) 弊所の血漿分画製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。 各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日）」に従い、ウシウイルス性下痢ウイルス（BVDV）、仮性狂犬病ウイルス（PRV）、ブタパルボウイルス（PPV）、A型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告したアレナウイルス属は、エンベロープの有無、核酸の種類等からモデルウイルスとしてはBVDVが該当すると考えられるが、上記バリデーションの結果から、BVDVの除去・不活化効果を有することを確認している。 また、これまでに当該製剤によるアレナウイルス感染の報告例は無い。 以上の点から、当該製剤はアレナウイルスに対する安全性を確保していると考える。

*現在製造を行っていない

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UNDIAGNOSED FATALITIES - SOUTH AFRICA ex ZAMBIA (10): ARENAVIRUS
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<http://www.nicd.ac.za/pubs/communique/2008/NICDCommOct08Vol07_10.pdf>

Arena virus outbreak, South Africa — Update

This updates all previous reports and includes available data as of 24 Oct 2008. An outbreak of infection due to an arenavirus was identified in South Africa in early October 2008. A total of 5 cases has been reported for the period 12 Sep to 24 Oct 2008.

The primary case (case 1) had onset of illness on 2 Sep 2008. An additional 3 secondary cases (case 2, 3 and 4) and 1 tertiary case (case 5) have been confirmed to have an arenavirus infection by laboratory testing. The primary case and 3 secondary cases have died. The tertiary case is currently hospitalized. Ages of cases ranged from 33 to 47 years. 4 cases were female and 1 male. The source of infection is, as yet, unknown for the primary case. The other 4 cases all had potential exposure to blood and/or body fluids of a primary or secondary case in the health-care setting.

The primary case was a safari booking agent resident in Zambia. The patient was flown to South Africa for medical care in a critically ill condition on 12 Sep 2008, and died on 14 Sep 2008. Case 2 was a paramedic who cared for case 1 during the transfer from Zambia on 12 Sep 2008 and case 3 was a nurse who cared for case 1 in the intensive care unit from 12-14 Sep 2008. Case 2 was admitted on 27 Sep 2008 and died on 2 Oct 2008 and case 3 was admitted on 30 Sep 2008 and died on 5 Oct 2008. On 14 Sep 2008, case 4 performed terminal cleaning of the room in which case 1 was hospitalized. The 5th patient is a nurse who cared for case 2 from 27 Sep 2008 to 2 Oct 2008. She became ill on 9 Oct 2008 and is currently critical but stable. Ribavirin has been used for treatment in this case based on good evidence of efficacy in patients with Lassa fever (an arenavirus infection). The estimated incubation period (interval from exposure to symptom onset) in secondary and tertiary cases ranges from 7 to 13 days. In 4 patients who died, the interval from onset of illness to death ranged from 9 to 12 days (Figure 1).

Only limited clinical data are currently available for case 4, who presented late in the course of illness with bleeding and confusion and died soon thereafter. Clinical features of the remaining 4 cases, for which more clinical data were available, are presented. All patients presented initially with a non-specific flu-like illness with symptoms of fever,headache and myalgia. The illness increased in severity over 7 days with all 4 patients developing diarrhoea and pharyngitis during the course of illness. A morbiliform rash on the face and trunk was reported in 4 cases on day 6 - 8 of illness. Facial swelling occurred in 3 patients. There appeared to be an initial clinical improvement after hospital admission in 3 patients, followed by clinical deterioration. Sudden and rapid deterioration

with respiratory distress, neurological signs and circulatory collapse were terminal features in all patients who died. Bleeding was not a prominent feature. However, one patient had a petechial rash and another had oozing of blood from venepuncture sites. Chest pain was reported in case 1.

At the time of admission all patients had thrombocytopenia (range: 42-104 X109/L). Liver transaminases (AST and ALT) were available for 4 of 5 cases and were variable at the time of admission, however all 4 patients had raised AST and ALT during the course of their illness. Leucopenia was present on admission in 2 patients and 3 patients had a normal white blood cell count on admission. 4 patients subsequently developed leucocytosis during the course of hospitalisation. All contacts (family members, friends and healthcare staff) are being monitored with twice daily temperature measurements for a period of 21 days after the last exposure to a known case. In addition, safe burial of the deceased has been supervised by environmental health officers. Full personal protective equipment (PPE) and isolation precautions as per VHF protocols have been instituted.

The causative agent in this outbreak was initially identified as an Old World arenavirus by immunohistochemical tests performed at the Infectious Diseases Pathology Branch of the Centers for Disease Control and Prevention in Atlanta, USA, and on autopsy liver and skin samples taken with biopsy needles and skin punches in the Special Pathogens Unit of the National Institute for Communicable Diseases, National Health Laboratory Service, Sandringham (SPU-NICD/ NHLS), South Africa, from cases 2 and 3 on 9 Oct 2008 under biosafety level 4 laboratory conditions. Subsequently, infection with an Old World arenavirus has been confirmed in all 5 cases by positive PCR results and virus isolation by SPUNICD/ NHLS and CDC. Analysis of sequencing data generated at SPU-NICD/NHLS, Columbia University, New York, and CDC, Atlanta appears to indicate that the current outbreak is caused by a unique Old World arenavirus.

There are currently no additional suspected cases. The outbreak appears to be contained and has been confined to individuals with very close contact in a health-care setting. Monitoring of contacts, active case finding and investigation and management of suspected cases will continue as needed. Further characterization of the causative agent is under way and investigation into the source of infection in the primary case is required. Additional studies to determine whether mild/asymptomatic infection occurred amongst close contacts and other exposed individuals would be essential in better characterizing the extent of this outbreak and clinical spectrum of disease.

Arenaviruses are a family of enveloped negative sense single-stranded RNA viruses. Members of the family are parasites of rodents, in which they establish chronic renal infection. High titres of virus are present in rodent urine, which can contaminate human food or house dust. Exposed humans may become infected as accidental hosts. The prototype of the family is lymphocytic choriomeningitis (LCM) virus and infection of humans with this virus may present as an influenza-like illness, aseptic meningitis or severe meningo-encephalomyelitis. Arenaviruses which cause a haemorrhagic fever syndrome are well documented in South America (New World arenaviruses, including Junin, Machupo, Sabia and Guanarito viruses). The so-called Old World arenaviruses include LCM which in fact has a worldwide distribution, and Lassa fever virus which affects up to 500 000 people annually in West Africa, specifically in Nigeria, Sierra Leone, Liberia and Guinea, but the virus is suspected to be more widely distributed in that region.

The clinical spectrum of Lassa fever virus infection ranges from inapparent, through mild febrile illness to fulminant haemorrhagic disease, and mortality rates vary from 1-2 percent among cases in the community at large, through 20 percent among hospitalized patients, to >40 percent in nosocomial outbreaks. The multimammate mouse (Mastomys natalensis), which is the most important host of Lassa fever virus, has a distribution extending from West Africa across to East Africa and from there southwards to the northeasterncorner of South Africa. Its distribution overlaps with that of other Mastomys species, and arenaviruses have been found in southern African rodents in the past, but there has been no previous association of these viruses with human disease despite sustained monitoring. Preliminary

testing indicates that the virus associated with the present nosocomial disease outbreak is a distinct new member of the family.

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[This update provides a definitive account of the recent outbreak of arenavirus-associated disease in South Africa. A primary case (case 1) had onset of illness on 2 Sep 2008. An additional 3 secondary cases (case 2, 3 and 4) and 1 tertiary case (case 5) have been confirmed to have an arenavirus infection by laboratory testing. Case 5 (not previously reported) is a nurse who cared for case 2 from 27 Sep 2008 to 2 Oct 2008. She became ill on 9 Oct 2008 and is currently critical but stable. Cases 1, 2, 3 and 4 did not survive infection.

Infection with an Old World arenavirus has been confirmed in all 5 cases by positive PCR results and virus isolation by SPUNICD/ NHLS and CDC. Analysis of sequencing data generated at SPU-NICD/NHLS, Columbia University, New York, and CDC, Atlanta, appears to indicate that the current outbreak is caused by a unique Old World arenavirus.

There are currently no additional suspected cases. The outbreak appears to be contained and has been confined to individuals with very close contact in a health-care setting. Monitoring of contacts, active case finding and investigation and management of suspected cases are continuing. Further characterization of the causative agent is under way, as is investigation into the source of infection in the primary case.
- Mod.CP]

[see also:
Undiagnosed fatalities - S. Africa ex Zambia (09): arenavirus 20081018.3300
Undiagnosed fatalities - S. Africa ex Zambia (08): arenavirus 20081013.3241
Undiagnosed fatalities - S. Africa ex Zambia (07): arenavirus 20081012.3234
Undiagnosed fatalities - South Africa ex Zambia (06): WHO 20081010.3211
Undiagnosed fatalities - South Africa ex Zambia (05) 20081008.3192
Undiagnosed fatalities - South Africa ex Zambia (04) 20081008.3188
Undiagnosed fatalities - South Africa ex Zambia (03) 20081007.3178
Undiagnosed fatalities - South Africa ex Zambia (02) 20081006.3157
Undiagnosed fatalities - South Africa ex Zambia: RFI 20081005.3139]
.....cp/ejp/dk

別紙様式第2-1

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 10. 17	該当なし	
一般的名称	人全血液		研究報告の公表状況	ABC Newsletter, No. 38. 2008 Oct 17.	公表国 イタリア	
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)					

研究報告の概要

○イタリアで久々に発生したWNV症例

2008年、イタリアで久々にヒトのウエストナイルウイルス(WNV)脳炎が2例報告された。

1例目は、最近ウマ(6例)のWNV確定症例およびトリ(13例)のWNV陽性が特定されているフェラーラとボローニャの間に位置する農村地帯在住の80歳代の女性患者である。患者に渡航歴はなく、9月5日に発熱および複数回の嘔吐を発症した後、高熱、嘔吐、意識障害、幻覚を呈し、9月19日にイモラの病院に入院したが救急室で痙攣状態となった。その後回復したが、ELISAによるWNV特異抗体検査で急性WNV感染が示され、さらに追加検査によりWNV特異抗体が確認された。10月9日のユーロサーベイランスレポートは、検査結果はWNVに対する抗体反応であり、WNV神経侵襲性感染の仮説を裏づけると述べている。患者の家から2、3km以内の場所には、数種類の鳥類集団が生息し、蚊(イエカ、ヒトスジシマカ)が発生している大きな沼がある。

神経浸襲性WNV疾患の2例目は、フェラーラ在住の60歳代後半の男性で、10月3日にボローニャで特定された。患者は、高熱を伴う急性髄膜脳炎の症状を発現し、血清および脳脊髄液検体はWNV特異IgG、IgM抗体陽性で、2回の血清RT-PCR検査は陽性だった。

WNV髄膜脳炎の積極的サーベイランスプログラムが開始され、当該地域で供血者スクリーニング用核酸増幅検査が導入された。また、イタリアの国立血液センターは、全血液センターに対し、当該地域に1日以上滞在したことのある供血者を28日間供血延期とするように指導した。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2008年、イタリアで久々にヒトのウエストナイルウイルス(WNV)脳炎が2例報告されたため、WNV髄膜脳炎の積極的サーベイランスプログラムが開始され、供血者スクリーニング用核酸増幅検査の導入、28日間供血延期措置がとら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問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、ウエストナイルウイルス感染の発生に備え、平成17年10月25日付血液対策課発事務連絡に基づき緊急対応の準備を進めている。今後も引き続き情報の収集に努める。

(9)

WNV Case in Italy is First There in Many Years

Two human cases of West Nile Virus (WNV) encephalitis have been reported in Italy in the last month, the first human cases in that country in many years.

On September 20, the laboratory of the Regional Reference Center for Microbiological Emergencies in Bologna, Italy, reported the detection of specific IgM and IgG antibodies against WNV in the serum of a female patient in her 80s who lives in a rural area between Ferrara and Bologna.

Six confirmed cases of WNV disease in horses have recently been reported in this area, and 13 birds (six crows and seven magpies) have been identified as positive for WNV. Subsequently, an active surveillance program for possible human cases of WNV meningoencephalitis began.

Nucleic acid amplification testing has been introduced for blood donor screening in the provinces of Bologna and Ferrara. The Italian National Blood Center also has instructed all blood centers to defer for 28 days donors who have been for at least one night in the subject areas.

No Travel Reported. The patient had fever and repeat vomiting episodes on September 5. A first diagnosis of suspected urinary tract infection was made and the patient was given medication, but the symptoms remained and the patient was admitted to an Imola hospital on September 19 with high fever, vomiting, impaired consciousness, and hallucinations. The patient went into convulsions in the emergency room. She has regained consciousness and has almost completely recovered, though she remains hospitalized as a safety precaution.

Serum samples were tested for WNV-specific antibodies using an enzyme-linked immuno-sorbent assay, which indicated an acute WNV infection. WNV-specific antibodies were further confirmed by additional serological tests on the first samples. The samples were tested for Japanese encephalitis virus (JEV) and tick-borne encephalitis virus (TBEV). "Results clearly demonstrated that the antibody response was mainly directed against WNV, thus corroborating the hypothesis of a WNV neuroinvasive infection," according to the *Eurosurveillance Report* (10/9/08).

The patient's relatives reported that she had not traveled outside the small village where she has lived for the past two years. The patient's home is located within a few kilometers from a large swamp that is home to a sizeable population of different bird species and is infested by mosquitos (both *Culex* and *Aedes albopictus*).

A second human case of WNV neuroinvasive disease was identified in Bologna on October 3 – a man in his late 60s who lived in the province of Ferrara where WNV-positive horses and birds have recently been identified. The patient suffered from symptoms of acute meningoencephalitis with high fever. Serum and cerebrospinal fluid samples of this patient have tested positive for IgG and IgM antibodies against WNV and two different RT-PCRs performed on the serum were positive, though confirmatory laboratory testing was still pending.

WNV has been reported in Europe, the Middle East, Africa, India, parts of Asia, and Australia. Human WNV disease has been reported in the Mediterranean Basin: in Algeria in 1994, Morocco in 1996, Tunisia in 1997 and 2003, Romania in 1996 through 2000, the Czech Republic in 1997, Israel in 1999 and 2000, Russia in 1999 through 2001, and France in 2003. Enzootics involving horses were reported in Morocco in 1996 and 2003, Italy in 1998, Israel in 2000, and southern France in 2000, 2003, and 2004. (Sources: *Eurosurveillance Report*, 10/9/08; European Commission response to European Blood Alliance query, 10/6/08) ♦

別紙様式第2-1

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日 2008. 11. 4	新医薬品等の区分 該当なし
一般的名称	人全血液		研究報告の公表状況	Furtner M, Gelpi E, Kiechl S, Knoflach M, Zangerl A, Gotwald T, Willeit J, Maier H, Ströbel T, Unterberger U, Budka H. J Neurol Neurosurg Psychiatry. 2008 Feb;79(2):229-31.	公表国 オーストリア
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)				

研究報告の概要

○ヒト成長ホルモンによる治療22年後に発症した医原性クロイツフェルト・ヤコブ病、臨床および放射線学的特徴
医原性のクロイツフェルト・ヤコブ病(iCJD)の多くは、プリオンに汚染されたヒト成長ホルモン(hGH)製剤の投与によるものである。
患者は、11歳でクッシング症候群と診断され、1984年9月から1985年11月まで死体から採取し市販用に製造されたhGH(クレスコモン、カビ社、現在は製造中止)の投与を受けていた。
2007年、神経学的兆候により入院後、状態は急速に悪化し、集中的な理学療法と言語療法にもかかわらず、患者は4カ月後に死亡した。
組織学的検査で海綿状の変化、神経細胞脱落、グリオーシスの特徴を示し、免疫組織学的検査は特異的なプリオン蛋白の沈着が見られた。医原性のリスクが認められたため、WHOの基準に従い確定iCJDに分類された。プリオン蛋白遺伝子(PRNP)には既知の突然変異は認められず、患者はPRNPコドン129、メチオニンホモ接合体であった。
疾患発症後の1、2、3カ月目に実施したMRIによる連続造影上の変化は、海綿状の変性を示しており、拡散強調画像の偽正常化は進行性の細胞死と関連していると推察された。
hGH投与22年後におけるCJD発症は、英国における一連のhGH-iCJD試験で推計された暴露後およそ20年というリスクのピークと一致する。
本症例は、hGHを投与された患者としては、オーストリアにおける初のCJD症例である。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

総合機構処理欄

報告企業の意見

ヒト(死体)由来のヒト成長ホルモン(hGH)製剤の投与を受けた患者が、22年後にクロイツフェルト・ヤコブ病を発症し、4カ月後に死亡し、確定医原性CJDに分類されたとの報告である。
なお、日本においては1995年以降には、すべてリコンビナントヒト成長ホルモン製剤に切り替わった。

今後の対応

日本赤十字社では、CJDのリスクのある血液を排除する目的から、献血時にhGH製剤投与の有無を確認し、該当するドナーを無期限に献血延期としている。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

(10)

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A novel mutation (c.64_65delGGinsAACC [p.G21fsX66]) in the *GTP cyclohydrolase 1* gene that causes Segawa disease

DYT5 dystonia (Segawa disease) is an autosomal-dominant inherited progressive dystonia that is evoked by mutations/deletions of the *GTP cyclohydrolase 1* (*GCH1*) gene,[1] which codes for the rate-limiting enzyme of tetrahydrobiopterin (BH_4) synthesis. Segawa disease is a rare disorder with an estimated prevalence of 0.5 per million. We report a clinical course caused by a novel mutation of the *GCH1* gene in a 25-year-old Caucasian female presenting in our outpatient clinic. The patient was born to healthy parents with no history or signs of neurological diseases. She described the development of a gait disturbance beginning at the age of 5 years. She was increasingly unable to walk at her soles, but was only walking at the outer edges of her feet (*pedes equinovarus*), causing a monstrous callus, within years. The feet cramped after only a few steps, which was relieved after some rest. Several stays in hospital did not reveal the final diagnosis, so that the gait disturbance was initially classified as a psychogenic disorder. The patient was then introduced to our movement disorder outpatient clinic just before an operation of the feet abnormalities. Clinical examination showed focal cramps of both feet with relevant relief only by inactivity. The feet were severely adducted and supinated. Neurophysiological examinations, including somatosensory and magnetic-evoked potentials, were normal. A magnetic resonance imaging scan of the cervical and thoracic spine revealed only a short hydromyelia with no signs of inflammation or neoplasma. Analyses of the biogenic amines and pterins in the cerebrospinal fluid, according to the methods of Curtius and Hyland,[2 3] revealed highly decreased dopamine (homovanillic acid 48 nmol/l; normal values: 115–455) and serotonine metabolites (5-hydroxyindoleacetic acid 20 nmol/l; normal values: 51–204). Similarly, all pterines were markedly reduced (tetrahydrobiopterin: below detection level [normal value: 18–53 nmol/l]; total neopterin: 6 nmol/l [normal value: 10–31]). Folate metabolites were normal. To confirm the diagnosis of Segawa disease, GTP-cyclohydrolase I (GTPCH) enzyme activity was determined in skin fibroblasts according to Bonafé *et al*,[4] which showed only 34% activity (0.99 μU/mg protein) compared with healthy controls (reference value: 2.6±0.53 μU/mg protein). Treatment with low doses of levodopa was capable of resolving the symptoms completely. Sequencing of exons 1–6 of the *GCH1* gene revealed a heterozygous deletion of two guanines at positions 64 and 65 and an insertion of 4 bases (AACC; fig 1), leading to a frameshift from amino acid 21 and subsequent termination of the protein after amino acid 66 within exon 1 (c.64_65delGGinsAACC [p.G21fsX66]). Multiplex ligation-dependent probe amplification (MRC, Amsterdam, The Netherlands) of the whole *GCH1* did not detect any further deletions. The clinically unaffected parents did not show any mutation in the *GCH1* gene (fig 1), confirming that the mutation in the patients represents a *de novo* mutation. This novel combined deletion-insertion mutation leading to protein truncation within exon 1 has not been reported before, despite up to more than 100 abnormalities of the *GCH1* gene being reported—including exon (start point change, missense, nonsense and frameshift mutations) and intron mutations, and deletions.

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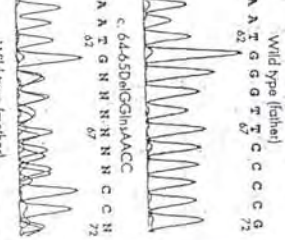

Figure 1 Genomic sequences of the index patient (middle panel) and both parents (father: upper panel; mother: lower panel), revealing a heterozygous deletion of two guanines at positions 64 and 65 and an insertion of the four bases AACC in the index patients, but wild-type sequences in both parents. The sequence abnormalities lead to a frame shift from amino acid 21 and subsequent termination of the protein after amino acid 66 within exon 1.

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Iatrogenic Creutzfeldt–Jakob disease 22 years after human growth hormone therapy: clinical and radiological features

Creutzfeldt–Jakob disease (CJD) is a human transmissible spongiform encephalopathy or prion disease. Although CJD is most frequently sporadic, numerous acquired or iatrogenic CJD (iCJD) cases have been reported, about half of which are attributable to prion-contaminated human growth hormone (hGH) preparations.[1] Cadaveric hGH was provided by public and commercial sources up to 1985, when recombinant GH became available. Incubation periods of iCJD peak at a median of 12 (range 5–30) years after exposure.[2 3]

We report the first Austrian case of hGH associated autopsy-proven iCJD and discuss clinical features and serial magnetic resonance imaging (MRI) findings.

CASE REPORT

Clinical history

A 39-year-old man presented with right-sided clumsiness and dysaesthesia, which had started in his leg 3 weeks prior to admission and had spread to his right arm. No impairment of cognitive function and no involuntary movements were present. There was no family history of neurological disease. The patient had been healthy until the age of 11 years, when progressive obesity and growth impairment had been noticed and a diagnosis of Cushing syndrome had been made. The patient moved to Austria at the age of 15 years (1982) and was subsequently diagnosed with a hormone-producing pituitary adenoma, which was removed by transsphenoidal hypophysectomy. The frontal skull base defect was covered with

autologous connective tissue (fascia lata). Due to persistent Cushing syndrome symptoms, bilateral adrenalectomy was performed. To promote body growth (height <3rd percentile), he received commercially manufactured cadaveric hGH (Crescormon®, Kabi Pharma, now discontinued) from September 1984 (2 IU IM three times per week, which was later reduced to 2 IU IM twice a week). The treatment was continued until November 1985 and resulted in an increase of body height of 13.5 cm.

In 2003, a recurrency of the pituitary adenoma causing Cushing symptoms was diagnosed and transsphenoidal resection was performed, again with an autologous fascia lata graft.

On admission, the patient's neurological exam showed coarse bilateral gaze nystagmus, vertical gaze palsy and mild right-sided hemiparesis. Tendon reflexes in both lower extremities were exaggerated, whereas pyramidal signs were negative. Gait was paraspastic, with a deviation tendency to the right, but unaided walking was still possible. Cerebellar tests revealed bilateral ataxia in the upper and lower limbs and dysdiadochokinesia of both hands. Testing for infectious, parainfectious, as well as neoplastic or paraneoplastic neurological diseases, was negative, as was metabolic screening.

Serial cerebral MRI was performed in months 1, 2 and 3 (fig 1). Electroencephalographic recordings (EEGs) in months 1 and 2 showed diffuse slowing with generalized delta activity and intermittent rhythmic delta-theta runs with a right fronto-central accentuation. EEG in month 3 revealed further slowing and some non-periodic bilateral sharp/slow wave complexes.

Cerebrospinal fluid (CSF) examinations in week 1 and week 6 after admission exhibited divergent results. In the first sample, 14-3-3 protein was undetectable; protein content, as well as cytology, were normal. In the second CSF sample, a strong signal in the molecular weight range of the 14-3-3 protein was detected.

Neuropsychological examination 3 weeks after admission showed reduction of attentive functions, whereas memory was unimpaired. Over 3 months of hospitalization, the patients condition rapidly deteriorated. Myoclonus of both arms and legs emerged; the patient became bedridden after about 6 weeks. Speech was increasingly dysarthric, and severe dysphagia ensued. Hypostatic pneumonia required antibiotic treatment. Despite intensive physiotherapy and speech therapy, the patient's condition continued to worsen. The patient died after an overall disease course of 4 months.

Neuropathology

Histology showed the characteristic triad of spongiform change, neuronal loss and gliosis. Immunohistochemistry revealed characteristic prion protein deposits in cerebral and cerebellar cortices, confirming the diagnosis of CJD. Due to the recognised iatrogenic risk (hGH), the disease was classified as definite iCJD according to World Health Organization (WHO) criteria.[1] Western-blot analysis of proteinase K restistant PrP was not performed due to lack of adequate material.

Genetic analysis

Sequencing of the entire coding region of the prion protein gene (*PRNP*) performed after isolation of genomic DNA from peripheral blood showed no known mutations. The patient was methionine homozygous at codon 129 of the *PRNP*.

DISCUSSION

This case of definite iatrogenic CJD 22 years after hGH medication exhibits several noteworthy features.

MRI studies 1, 2 and 3 months after manifestation of disease revealed early bilateral cortical involvement of the mesial frontal lobes. Diffusion-weighted imaging (DWI) hyperintensities progressed to adjacent cortical areas and to the striatum, in line with clinical deterioration (fig 1). DWI has been recommended as the most sensitive test for early diagnosis of CJD,[2] but is not suggestive of a specific form of disease. HGH-iCJD cases have exhibited DWI hyperintensities mainly in the basal ganglia. Cerebellar malfunction is one of the most common early signs of iCJD after hGH treatment[1] and was one of the main clinical disturbances at disease onset in our patient. However, no corresponding MRI abnormalities were detected in the cerebellum. To our knowledge, no other hGH-iCJD case has been documented with early frontomesial DWI changes and progressive bilateral striate hyperintensities.

CSF 14-3-3 protein was negative on first testing and turned positive 4 weeks later. Of interest, DWI changes preceded CSF 14-3-3 protein conversion by weeks and had spread from the cortical distribution shown in figure 1A/B to a striatal DWI pattern that is commonly associated with sporadic CJD (fig 1B). It has been speculated that these changes on serial imaging indicate spongiform degeneration, but that the neurons are still viable in the early disease stages, and that a subsequent DWI pseudonormalization is related to progressive cell death.[4]

The clinical presentation, with paraspastic gait as one of the first striking features, also requires attention. This correlates well with the imaging findings and represents a bilateral parietal edge syndrome—that is, first motoneuron dysfunction in the leg areas of both precentral gyri.

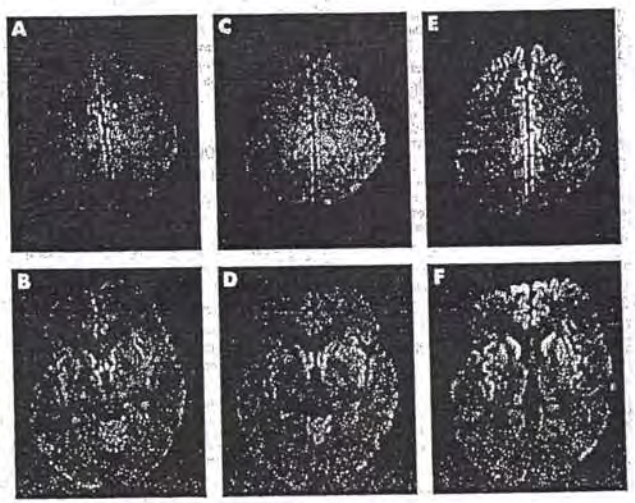

Figure 1 Magnetic resonance imaging (MRI) 1 month (panels A and B), 2 months (C, D) and 3 months (E, F) after onset. Diffusion weighted imaging (DWI) 1 month after onset revealed bilateral frontomesial hyperintensities (A), and moderate DWI signal increases in the medial portion of both caudate heads (B). Two months after onset, the bifrontal hyperintensities showed slight enlargement (C), and DWI signals were elevated in both caudate heads, the adjacent putamina and insular cortices (D). On follow-up MRI 1 month later, there was increased DWI signal in the frontomesial and frontopolar cortex (E,F) and marked DWI hyperintensity in both caudate heads, both putamina with accentuation in their rostral parts, and both insular ribbons (F). ADC maps and FLAIR images were inconspicuous (data not shown).

Occurrence of CJD 22 years after hGH administration is in line with the peak risk approximately 20 years after exposure calculated from a large hGH-iCJD series in the UK,[3] whereas the mean incubation period in French hGH recipients was considerably shorter at 9–10 years.[5] Differences of infectivity in hormone lots have been suggested as an explanation for this finding.

Some unusual circumstances and clinical features also deserve comment. First, iCJD associated with hGH has, so far, only been reported after administration of non-commercial hormone. The reports available, however, have excluded patients treated with commercially prepared hormone; hence, there are insufficient data on the CJD rate in these patients.[2 3] Second, the administration period of hGH and disease duration were both short for iCJD patients even though comparable cases have been reported in previous literature.[1 7]

In summary, this is the first CJD case from Austria in a patient having received hGH and only the third iatrogenic case detected in this country. The recognised iatrogenic risk (cadaveric hGH 22 years before onset) and the neuropathological confirmation of CJD meet the WHO criteria for definite iCJD, although the possibility of a sporadic methionine-homocygous juvenile CJD case without causal relation to hGH treatment cannot be definitely ruled out.

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APPENDIX

Histopathological examination

The total fixed brain weight was 1408 g. Macroscopically, moderate diffuse cerebral and cerebellar atrophy was observed. In addition, there were signs of diffuse oedema. On coronal sections, the cortical ribbon of the insular and parietal cortices was narrowed. Histology showed characteristic spongiform change, moderate neuronal loss and gliosis in cerebral cortex and basal ganglia (see Supplementary figure). The cerebellar cortex was severely affected with marked spongiform change of the molecular layer and neuronal loss of the granule cell layer (see Supplementary figure). The Purkinje cells and brain stem nuclei were comparatively better preserved. Immunohistochemistry using the antibody 12F10 (Cayman, Ann Arbor, Michigan, USA) revealed strong pathological prion protein (PrP^{res}) deposits in cerebral and cerebellar cortices, and basal ganglia in a diffuse synaptic pattern (see Supplementary figure). In the brain stem nuclei, only discrete PrP^{res} deposits were demonstrable. There were no PrP^{res} plaques neither in the cerebellum nor in the cerebral cortex or white matter. These features confirmed the diagnosis of Creutzfeldt–Jakob disease (CJD). Due to the recognised iatrogenic risk (due to human growth hormone), the disease was classified as definite iatrogenically transmitted CJD, according to World Health Organisation criteria.

Skin reactions after intramuscular injection of Botulinum toxin A: a rare side effect

The use of Botulinum toxin (BTX) has been constantly increasing over the past years, not least on account of obtaining the license for the treatment of facial lines. It has proven a safe drug with only a few adverse effects. Local irritations at the injection site are not uncommon, whereas more widespread and generalised exanthemas were first described in 1992.[1] One dramatic case documents a lethal outcome due to treatment with a mixture of BOTOX® (BTX-A) and lidocaine.[2] In accordance with databases from the companies Allergan and Ipsen (SPC BOTOX®, Allergan, December 2005; SPC, DYSPORT®, Ipsen Pharma, April 2006), skin reactions seem to be a rare phenomenon with a frequency of less than 1:1,000. The Ipsen database (January 2007) mentions 5 cases of local and 4 cases of more widespread redness, bulging and pruritus in Germany, as well as 11 cases abroad. Here, we report on two further cases of rapid-onset skin reactions after injection of two different BTX-A products.

CASE 1

A 49-year-old woman developed a left-sided spastic hemiparesis after cavernoma exstirpation in 1997. Successful treatment of the spastic arm muscles was carried out with BOTOX® for about 5 years and with DYSPORT® for the last 4 years. She did not receive any other medication. Injection intervals ranged from 3 to 9 months. During the treatment session in April 2006, we applied a total dose of 1,000 Units DYSPORT® (250 MU into the left biceps muscle, 250 MU into the left flexor pollicis longus and extensor carpi radialis muscles, 500 MU into the left flexor digitorum superficialis muscle). Within 6 hours after intramuscular injection of BTX-A, a segmental or "pseudosegmental" fine-spotted pruriginous exanthema emerged in the region of the entire left shoulder, arm and left breast. Fever or other additional symptoms did not occur. Allergological tests, such as prick tests, and an intracutaneous test were normal. Treatment with DYSPORT® was repeated 3 months later with a dose reduction of 50% without any adverse effects. At a later visit, she received 1,000 Units DYSPORT®, which was well tolerated.

CASE 2

A 63-year-old man presented with right-sided limb spasticity due to a stroke 7 years ago. The patient received a stable medication consisting of gabapentine, tramadole, tetrazepam, clopidogrel and atorvastatin. From 2003, he was successfully treated with injections of 900–1,100 Units DYSPORT® at regular intervals of 3 months. In 2006, the therapy was changed to BOTOX®. Within

Figure 1 Photograph of the skin reaction as described in Case 2 about 1 hour after injection into the right brachial muscle. Informed consent was obtained for publication of this figure.

別紙様式第２－１

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	Laboratory Hematology (United States) 2007, 13 (1) p34-8	公表国 米国	
販売名（企業名）	－					

研究報告の概要

重症筋無力症の治療として行ったアルブミンを交換液とした血漿交換の後に、パルボウイルス B19（以下「B19」）感染による赤芽球癆を発症した女性の症例を報告する。
アルブミン投与から 2 週間後に、患者は網状赤血球欠乏性貧血を発症し、骨髄穿刺を行ったところ、多数の巨大な前正赤芽球欠乏を伴う顕著な一連の低形成赤血球が示され、重度網状赤血球減少症を伴う貧血および骨髄の形態によって、B19 感染が原因の赤芽球癆が疑われ、IgM および IgG 型抗 B19 抗体により確認された。
患者は免疫グロブリン（0.4g/kg、4 日間）で治療したところ、貧血は徐々に回復した。
アルブミン、凝固因子、免疫グロブリンなどの血液製剤の感染性は除外できず、血液成分による B19 感染は依然未解明の問題である。
B19 はエンベロープを有さないウイルスであるため、溶媒－界面活性剤処理には抵抗性であるが、60℃で 10 時間低温殺菌すると迅速に不活化することを示したとの報告もある。
ウイルス不活化の新たな方法や B19 陽性単位の棄却などの多くの戦略は、血液製剤の安全性を増すのに有用である。

報告企業の意見	今後の対応
アルブミン投与後にパルボウイルス B19 感染が疑われた症例の報告である。 当社血漿分画製剤は最終製品において NAT 検査を行い、パルボウイルス B19DNA 陰性であることを確認している。	今後ともパルボウイルス B19 に関する血漿分画製剤の安全性に関する情報に留意していく。

使用上の注意記載状況・その他参考事項等

慎重投与（次の患者には慎重に投与すること）
・溶血性・失血性貧血の患者［ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。］
・免疫不全患者・免疫抑制状態の患者［ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。］

重要な基本的注意
(1) 本剤の原材料となる‥［スクリーニング項目、不活化・除去工程］‥投与に際しては、次の点に十分注意すること。
1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。

妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。［妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。］

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CASE REPORT

Parvovirus B19 Infection after Plasma Exchange for Myasthenia Gravis

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ABSTRACT

We describe a case of pure red cell aplasia caused by a B19 parvovirus infection in a female myasthenic patient treated with plasma exchange, corticosteroids, and cholinesterase inhibitors. Two weeks after albumin infusion, she developed anemia with an absence of reticulocytes. A bone marrow aspirate was performed, showing a markedly hypoplastic erythroid series with numerous giant pronormoblasts. Anemia with severe reticulocytopenia and morphology of bone marrow suggested a diagnosis of pure erythroblastopenia due to parvovirus B19 infection, which was confirmed by positive immunoglobulin (Ig)M and IgG anti-B19 virus. The patient successfully responded to IVIG treatment with a complete remission. In this case, we could not confirm whether an albumin-derived infection combined with a concomitant immunocompromised condition due to myasthenia and immunosuppressive treatment was responsible for the disease. Although human B19 DNA content does not reflect infectivity, it is not possible to exclude that blood derivates, such as albumin, clot factors, and immune globulin may be infectious. Actually, blood component B19 infection is still an unresolved problem. Many strategies such as new methods for viral inactivation and discarding positive B19 units may help to increase blood product safety. *Lab Hematol* 2007;13:34-38.

KEY WORDS: Parvovirus B19 • Pure red cell aplasia • Albumin • Myasthenia gravis • Plasma exchange

INTRODUCTION

Parvovirus B19 is a single-stranded DNA virus, forming small capsides and lacking a lipid envelope. Its genome encodes 3 major viral proteins, VP1 and VP2, the viral capsid proteins, which lead to self-assembly of viral particles, and NS1, a nonstructural protein, which is responsible for cytotoxicity. It has a peculiar tropism for human erythroid progenitors, with inhibition of erythroid colony growth and cytopathic effect [1-2].

B19 parvovirus is a common infection in humans, and about 50% of adults have immunoglobulin (Ig)G antibodies against the virus. Parvovirus infection is common in childhood and continues at a low rate throughout adult life. Most cases of parvovirus infection are asymptomatic. The most common clinical presentation is fifth disease of childhood, characterized by typical exanthema, fever, and flu-like symptoms. Acute or chronic arthropathy due to deposition of immune complexes may occur in adults. In patients with chronic hemolytic anemia, such as hereditary spherocytosis and sickle cell disease, acute parvovirus B19 infection can cause an abrupt cessation of red cell production, with transient aplastic crisis. In patients with immunodeficiency states, such as congenital immunodeficiencies or AIDS and patients receiving cytotoxic chemotherapy or immunosuppressive drugs, such as administered after an organ transplantation, there can be a failure to produce neutralizing antibodies. In these cases, pure red cell apla-

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sia can develop, with an absence of circulating reticulocytes and giant pronormoblasts in the bone marrow, without maturing normoblasts. Hydrops fetalis from transplacental infection and usually transitory hemophagocytic syndrome are other clinical disorders caused by B19 [3].

Parvovirus B19 transmission by blood products and plasma derivates, such as albumin, clotting factor concentrates, and intravenous immunoglobulin (IVIG) has been repeatedly demonstrated [4]. Transmissibility in coagulation products has occurred among patients who received heat-treated, pasteurized, monoclonally purified and solvent-detergent–treated concentrates [5]. Infection with B19 due to transfusion with cellular blood products is a rare event, but it has been reported twice with red blood cells and once with platelets [6-8]. We report a case of a myasthenic patient with pure red cell aplasia due to a parvovirus B19 infection.

CLINICAL CASE DESCRIPTION

In 1997, a 29-year-old woman complained of intermittent speaking difficulty (dysarthria). In April 1998, 10 days before the full-term delivery of her second healthy baby, more severe symptoms appeared, such as facial nerve and oro-pharyngeal deficit and weakness of the arms and legs. Ten days after delivery, the patient was admitted to a hospital for a typical myasthenic crisis with severe weakening of respiratory muscles, requiring a respirator to assist ventilation. Treatment was started with 4 consecutive plasma exchanges and administration of corticosteroids and cholinesterase inhibitors (pyridostigmine bromide) with marked clinical improvement. In August 1998, the patient withdrew from medical therapy, which led to a worsening of symptoms and a new hospitalization in a different institution. There she was treated with 5 therapeutic plasma exchanges using albumin as replacement fluid. Medical treatment was started again. On August 31, she had a deep vein thrombosis, treated with IV heparin. On September 3, she was admitted to the Neurology Department of our hospital. At admission, the patient had normochromic-normocytic anemia (hemoglobin [Hgb], 97 g/L), with normal platelet and white blood cell counts.

Two weeks later, anemia worsened and was associated with thrombocytopenia (Hgb, 81 g/L; platelets, 57×10^9/L) (Figure 1). Schistocytes were absent. A diagnosis of heparin-induced thrombocytopenia was made. Heparin tapering was started, and the platelet count improved. A few days later, since anemia was still severe (Hgb, 80 g/L) and of an aregenerative type with an absence of reticulocytes, a bone marrow aspirate was performed. This showed many moderate hypercellular marrow particles and an increased number of megakaryocytes. An erythroid series was markedly hypoplastic with complete maturative arrest. The only visible erythroid precursors were giant pronormoblasts with vacuolated deep basophilic cytoplasm, sometimes grouped in clusters simulating metastatic cells (Figures 2 and 3). Anemia with severe reticulocytopenia and morphology of bone marrow suggested a diagnosis of pure erythroblastopenia due to parvovirus B19 infection, which was confirmed by positive tests for IgM and IgG anti-B19 virus. Increased megakaryocytes tended to confirm that thrombocytopenia was heparin-induced. The patient was treated with immune globulin (0.4 g/kg for 4 days). Reticulocytosis appeared on September 30 (202×10^9/L; normal values, $30\text{-}90 \times 10^9$/L). Anemia recovered slowly (Hgb, 92 g/L at discharge), and thrombocytopenia completely regressed. The patient was admitted again to

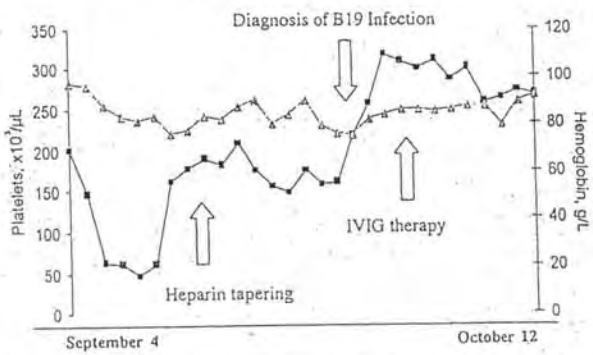

FIGURE 1. Hematological values and clinical course of the patient from admission (September 4, 1998) to discharge (October, 12 1998). Triangle indicates platelet count; square, hemoglobin concentration.

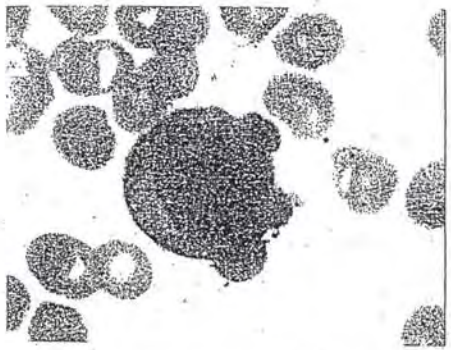

FIGURE 2. Basophilic giant pronormoblast with pseudopodia or "dog ears."

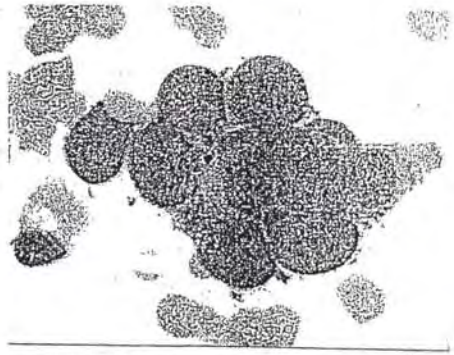

FIGURE 3. A cluster of pronormoblasts with maturative arrest.

the hospital in May 1999 for surgical resection of a thymoma. At that time, her full blood count was normal, IgM anti-B19 was negative, and IgG anti-B19 was still positive.

DISCUSSION

We described a case of pure red cell aplasia caused by parvovirus B19 in a patient with myasthenia gravis treated with plasma exchanges using albumin, corticosteroids, and cholinesterase inhibitors.

Parvovirus B19 has a particular tropism for erythroid progenitors. The cellular receptor for B19 is erythrocyte P antigen, a globoside that consists of a long-chain fatty acid on a ceramide back-bone structure with 4 sugar residues ending with terminal N-acetyl galactosamine. The P antigen is a common erythrocyte and erythroblast antigen, and it is expressed in almost all subjects. People who lack the P antigen are resistant to infection [1]. In this case, the patient had P_1 phenotype, which is the most common phenotype among Caucasians (79%) and Africans (94%). P_2 phenotype is more common among Asian people, such as Cambodians and Vietnamese. [9].

P antigen is also expressed on megakaryocytes, endothelial cells, synovium, villous trophoblast cells of placental tissues, fetal liver, and heart cells. B19 infection may also be responsible for thrombocytopenia, and megakaryocytes may be lysed by restricted expression of viral proteins in the absence of viral propagation [10]. In this case, thrombocytopenia was heparin-induced, confirmed by an increase of the peripheral platelet count when heparin tapering was started (Figure 1). Heparin-induced thrombocytopenia is more often reported after orthopedic, cardiac, or vascular surgery, but it may develop in any patient exposed to unfractionated heparin or low molecular weight heparin [11]. Furthermore, the patient's bone marrow showed increased megakaryocytes, which tended to confirm that thrombocytopenia was heparin induced.

After binding with P antigen, the virus enters the targeted cells, probably because of the VP1 phospholipase activity, and starts to synthesize viral components. It has been demonstrated that B19 is a potent inhibitor of erythroid cell differentiation, and it is cytotoxic for erythroid precursors. It acts by inducing apoptosis through the activation of the caspase pathway or direct lytic effect on erythroid cells. Apoptosis is mediated by NS1 expression, which induces activation of caspase-3, caspase-6, and caspase-8 in a cellular model [12,13].

The virus is also responsible for a cytopathic effect on cells causing a maturative arrest in the erythroid cell line. In smears from bone marrow aspirate, the pathognomonic cell for B19 infection is the giant proerythroblast, which is a large cell, from 25 to 32 µm in diameter, with a high nucleo-cytoplasmic ratio; the nucleus is round and it has a fine and uncondensed chromatin pattern with irregular, indistinct purple-colored inclusions. A giant proerythroblast has a dark blue vacuolated cytoplasm with small broad-based cytoplasmic pseudopodia, named "dog-ear" projections. Sometimes they are grouped in clusters simulating metastatic cells [14]. As shown in Figures 2 and 3, the patient's bone marrow was characterized by the presence of large numbers of these immature erythroid cells. This accounts for anemia with severe reticulocytopenia, sometimes requiring red blood cell transfusions.

In patients with chronic hemolytic disorders, such as sickle cell disease and spherocytosis, B19 may cause transient aplastic crisis characterized by aregenerative acute anemia, sometimes associated with pancytopenia. Persisting B19 infection can occur in a wide variety of conditions, including congenital immunodeficiencies, HIV infection, lymphoproliferative disorders, and transplantation. In these cases, patients may have chronic pure red cell aplasia and more

rarely pancytopenia [15]. In pregnant women, parvovirus B19 may be transmitted to the fetus and may lead to miscarriage or hydrops fetalis [16].

Although the presence of giant proerythroblasts is suggestive of B19 infection, the diagnosis should be made by serological detection of antibodies or molecular detection of viral components. Serological determination of antibodies may be performed by enzyme-linked immunosorbent assays that are able to identify IgM and IgG antibodies. IgM antibodies remain detectable for 2 or 3 months following the infection, as opposed to IgG antibodies which appear 2 weeks after the infection but persist for life. Immunocompromised patients sometimes are not able to produce IgM, and in these cases molecular tests, such as direct hybridization and gene-amplification methods, may be helpful to confirm a clinical suspicion [2].For our patient, tests gave positive results for IgG and IgM at the time of the diagnosis. Some months later, because of a further admission, her test results for IgM anti-B19 were negative, while those for IgG anti-B19 were still positive. At that time, molecular tests were not performed.

In children and immunocompetent adults, B19 infection does not require any treatment. In patients with immunodeficiencies or pure red cell aplasia, treatment with IVIG may be helpful and should be associated with discontinuing immunosuppressive drugs. Generally a 5- or 10-day course of IVIG (0.4 g/kg of body weight) causes a rapid virus elimination associated with reticulocytosis and elevation of Hgb concentration [17].

B19 may be transmitted by respiratory droplets, but secondary infection among households and nosocomial infection have been described [18,19]. B19 transmission by blood products and derivates, such as IVIG [20], solvent-detergent–treated pooled plasma [21], and clotting factor concentrates [5] has been repeatedly demonstrated, even after viral inactivation methods.

B19 is an envelope-free virus and therefore resistant to solvent-detergent treatment. This treatment is effective for clearance of HBV, HCV, and HIV, but it is not effective for HAV and B19, both of which lack the envelope. B19 resistance to heat is controversial. The virus is relatively heat stable [21], but Blümel et al [22] showed that pasteurization for 10 hours at 60°C rapidly inactivates B19. Although human B19 DNA content does not reflect infectivity, we cannot exclude the possibility that blood derivates, such as albumin, clot factors, and immune globulin may be infectious. In our patient, we could not confirm whether an albumin-derived infection combined with a concomitant immunocompromised condition due to myasthenia and immunosuppressive treatment was responsible for the disease. Blood component B19 infection is still an unresolved problem. Many strategies such as new methods for vital inactivation and discarding positive-B19 units [23-25] may help to increase blood product safety.

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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
			2008. 9. 18	該当なし	
一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Kantele A, Marti H, Felger I, Müller D, Jokiranta TS. Emerg Infect Dis. 2008 Sep;14(9):1434-6.	公表国 フィンランド	
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

○マレーシアから帰国したヨーロッパ人旅行者におけるサルマラリア
2007年にマレー半島でフィンランドの旅行者が*Plasmodium knowlesi*に感染した。
患者は53歳男性で、マレー半島を4週間旅行してフィンランドに帰国した3日後に高熱を発症し、翌日受診した。患者ははじめの2週間クアラルンプールに滞在し、周辺地域を数日間旅行した。その後自動車で北西の海岸部に向かい5日間イポー近くのジャングルで過ごした。この間蚊帳のない家に泊まり防虫剤は使用していなかったが、蚊に刺されたという報告はなかった。最後の週はランカウイ・ビーチの高級ホテルに滞在していた。
血液塗抹検査でマラリア原虫が陽性となり、入院後塩酸キニーネとドキシサイクリンを合計10日間投与された。回復後12ヶ月間のフォローアップ期間中に再発は見られなかった。PCR産生物のヌクレオチド配列解析を行ったところGenBankに登録されていた*P. knowlesi*と一致した。
*P. knowlesi*は通常サルにマラリアを引き起こす寄生虫であるが、ヒトマラリアを引き起こす可能性がある第5のマラリア原虫(*Plasmodium* species)とされている。当該疾患はヒトの生命を脅かす恐れがあり、臨床医や臨床検査技師は、旅行者の当該病原体についての認識を高めるべきである。

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
2007年にマレー半島でフィンランドの旅行者が、通常サルにマラリアを引き起こす*Plasmodium knowlesi*に感染し、帰国後に発症したとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の献血を一定期間延期している(1～3年の延期を行うとともに、帰国(入国)後マラリアを思わせる症状があった場合は、感染が否定されるまでの間についても献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

(2)

DISPATCHES

Monkey Malaria in a European Traveler Returning from Malaysia

Anu Kantele, Hanspeter Marti, Ingrid Felger, Dania Müller, and T. Sakari Jokiranta

In 2007, a Finnish traveler was infected in Peninsular Malaysia with *Plasmodium knowlesi*, a parasite that usually causes malaria in monkeys. *P. knowlesi* has established itself as the fifth *Plasmodium* species that can cause human malaria. The disease is potentially life-threatening in humans; clinicians and laboratory personnel should become more aware of this pathogen in travelers.

Traditionally, only 4 *Plasmodium* species have been known to cause malaria in humans: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*, although >26 *Plasmodium* species are known to circulate among primate populations (*1*). Some of these species have been implicated in symptomatic human malaria after experimental or accidental infection (*2*). Only a few reports of naturally acquired monkey malaria in humans are currently available (*1*,*3*–*9*). The lack of data may be because light microscopy has been used as the sole diagnostic method and an atypical *Plasmodium* species may have been misidentified as one of the 4 traditional *Plasmodium* species causing human malaria.

P. knowlesi was first described in 1931 in a long-tailed macaque imported from Singapore to India; in 1932, *P. knowlesi* was experimentally shown to be infectious to humans (*10*). The first natural infection of *P. knowlesi* in humans was reported in 1965 in a man returning to the United States after a visit to Peninsular Malaysia (*11*). Subsequently, in 1971, there was a report of a presumed natural infection in a citizen of Malaysia (*6*). Despite extensive studies in Malaysia in the 1960s (*2*), no other reports were published on naturally acquired *P. knowlesi* infections in humans until 2004, when Singh et al. studied PCR-negative *P. malariae* cases in the Kapit division in Sarawak, Malaysia (*3*). A different PCR analysis showed that *P. knowlesi* caused 58% of the 208 malaria cases studied. Further cases reported from China (*4*), Thailand (*5*), Philippines (*8*), and Singapore (*12*) show that *P. knowlesi* infections in humans are not found exclusively in Malaysia. Recently, Cox-Singh et al. reported that *P. knowlesi* is widely distributed among inhabitants of Malaysia (*7*).

The Study

A 53-year-old Finnish man was admitted to a local hospital in Finland in March 2007 with fever after 4 weeks of travel in Peninsular Malaysia. He had not taken any antimalarial prophylaxis. In Malaysia, he spent 2 weeks in Kuala Lumpur and made a few day trips to surrounding rural areas. Thereafter, he traveled by car to the northwestern coast and stayed for 5 days in the jungle ≈80 km south of Ipoh. While in this area, he slept in a house without mosquito screens or nets and did not use any repellents; he did not report any mosquito bites. The last week of his travel was spent in the Langkawi Beach area where he stayed at a high-quality hotel. During his trip he occasionally had some minor abdominal problems, but these symptoms subsided spontaneously after his return to Finland. High fever (38.8°C axillary temperature) occurred 3 days after his return to Finland but abated quickly. On the fourth day, the fever returned and he sought medical care at a local hospital. Laboratory tests showed the following results: C-reactive protein 2.0 mg/dL (normal range <1.0 mg/dL), hemoglobin 15.2 g/dL (normal range 13.4–16.7 g/dL), leukocyte count 2.6 × 10⁹/L (normal range 3.4–8.2 × 10⁹/L), and thrombocytes 143 × 10⁹/L (normal range 150–360 × 10⁹/L). Blood smear was positive for *Plasmodium* organisms, and the causative agent was identified as *P. falciparum* with levels of parasitemia <1.0%. The patient was admitted to the hospital and given intravenous (IV) quinine dihydrochloride and oral doxycycline.

On day 2 of the patient's hospital stay, fever returned and he was transferred to the Helsinki University Central Hospital (Department of Infectious Diseases at Aurora Hospital). Blood smears obtained there showed *Plasmodium* parasites that were considered atypical, and the laboratory reported suspicion of a co-infection (*P. falciparum* and *P. malariae*) (Figure). The IV quinine dihydrochloride was replaced with oral quinine hydrochloride, and doxycycline was continued. During treatment, the patient experienced an attack of hypoglycemia (electrocardiogram and blood pressure was normal during this attack), transient mild visual and hearing loss, and transient lymphopenia (a low of 0.46 × 10⁹/L). He received quinine hydrochloride and doxycycline for a total of 10 days.

Because identification of the *Plasmodium* species was difficult, a blood sample was drawn for PCR analysis on day 2 of hospitalization. First, a nested PCR was performed according to a standard protocol with rOval and rPLU2 primers (template DNA purified in Basel from 200 μL of erythrocytes by QIAamp DNA Mini Blood Kit (QIAGEN,

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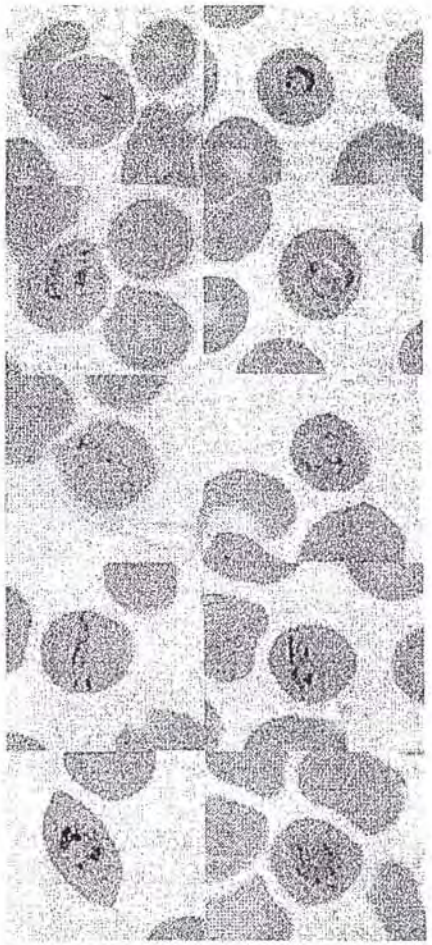

Figure. Microscopic findings in the thin blood smears of a patient with *Plasmodium knowlesi* malaria. Early ring forms are shown in the first row, later trophozoites in the second and third rows, trophozoites resembling band forms in the fourth row, and putative early gametocytes or schizonts in the fifth row. Size of the infected erythrocytes is normal. Antimalarial medications, given 8 hours before the blood shown in the smear was drawn, could have affected morphology. (Original magnification ×1,000.)

Helsinki, Finland) (*13*,*14*), but the reaction did not yield any amplification product. Nested PCR was repeated with an alternative primer pair (rPLU6 and rPLU2) (*14*) derived from a conserved region of the 18S rRNA marker gene, and an amplicon was obtained. Failure of PCR amplification has been reported for some *P. ovale* isolates (*15*); therefore, a *P. ovale* infection was suspected, and the patient was given primaquine phosphate for 14 days as an outpatient to eradicate possible liver hypnozoites. The PCR product was subjected to direct nucleotide sequencing (GenBank accession no. FJ009511) and found to be identical to 2 *P. knowlesi* sequences previously submitted to GenBank, 1 human isolate from Malaysian Borneo (AY327556) and a *Macaca mulatta* isolate from Columbia (U72542). Six other published *P. knowlesi* sequences differ from our sequence only by 1 nucleotide (99% identity). In contrast, a number of differences were seen between our sequence and the *P. ovale* sequences (*15*). The sequence from our case showed only 50% identity to the ovale primer; therefore, we concluded that our patient was infected with *P. knowlesi*. During the 12-month follow-up period, the patient showed no signs of relapse.

Conclusions

We suggest that *P. knowlesi* infection should be considered in malaria patients who have a history of a travel to forested areas in Southeast Asia, especially if *P. malariae* malaria is diagnosed or atypical plasmodia are seen with microscopy. The asexual stages of various species of *P. knowlesi* can easily be misidentified as *P. malariae* in light microscopic examination (Figure) (*3*,*7*,*10*). Because most laboratories diagnose malaria by light microscope examination only, numerous cases of *P. knowlesi* malaria may have been misdiagnosed as ordinary *P. malariae* malaria; monkey malaria may be more widespread among humans than was previously thought. As the disease is potentially dangerous, a proper identification of the malaria species is crucial. If PCR assays for malaria detection are used, PCR primers specific for *P. knowlesi* (*3*) should be included to provide valuable diagnostic information.

P. knowlesi has established itself as the fifth species of *Plasmodium* that causes human malaria (*3*,*7*,*12*). Because the disease is potentially life-threatening in humans, laboratory clinicians and physicians (especially those taking care of travelers) should become more aware of this disease; it is easily misdiagnosed as a less severe form of malaria.

Acknowledgments

We thank the patient for allowing us to publish his case, Heli Siikamäki for helpful discussions, and personnel of the Unit of Parasitology, Helsinki University Central Hospital Laboratory, for recognizing the atypical nature of *Plasmodium* parasites in the patient's thin blood smears.

The research of T.S.J. is financially supported by the Academy of Finland (projects 201506 and 202529), the Helsinki University Central Hospital Funds, and the Sigrid Jusélius Foundation; the work of A.K. is supported by the Finnish Medical Foundation and the special Finnish governmental subsidy for health sciences research.

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別紙様式第2-1

No. 22

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 9. 18	該当なし	
一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	野崎一朗, 浜口毅, 篠原もえ子, 中村好一, 北本哲之, 佐藤猛, 水澤英洋, 森若文雄, 志賀裕正, 三條伸夫, 黒岩義之, 西澤正豊, 武田雅俊, 葛原茂樹, 黒田重利, 村井弘之, 村山繁雄, 立石潤, 山田正仁. 2008年プリオン研究会; 2008 Aug 29-30; 新得町.	公表国 日本	
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

○わが国におけるヒトのプリオン病の発症状況:最近9年間のサーベイランスデータ

【背景・目的】わが国のプリオン病の病型は多彩であり、その発症動向を把握することは重要な課題と考えられる。

【方法】現行のサーベイランスシステムが開始された1999年4月から2008年2月までの9年間に、プリオン病の疑いとして情報収集された1339例を検討した結果、プリオン病と判定された症例について、その内訳、発症状況などを検討した。

【結果】1069例がプリオン病と判定された。プリオン病の発症数は、年間120例前後で推移していた。病型別では孤発性CJDが821例(76.8%)、遺伝性プリオン病が171例(16.0%)、硬膜移植後CJD74例(6.9%)、変異型CJDI例(0.1%)、分類不能2例(0.2%)であった。プリオン病の剖検率については、全体で19.1%と欧米諸国の平均よりも著明に低く、最も多く検索されていた硬膜移植後CJDにおいても37%と低かった。病型が判明している孤発性CJD32例では、MM1が最も多く、次にMM2が皮質型、視床型ほぼ同数で欧米と比較すると多い結果となった。MV1、VV1は1例も確認されなかった。遺伝性プリオン病の変異別頻度はV180I、P102L、E200K、M232R他の順で、欧米諸国のデータとは異なっていた。硬膜移植後CJDの発生は2002年以降減少傾向にあり、現在までに132例が確認された。変異型CJDに関しては、2001年に発症した1例のみであった。

【結論】わが国のプリオン病剖検率は欧米諸国に比較し著明に低率であった。孤発性CJDについては、わが国では欧米に比較してMM2型が多かった。硬膜移植後CJDの発生は2002年以降は減少傾向であった。遺伝性プリオン病の変異別頻度は欧米諸国の割合と著しく異なっていた。

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
CJDサーベイランス委員会による調査では過去9年間に日本国内で1069例がプリオン病と判定された。また、我が国では剖検率が欧米諸国より著明に低く、病型は欧米諸国と大きく異なっているとの報告である。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980~96年に1日以上の英国滞在歴のある方からの献血を制限している。加えて、CJDの感染防止の目的から、プリオン病家族歴、硬膜移植歴について問診を行い、該当するドナーを無期限に献血延期としている。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

(13)

Poster-33

JRC2008T-060

わが国におけるヒトのプリオン病の発症状況:最近9年間のサーベイランスデータ

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【背景・目的】わが国では、通常の孤発性 Creutzfeldt-Jakob 病 (CJD)、硬膜移植後 CJD に加え、ウシ海綿状脳症からの感染が疑われる変異型 CJD も確認されている。プリオン病の病型は多彩であり、その発症動向を把握することは重要な課題と考えられる。

【方法】「プリオン病及び遅発性ウイルス感染症に関する調査研究班」・CJD サーベイランス委員会による現行のサーベイランスシステムは 1999 年 4 月より開始され、2008 年 2 月までの 9 年間にプリオン病の疑いとして情報収集された 1339 例が検討された。CJD サーベイランス委員会での検討の結果、プリオン病と判定された症例について、その内訳、発症状況などを検討した。

【結果】1069 例がプリオン病と判定された。プリオン病の発症数については、2007 年はまだ情報収集不足で少ないが、それ以外は年間 120 例前後で推移していた。病型別では孤発性 CJD が 821 例 (76.8%)、遺伝性プリオン病が 171 例(16.0%)、硬膜移植後 CJD 74 例 (6.9%)、変異型 CJD 1 例 (0.1%)、分類不能 2 例(0.2%)であった。プリオン病の剖検率については、全体で 19.1%と欧米諸国の平均よりも著明に低かった。分類別では、最も多く検索されていたのは硬膜移植後 CJD であったが、それでも 37%と低い割合にとどまっていた。孤発性 CJD におけるプリオン蛋白遺伝子コドン 129 多型とプロテアーゼ抵抗性プリオン蛋白ウェスタンブロット解析パターンの組み合わせによる病型が判明しているものは 32 例であった。最も多いのは MM1 であったが、次に MM2 が皮質型、視床型ほぼ同数あり、欧米のデータと比較すると多い結果となった。MV1、VV1 は 1 例も確認されなかった。遺伝性プリオン病の変異別頻度は V180I、P102L、E200K、M232R 他の順であった。欧米諸国のデータと比較すると、日本で 4 割を占める V180I は欧米諸国ではまれで、4 番目に多い M232R については欧米では 1 例も認められなかった。一方欧米で 2 番目に多い V210I はわが国では確認されなかった。硬膜移植後 CJD の発生は 2002 年以降減少傾向にあり、現在までに 132 例が確認された。変異型 CJD に関しては、2001 年に発症した 1 例のみであった。

【結論】わが国のプリオン病剖検率は欧米諸国に比較し著明に低率であった。孤発性 CJD については、わが国では欧米に比較して MM2 型が多かったが、剖検率自体が低く非典型例が多く剖検されている可能性が考えられた。硬膜移植後 CJD が多発しているが、2002 年以降はその発生は減少傾向であった。遺伝性プリオン病の変異別頻度は V180I、P102L、E200K、M232R 他の順で、これは欧米諸国の割合と著しく異なっていた。

別紙様式第2-1

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 9. 18	該当なし	
一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	前野英毅, 村井活史, 武田芳於, 室塚剛志, 脇坂明美, 沼田芳彰, 堀内基広. 2008年プリオン研究会; 2008 Aug 29-30; 新得町.	公表国 日本	
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

○ウイルス除去膜濾過による異常型プリオン蛋白質(PrP^{Sc})の除去

【目的と意義】血漿分画製剤の濾過工程におけるPrP^{Sc}除去効果をワーストケースとして評価するため、最も感染性があると報告されている17-27nmの小さなPrP^{Sc}を使用し、日本赤十字社血漿分画センターで製造しているウイルス除去膜濾過工程を含んでいる2つの製剤(血液凝固第VIII因子製剤[FVIII]:プラノバ20N(平均孔径19nm)濾過、抗HBs人免疫グロブリン製剤[HBIG]:プラノバ35N(平均孔径35nm)濾過)についてその除去効果を検証した。

【材料と方法】263K株に感染したハムスターの10%脳乳剤よりスパイクマテリアルを作成し、プラノバ20N(平均孔径19nm)で濾過してスパイクマテリアル中の19nmより小さいPrP^{Sc}の量を確認した。製剤の濾過前液に相当する溶液にスパイクマテリアルを添加し、30分撹拌後、製造と同じ条件にてプラノバ20N及びプラノバ35Nで濾過した。濾過前後の液をProtein Misfolding Cyclic Amplification(PMCA)でPrP^{Sc}を増幅後、プロテアーゼK抵抗性プリオン蛋白質をウェスタンブロットで検出した。各検体を3回測定し、50%の確率で検出できる希釈倍率からPrP^{Sc}濃度を算出して対数減少率(LRV)を計算した。

【結果・考察】濾過によるPrP^{Sc}の対数減少率(LRV)は、FVIIIで≧5.3、HBIGで1.5であった。濾過膜の孔径より小さな材料をスパイクマテリアルとしているにもかかわらず、PrP^{Sc}がプラノバ35Nやプラノバ20Nで除去されたのは、PrP^{Sc}が凝集や膜へ吸着したためと考えられる。

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
日本赤十字社が血漿分画製剤製造に用いているウイルス除去膜濾過により、263K株に感染したハムスターより得たスパイクマテリアル中のPrP^{Sc}が除去されたとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努めるとともに、血漿分画製剤の製造工程における病原因子の除去・不活化技術の向上に努める。

(14)

Poster-11

演題名 ウイルス除去膜濾過による異常型プリオン蛋白質(PrP^{Sc})の除去

演者名 ○前野英毅[1]、村井活史[1]、武田芳於[1]、室塚剛志[1]、脇坂明美[1]、
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【目的と意義】血漿分画製剤の vCJD に対する安全性を評価するために、プリオン病感染動物の脳乳剤を工程液に添加して、PrP^{Sc}の除去効果を検証することが一般的に行われている。しかし、血漿中のPrP^{Sc}が脳内のPrP^{Sc}と同様に凝集しているのかは不明であり、血漿中のPrP^{Sc}が小さなものであった場合には、濾過工程におけるPrP^{Sc}除去効果を過大に評価してしまう可能性がある。Silveira らはスクレイピー263K 株に感染したハムスターの脳乳剤を Sodium Undecyl Sulfate(SUS)で処理し、最も感染性があるPrP^{Sc}は 17-27nm であると報告したが、この様な小さなPrP^{Sc}を用いれば濾過工程のPrP^{Sc}除去効果をワーストケースとして評価できると考えた。そこで、日本赤十字社血漿分画センターで製造しているウイルス除去膜濾過工程を含んでいる2つの製剤(血液凝固第 VIII 因子製剤[FVIII]:プラノバ 20N 濾過、抗 HBs 人免疫グロブリン製剤[HBIG]:プラノバ 35N 濾過)について、SUS で処理したPrP^{Sc}を用いてその除去効果を検証した。

【材料と方法】263K 株に感染したハムスターの 10%脳乳剤に Sarkosyl を 1%となるように添加し、100,000×g、30 分の超遠心により沈殿画分を得た。沈殿画分を PBS で溶解後、1%となるよう SUS を加え、37℃で 1 時間放置した。これをプラノバ 20N(平均孔径 19nm)で濾過し、スパイクマテリアルとした。また、プラノバ 20N(平均孔径 19nm)で濾過してスパイクマテリアル中に含まれる 19nm より小さいPrP^{Sc}量を確認した。スパイクマテリアル 1mL を FVIII 濾過前液に相当する溶液 20mL に添加し、30 分撹拌後、製造と同じ条件にてプラノバ 20N で濾過した。また、HBIG については、濾過前液 20mL に 0.2mL のスパイクマテリアルを添加し、30 分撹拌後、プラノバ 35N で濾過した。濾過前後の液を 10%正常ハムスターの脳乳剤で段階希釈し、Protein Misfolding Cyclic Amplification(PMCA)でPrP^{Sc}を増幅した。増幅後、プロテアーゼK抵抗性プリオン蛋白質をウェスタンブロットで検出した。各検体を 3 回測定し、50%の確率で検出できる希釈倍率からPrP^{Sc}濃度(このPrP^{Sc}濃度を$PMCA_{50}$/mL と定義)を算出した。

【結果・考察】スパイクマテリアルの濃度は$\geq 10^{11.3}$ $PMCA_{50}$/mL であり、この内 19nm 以下のPrP^{Sc}は$10^{9.9}$ $PMCA_{50}$/mL であった。スパイクマテリアルを FVIII に添加した濾過前液のPrP^{Sc}量は$10^{10.6}$ $PMCA_{50}$、プラノバ 20N 濾過後液では検出限界($\leq 10^{5.3}$ $PMCA_{50}$)以下となり、対数減少率(LRV)は≧5.3 であった。一方、HBIG では濾過前液のPrP^{Sc}量は$10^{10.4}$ $PMCA_{50}$、プラノバ 35N 濾過後液は$10^{8.9}$ $PMCA_{50}$であり、LRV は 1.5 であった。濾過膜の孔径より小さな材料をスパイクマテリアルとしているにもかかわらず、PrP^{Sc}がプラノバ 35N やプラノバ 20N で除去されたのは、PrP^{Sc}が凝集や膜へ吸着したためと考えられるが、現在、その除去の機構を明らかにしているところである。

別紙様式第2-1

No. 27

医薬品 研究報告 調査報告書

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一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	津久井和夫, 湯川眞嘉, 小野寺節. 2008年プリオン研究会; 2008 Aug 29-30; 新得町.	公表国 日本	
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

○スクレイピー実験感染による血中PrP^{res}経時的変化の追跡

背景:昨年本シンポジウムにおいて酸性SDS沈降法(仮称)により血漿中PrP^{res}と思われる蛋白の検出を報告した。この蛋白は、PK抵抗性で且つ血漿中で糖鎖を介して凝集していると思われた。

方法:263K感染ハムスター脳乳剤を脳内接種した8週齢ゴールデンハムスター5匹(感染群)と同週齢の5匹のハムスター(非感染群)から、2週に一度の割合で経時的に採血し、血漿を分離した。血漿検体はPK処理後、酸性SDS沈降法により部分精製・濃縮し、一次抗体を3F4として、イムノブロットによる反応性蛋白を化学発光で検出した。

結果:PK抵抗性3F4反応性蛋白バンドは、感染後4週から6週で認められ、10週ではほぼ消失した。PrP^{res}に特有と思われる25KDaバンドはピーク時のみで認められ、後に低分子量フラグメントに移行する様相を見せた。また、発症末期では、PrP^{res}と見られる血漿中蛋白バンドは認められなかった。

考察:血中PrP^{res}と思われる分子は、感染後定常的に蓄積するのではなく、発現と同時に暫時分解されて行くと思われた。これは他で報告されたPrP^{res}の脾臓による動態と近似しており、血中PrP^{res}が脳病変に由来するのではなく末梢組織(脾臓等)病変に由来していることを示唆している。この結果から、PrP^{res}をマーカーとした血液検査は、感染後発症前~発症中期までに限定されるという可能性が示唆された。

使用上の注意記載状況・その他参考事項等
解凍赤血球濃厚液「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
ハムスターを使用した感染実験において、血中PrP^{res}を対象とした血液検査は、感染後発症前~発症中期までに限定されるという可能性が示唆されたとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努めるとともに、検査法の確立に向けた基礎研究を継続していく。

(15)

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Poster-18

スクレイピー実験感染による血中 PrPres 経時的変化の追跡

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目的:

スクレイピー263K 株実験感染による血中 PrPres の感染後発現動態の解析

背景:

vCJD の血液による二次感染が起こることがほぼ確定した現在、感染者の発病前診断をすることにより、血液を介した感染拡大を阻止することが必要である。このため、発病前キャリアー状態の感染者を検出するために、血液検査システムの確立がプリオン研究の緊急課題として強く求められている。我々は、昨年本シンポジウムにおいて酸性 SDS 沈降法 (仮称) により血漿中 PrPres と思われる蛋白の検出を報告した。この蛋白は、PK 抵抗性で且つ血漿中で糖鎖を介して凝集していると思われた。

方法:

1、8 週齢ゴールデンハムスター5 匹に 263K 感染ハムスター脳乳剤を脳内接種により投与し感染群とした。同週齢のハムスター5 匹を非感染群として対照とした。感染群・非感染対照群各ハムスターは、眼窩静脈叢穿刺により 2 週に一度の割合で経時的に採血し、血漿を分離した。

2、血漿検体を直ちに 37℃で 1 時間の PK 処理をし、次いでベファブロックで PK 反応を止めた後、SDS を終濃度 3%及び DTT を終濃度 50mM 加え 100℃10 分の加熱処理により不活化して−80℃に保存した。保存した血漿検体は、室温で溶解し、酸性 SDS 沈降法 (昨年本シンポジウムで報告) により部分精製・濃縮し、一次抗体を 3F4 として、イムノブロットによる反応性蛋白を化学発光で検出した。

結果:

1、PK 抵抗性 3F4 反応性蛋白バンドは、感染後 4 週から 6 週で認められ、10 週ではほぼ消失した。

2、検出された蛋白バンドは、PrPres に特有と思われる 25KDa バンドはピーク時のみで認められ、後に低分子量フラグメントに移行する様相を見せた。

3、発症末期では、PrPres と見られる血漿中蛋白バンドは認められなかった。

考察:

血中 PrPres と思われる分子は、感染後定常的に蓄積するのではなく、発現と同時に暫時分解されて行くと思われた。このため、血漿中 PrPres の検出は一時的な検出陽性期間 (4 週~8 週?) で可能であり、末期では検出困難となると推定された。これは、井上らの報告 (Jpn.J.Infect.Dis., 58,78-82, 2005) による PrPres の脾臓による動態と近似しており、血中 PrPres が脳病変に由来するのではなく末梢組織 (脾臓等) 病変に由来していることを示唆している。この結果から、PrPres をマーカーとした血液検査は、感染後発症前~発症中期までに限定されるという可能性が示唆された。

謝辞:

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医薬品 研究報告 調査報告書

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一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Houston F, McCutcheon S, Goldmann W, Chong A, Foster J, Siso S, Gonzalez L, Jeffrey M, Hunter N. Blood. 2008 Jul 22.	公表国
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研究報告の概要

○プリオン病はヒツジにおいて輸血により効率的に伝播する

ウシ海綿状脳症(BSE)のエピデミックに続く変異型クロイツフェルトヤコブ病(vCJD)の出現により、当該疾患の輸血による医原性伝播リスクの可能性が懸念され、血液供給を保護するために費用のかかる制御措置がとられることとなった。以前我々は、BSEおよび自然発生スクレイピーが輸血により伝播することをヒツジにおいて示した予備データを報告した。本稿で報告する当該実験の最終結果は、予想以上に高い輸血伝播率(BSE36%、スクレイピー43%)を示している。輸血によりBSE感染した受血ヒツジの一部(3/8)は、疾患の臨床症状を示すことなく、最高7年間生存した。大多数の伝播は、推定潜伏期の50%を超えたヒツジから採取された血液から生じた。この伝播率の高さ、および臨床症状を示す受血ヒツジの潜伏期が比較的短く一定であることから、血中の感染価が高いこと、および(または)輸血により効率的に伝播することが示される。当該実験により、血液製剤によるヒトでのvCJD伝播の調査に関して、ヒツジの使用が有用なモデルであることが示された。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「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
ヒツジを用いた感染実験において、BSEは36%、スクレイピーは43%と予想以上に高い輸血伝播率を示し、TSEが輸血により効率的に伝播すること、血液製剤によるヒトでのvCJD伝播の調査に関して、ヒツジが有用なモデルであることが示されたとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

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Prion diseases are efficiently transmitted by blood transfusion in sheep

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Prion diseases are efficiently transmitted by blood transfusion in sheep.

Running title: Transmission of sheep TSEs by blood transfusion

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Abstract

The emergence of variant Creutzfeld-Jakob disease (vCJD), following on from the bovine spongiform encephalopathy (BSE) epidemic, led to concerns about the potential risk of iatrogenic transmission of disease by blood transfusion and the introduction of costly control measures to protect blood supplies. We previously reported preliminary data demonstrating the transmission of BSE and natural scrapie by blood transfusion in sheep. The final results of this experiment, reported here, give unexpectedly high transmission rates by transfusion of 36% for BSE and 43% for scrapie. A proportion of BSE-infected tranfusion recipients (3/8) survived for up to 7 years without showing clinical signs of disease. The majority of transmissions resulted from blood collected from donors at >50% of the estimated incubation period. The high transmission rates and relatively short and consistent incubation periods in clinically positive recipients suggest that infectivity titres in blood were substantial and/or that blood transfusion is an efficient method of transmission. This experiment has established the value of using sheep as a model for studying transmission of vCJD by blood products in humans.

Introduction

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases, which include Creutzfeld-Jakob disease (CJD) in man, scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle. A new variant of CJD (termed vCJD) was recognised in the United Kingdom in the mid-1990s, apparently as a result of transmission of BSE to humans[1]. To date, there have been 166 cases of vCJD recorded in the UK, as well as several cases in other countries. Human TSEs are characterised by long asymptomatic incubation periods (usually several years), and there is no reliable test for detecting infection before the onset of clinical disease. It is not known how many people in the UK harbour vCJD, although estimates based on screening of tonsil and appendix samples suggest there could be up to 4000[2]. These infected individuals pose a risk of human-to-human transmission *via* blood transfusion or contaminated surgical instruments.

In patients with vCJD there is widespread replication of the infectious agent and deposition of PrP^{Sc} (disease-associated form of prion protein) in lymphoreticular tissues such as the tonsil, spleen and lymph nodes, in contrast to sCJD, where lymphoreticular involvement is minimal[3]. The fact that lymphocytes continually recirculate between blood and lymphoreticular tissues strongly suggests that the blood of vCJD patients is likely to be infectious. Data from rodent TSE models had shown that the highest levels of infectivity in blood were associated with leukocytes and, to a lesser extent, plasma[4]. As a result, costly control measures such as leucodepletion (filtration of blood and blood products to remove leukocytes) and importation of plasma were introduced to protect UK blood supplies, despite the limited data that were then available to judge the size of the risk and the efficacy of the control measures.

The potential for using sheep as a model for studying the risks of vCJD transmission by blood transfusion was highlighted by the similarity between the distribution of infectivity and PrP^{Sc} in sheep infected with TSEs and humans infected with vCJD[5-7]. One factor limiting the successful transmission of TSEs by blood in rodent models

was the small volumes of blood that could be injected. In contrast, the relative similarity in size of sheep and humans means that volumes of blood comparable to those used in human transfusion practice can be collected from and transfused into sheep. Using this model, we previously reported preliminary results showing that both BSE and natural scrapie could be transmitted between sheep by blood transfusion[8,9]. Although scrapie is not thought to be transmissible to humans, it was included as a representative of infection acquired under field conditions, which may give different results to those obtained from experimentally infected animals. Our blood transfusion experiment in sheep is complete after nine years, and this paper presents the full data from the study. The overall transmission rates for both scrapie and BSE are surprisingly high when factors such as the stage of infection and genetic background are taken into account, suggesting that blood transfusion represents an efficient route of transmission.

Materials and Methods

Donor and recipient sheep

The animal work was reviewed and approved by internal Ethical Review procedures at the Institute for Animal Health, UK, and carried out under the authority of Home Office Project Licences.

PrP genotypes of all sheep were confirmed by sequencing the coding region of the PrP gene[10], and are represented by single letter amino acid code for codons 136, 154 and 171, which have been linked to scrapie susceptibility (e.g. ARQ represents alanine, arginine and glutamine respectively at codons 136, 154 and 171).

All donor sheep were from the Edinburgh NPU Cheviot flock, which has endemic natural scrapie. The recipient sheep (including scrapie negative control donors) were Cheviots derived from the DEFRA scrapie-free (DEFRA/SF) flock of New Zealand origin. Transfusion recipients, positive and negative controls were housed in a purpose-built isolation unit on a different site to the donors, with strict procedures in place to minimise the risk of cross-contamination between groups, as described[9]. The sheep were scored at weekly intervals for clinical signs of TSEs, and killed when they reached humane end points agreed with the Home Office. For experimentally inoculated animals (BSE donors, positive controls and transfusion recipients), the incubation period (IP) in clinically positive sheep was defined as the period between the date of inoculation and the date of death. For scrapie-exposed donors, the IP in clinically positive sheep was defined as the age at death (i.e. they were assumed to have become infected immediately after birth).

Blood collection and transfusion

Procedures for blood collection/transfusion were as previously described[9]. Briefly, venous blood (450-500ml = 1 unit) was collected into sterile collection bags (NBPI-Fresenius, Emmer-Compascuum, NL) containing citrate phosphate dextrose adenine solution as anticoagulant. From donors that were about to be euthanased, 2 units were collected just before post-mortem, while from donors that were to be left alive, separate collections of 1 unit were made at least 28 days apart. However, for practical reasons it was not always possible to collect 2 units of blood from every donor sheep.

From www.bloodjournal.org at Central Blood Institute c/o Japanese Red Cross Society on October 16, 2008. For personal use only.

In most cases where 2 units of blood were obtained, one was transfused as whole blood (without leucodepletion) and the other was used to prepare a buffy coat fraction.

BSE blood transfusions

Fifteen sheep experimentally inoculated either orally (14) or intracerebrally (1) with 5g or 0.05g respectively of BSE-infected cattle brain homogenate were used as blood donors. The donor PrP genotypes were ARQ/ARQ (n = 3), ARQ/AHQ (n = 5) or AHQ/AHQ (n = 7), which are resistant to natural scrapie in the NPU flock, but produce the shortest IPs after inoculation with BSE. Two sheep previously reported as donors[9] were excluded from the study (along with their recipients) when re-genotyping showed them to be ARQ/ARR and VRQ/AHQ respectively, genotypes which result in relative resistance to oral infection with BSE.

Eleven donor sheep provided blood for transfusion at the preclinical stage of infection. Eight of these were culled at the time of donation as part of a separate time course pathogenesis experiment. The remaining three pre-clinical donors went on to develop clinical signs of BSE, with respective IPs of 629, 761 and 2131 days post infection. Four sheep were used as blood donors once they had developed clinical signs of BSE at 561-671 days post infection. PrP^{Sc} deposits in brain and/or in peripheral tissues were confirmed in all clinically affected donors by immunohistochemistry (IHC). In two donors culled at the pre-clinical stage, sparse PrP^{Sc} deposits were found in only one tissue in each sheep: Peyer's patch (58x81) and dorsal root ganglion (60x49). However, a negative result was obtained when the same tissues were immunostained in another laboratory. There were 15 ARQ/ARQ recipients of whole blood and 7 ARQ/ARQ recipients of buffy coat from BSE-infected donors. Figure 1 gives a summary of the experimental design, while details of the donor and recipient sheep are in Table 1.

Scrapie blood transfusions

The donors for this experiment were ten VRQ/VRQ and one VRQ/ARQ Cheviot sheep from the Edinburgh NPU flock, where sheep of these genotypes show a disease incidence approaching 100%. Epidemiological and pathological evidence suggests that infection occurs around the time of birth. Blood collections were made from animals in 3 different age groups (200-250 days, 450-500 days, 700-850 days) to represent donors at different pre-clinical stages of disease, as well as from one clinical case. Seven donors were culled after developing clinical signs of scrapie at ages ranging from 1081 to 1556 days, and were confirmed positive by histopathology and IHC. Two donors were culled before the onset of clinical signs at 1197 and 1350 days of age respectively, but PrP^{Sc} was detected in their tissues by IHC. Two donors died prematurely at 349 and 974 days of age: one was IHC negative, in the other, the tissues were too decomposed to allow analysis. There were 21 recipients (all VRQ/VRQ PrP genotype) of blood from scrapie-exposed donors; eleven were transfused with buffy coat and ten with whole blood. See Figure 1 for a summary of the experimental design, and Table 2 for details of donor and recipient sheep.

Positive and negative controls

Seven ARQ/AHQ and three ARQ/ARQ sheep were infected intravenously with 0.2g of the same BSE-infected cattle brain homogenate as given orally to the blood donors, and served as positive controls. No positive controls were used in the scrapie transfusion experiment. As negative controls for the BSE transfusion experiment, 12 ARQ/ARQ recipients were given transfusions of whole blood (6) or buffy coat (6) from 7 uninfected donors (6 ARQ/AHQ, 1 ARQ/ARR). Two recipients died at 633 days and 1181 days post transfusion respectively, and the remaining 10 recipients were culled between 2462 and 2586 days post transfusion. As negative controls for the scrapie experiment, 16 VRQ/VRQ sheep received either whole blood (8) or buffy coat (8) collected from 8 uninfected VRQ/VRQ donors. There were two intercurrent deaths at 397 days and 464 days post transfusion, and the other 14 animals were culled between 2052 and 2409 days post transfusion. None of the negative controls for the BSE or scrapie experiments showed clinical signs of TSEs and all were IHC negative for PrP^{Sc}.

PrP^{Sc} detection by immunohistochemistry (IHC)

Tissue samples from the brain, spleen, mesenteric lymph node and palatine tonsil of the sheep under study were fixed in formaldehyde and processed according to standard procedures. Sections were immunolabelled for PrP^{Sc} detection by IHC with primary antibody R145, which recognizes the 222-226 amino acid sequence of ovine PrP[11], as described previously[12,13].

Results

1) BSE transfusion experiment

A total of five transfusion recipients showed clinical signs of TSEs, and were confirmed positive by IHC and/or Western blot (see Table 1 & Figure 2). These included two (F19 and D505) out of twelve sheep transfused with whole blood from donors in the pre-clinical phase of infection (at 45% and 50% of estimated IP, respectively), as reported previously[8,9]. Two out of three recipients of whole blood and one out of two recipients of buffy coat from donors clinically affected by BSE developed clinical BSE. The IPs in the five clinically positive recipient sheep ranged from 531 to 610 days post transfusion (mean ± SD = 565 ± 35 days), and there was no obvious difference in the IPs of those that received blood from pre-clinical or clinical donors.

One recipient (D452) of whole blood from a pre-clinical donor died of unrelated causes at 1139 days post transfusion, but had PrP^{Sc}-positive IHC labelling in brain and other tissues. One of three recipients of whole blood (G92) and one of two recipients of buffy coat (G61) from clinical donors showed weak PrP^{Sc} deposition in the brain and lymphoid tissues after being culled at 2003 and 2497 days post transfusion respectively, in the absence of clinical signs. Full sequencing of the PrP gene of these sheep revealed that they carried an additional proline (P) to leucine (L) substitution at codon 168[14,15], which appears to be associated with the prolonged survival of these infected sheep. The polymorphism was also identified in two recipients of blood from a pre-clinical BSE-challenged donor, neither of which showed evidence of infection.

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Taking the results for all 22 recipients of blood from BSE-exposed donors, five clinical cases and three sheep showing evidence of infection in the absence of clinical signs were identified, giving an overall transmission rate of 36%.

One recipient was culled for health reasons at 1444 days post transfusion, two were culled with suspected TSE clinical signs at 2480 and 2160 days post transfusion respectively, and the remaining clinically negative sheep were culled between 2239 and 3068 days post transfusion. With one exception, examination of the tissues by IHC did not find evidence of infection. The exception (D337) was culled at 3018 days post transfusion and showed positive PrP^{Sc} labelling in the brain, but with a pattern distinct from that observed in other BSE-infected sheep. The brain PrP^{Sc} distribution involving major white matter tracts and sparing the dorsal motor nucleus of the vagus was similar to that of Nor98 (or "atypical" sheep scrapie) and therefore unlikely to be transfusion-related. No other sheep in the present study showed evidence of being infected with atypical scrapie.

Out of the ten sheep that were infected intravenously with BSE as positive controls, eight developed clinical signs confirmed by IHC, with an average IP of 702 days (± 61 days standard deviation). The remaining two animals were culled at 2591 days post infection and, although not demonstrably clinically affected, IHC showed PrP^{Sc} deposition in the brains and lymphoid tissues of both animals. These two sheep were heterozygous (PL_{168}) for the PrP polymorphism P168L (see above), while the other eight were homozygous (PP_{168}).

The PrP^{Sc} profile obtained by IHC from BSE positive recipients was the same as that found in the orally inoculated donors and in the positive controls[16]. In addition, characteristic BSE glycoform patterns were obtained by Western blot analysis of PrP^{Sc} positive donor and recipient sheep (data not shown; see[9]), and inoculation of brain homogenates from infected donors and recipients into a panel of inbred mouse strains produced IPs and lesion profiles characteristic of BSE (data not shown). Taken together, these results confirm that the strain characteristics were not altered following transmission *via* blood.

2) Scrapie transfusion experiment

Four out of ten recipients of whole blood and four out of ten recipients of buffy coat from donors in the pre-clinical phase of scrapie infection developed clinical signs of scrapie, which were confirmed by positive IHC results. One sheep transfused with buffy coat from the single clinical donor was also clinically affected and IHC positive (see Table 2 & Figure 2). Four of these cases (F144, F153, F141 & F143) were reported previously[9]. There were four intercurrent deaths at 354, 753, 1237 and 1615 days post transfusion respectively, and the eight remaining recipients were culled between 2329 and 2484 days post transfusion. These twelve animals were clinically negative at the time of death, and showed no detectable PrP^{Sc} by IHC. Thus, nine out of 21 recipients of blood from scrapie-exposed sheep developed clinical scrapie, giving an overall transmission rate of 43%.

The majority of confirmed scrapie cases in recipients (n = 7) occurred in the groups that received transfusions from donors in the late pre-clinical (>50% of estimated IP) or clinical phase of infection. Only 2 out of 9 recipients in these groups remained free

of infection. The other two positive recipients were in the group of 6 sheep that received transfusions from donors at 28-37% of estimated IP, and their IPs were much longer than the rest (1101 and 1138 days post transfusion compared to a range of 575-853 days in recipients of blood from donors at >50% of estimated IP). No disease was confirmed in the 6 recipients that received blood from donors at ≤20% of estimated IP.

The PrP^{Sc} profile obtained from brains of donors and recipients highlighted some differences in terms of presence of vascular plaques or glia-associated PrP^{Sc} in donors but not in recipients, or *vice versa* (unpublished data). Such discrepancies were interpreted as presence of more than one natural scrapie strain in the flock of origin.

Discussion

The outcome of the blood transfusion experiments showed that two different TSE agents, scrapie and BSE, could be efficiently transmitted between sheep by blood transfusion, using volumes similar to those employed in human transfusions. The overall transmission rates (percentage of all recipients that became infected) were 36% for BSE and 43% for scrapie. For BSE, the figure was much higher than anticipated because three of the eight BSE-infected recipients survived for long periods without showing clinical signs, whereas all the scrapie-infected recipients identified by IHC were also clinically positive. The greater probability of sub-clinical infection in recipients of blood from BSE-exposed donors is largely due to variability in the genetic susceptibility to infection among sheep used in the BSE experiment, which will be discussed below. The results are consistent with the known facts about transmission of vCJD by blood transfusion in humans[17]. Sixty-six individuals known to have received labile blood products from 18 donors who subsequently developed vCJD were followed up in an on-going study. Three of these recipients have been confirmed clinically and pathologically as vCJD cases, with intervals between transfusion and the development of clinical signs ranging from approximately 6½ years to 8½ years[18-20]. Another individual, who died of unrelated causes 5 years post transfusion, showed PrP^{Sc} deposits in lymphoid tissues but not brain at post mortem, and is thought to represent pre-clinical or sub-clinical infection[21]. These four individuals represent 6% of the total recipients, or 12.5% of recipients surviving longer than 5 years.

Various factors influence the transmission rate by transfusion in both sheep and humans, including: (i) the interval between blood donation and the onset of clinical signs in the donors, (ii) genetic variation in susceptibility of donors and recipients, and (iii) the blood component transfused.

1) Stage of incubation period of the donors at the time of blood donation.

The effect of the stage of incubation can best be deduced from the results of the scrapie transfusion experiment, since the PrP genotype of the sheep used (VRQ/VRQ) renders them almost 100% susceptible to natural and experimental infection[22]. The stage of incubation of the donor has a strong influence on the probability of transmission to the recipient (Figure 2). When donations were made at ≤20% of the estimated IP, there was no disease transmission, while donations made at >50% of the estimated IP produced an 80% transmission rate, with a mean IP of 729 days (SD ±

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99) in the recipients. Blood collected at 28-37% of the estimated IP transmitted infection at a lower rate of approximately 33%, and with longer IPs in the recipients of >1000 days. The data are consistent with a gradual increase in infectivity in the blood, from approximately 30-50% of IP until the clinical phase.

In the BSE transfusion experiment, the correlation between stage of infection and transmission is not clear-cut, but shows the same general trend of increasing probability of transmission to recipients as infection progresses in the donors (Figure 2). Possible explanations for the lower transmission rates from pre-clinical BSE-infected blood donors compared to pre-clinical scrapie-infected donors include:

a) Variation in susceptibility to infection of both donor and recipient sheep. This will be discussed below.
b) Differences in the pathogenesis of natural scrapie and experimental BSE. VRQ/VRQ sheep naturally infected with scrapie have detectable PrP^{Sc} deposits in lymphoid tissues early after infection (i.e. <50% estimated IP)[23,24]. Time course studies of ARQ/ARQ sheep orally infected with BSE showed that PrP^{Sc} was not consistently detected in lymphoid tissues before at least 65% of the average IP[7]. If infectivity in blood correlates with its presence in lymphoid tissues, this could explain the differences observed in the two transfusion experiments.

The probability of transmission from pre-clinical donors is of greatest relevance to the human situation. In the case of the four transfusion-related transmissions of vCJD, the donors developed clinical signs between 17-42 months after donation. The mean IP for vCJD has been estimated to be 16.7 years, with a lower 95% confidence interval of approximately 12.4 years[25]. Therefore, it is likely that the transfusion-related vCJD cases resulted from donations made at least half-way through the IP, which is in agreement with the data from the sheep experiments. In vCJD cases, the timing of detectable lymphoid replication in the pre-clinical stages of disease is unknown; therefore it is not clear whether the peripheral pathogenesis more closely resembles BSE or natural scrapie in sheep.

2) Effect of genetic variation in susceptibility.

A small proportion of sheep with $A_{136}Q_{171}/A_{136}Q_{171}$ PrP genotypes do not succumb to infection following natural or experimental exposure to scrapie and BSE, or have very prolonged incubation periods[26-28]. The reasons for this variability in response are not clearly understood, but it can be predicted to reduce infection rates in both donor and recipient sheep in the BSE transfusion experiment. The majority of pre-clinical donor sheep (8/11) in the BSE transfusion experiment were killed at, or shortly after, the time of donation, and none showed conclusive evidence of infection, although two transmitted infection to their respective transfusion recipients. It is potentially significant that donors that failed to transmit infection were heterozygous at PrP codon 154, while those that did transmit infection were homozygous. Thus, variable susceptibility to infection among the donor sheep may be the result of a protective effect of codon 154 heterozygosity to oral challenge with BSE, although more data are required to confirm this association.

A novel polymorphism, resulting in a proline to leucine substitution at codon 168 of the PrP gene, was identified in four BSE transfusion recipients and two positive

control sheep inoculated intravenously with BSE[14]. All six survived >2000 days without developing clinical signs of BSE, but on post mortem examination four showed PrP^{Sc} deposition in brain and lymphoid tissues. This suggests that the P168L polymorphism can protect against clinical disease, but does not prevent infection by the intravenous route. This polymorphism has not been identified in the Edinburgh NPU Cheviots used as donors in the BSE experiment, nor in sheep with the VRQ/VRQ genotype.

Although the genetic basis of susceptibility to BSE infection in sheep and humans is not directly comparable, the variability in response to BSE found in ARQ/ARQ sheep provides a more realistic reflection of the situation with vCJD in the human population than the very uniform susceptibility of VRQ/VRQ sheep to scrapie infection. In addition, the survival of BSE-infected transfusion recipients for up to 7 years without clinical signs demonstrates that prolonged secondary incubation periods and/or a sub-clinical/"carrier" state are possible following transfusion in sheep. The existence of such sub-clinical or prolonged pre-clinical infection states in humans is recognised as one of the important factors influencing the probability of onward transmission, and thus the potential size of the vCJD epidemic[29]. Susceptibility to human TSEs has been linked to codon 129 of the PrP gene, which can encode either methionine (M) or valine (V). Until recently, all clinical cases of vCJD (including the 3 transfusion-related cases) that have been tested have been homozygous for methionine at 129 (129MM). Interestingly, the "pre-clinical" individual believed to have been infected by transfusion was heterozygous (129MV)[21]. There is accumulating evidence to suggest that all human 129 genotypes may be susceptible to vCJD infection, with apparently greater likelihood of sub-clinical infection in 129MV and 129VV individuals[30-32].

3) Effect of blood component.

The four transfusion-related vCJD infections occurred in individuals who received transfusions of red cells that had not been leucodepleted. Leucodepletion was introduced in the UK in 1999 to control the risk of transmission of vCJD by blood transfusion, because previous studies in rodents had shown that infectivity appeared to be concentrated in the buffy coat, which contains most of the blood leukocytes[4]. Subsequently, leucodepletion of blood from scrapie-infected hamsters was shown to remove up to 72% of infectivity[33,34]. In the sheep experiments, only whole blood and buffy coat were transfused, because we were seeking to establish proof of principle of transmission of TSEs by blood transfusion, and assessing whether infectivity appeared to be concentrated in the buffy coat. The effect of leucodepletion was not investigated, but is being addressed in a follow-up study, along with estimates of the distribution of infectivity among other blood components, including plasma, platelets and red cells.

In our experiments, transmission rates did not appear to be significantly different in recipients receiving whole blood compared to recipients transfused with buffy coat. The number of sheep transfused with buffy coat in the BSE experiment was too small to allow statistical analysis. In the scrapie experiment, five of the positive recipients were transfused with buffy coat, and four with whole blood. The similarity in transmission rates for both components suggests that they contain approximately equivalent amounts of infectivity.

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We have shown that, for sheep infected with scrapie and BSE, high transmission rates can be achieved using blood transfusion, particularly when donors are at >50% of incubation period. The results also revealed the possibility of prolonged incubation periods and/or sub-clinical infections in some recipients of BSE-infected blood, which is at least partly due to genetic variation in the sheep PrP gene. The suggestion of relatively high titres of infectivity in blood is perhaps surprising in view of the need for ultra-sensitive methods of detection for PrP^{Sc} in blood[35,36]. It may be that, in blood, infectivity is not closely correlated with levels of protease-resistant PrP, but comparative titrations of brain and blood-borne infectivity in sheep will be required to further define the relationship. The results of our sheep transfusion experiments are consistent with what is known about transfusion-associated vCJD transmission in man, and support the use of sheep as an experimental model in which to study the risks associated with different blood products, the effectiveness of control measures and the development of diagnostic and screening tests.

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Author contributions

F.H. designed the study, performed transfusions and post-mortems on recipient sheep, analyzed data and wrote the paper. A.C. and S.McC. performed Western blots, and S.McC. reviewed the report. J.F. coordinated collection of blood and post-mortems on donor sheep. W.G. analyzed and interpreted PrP genotype data and reviewed the report. S.S. and L.G. examined tissues, interpreted IHC results, analyzed data and reviewed the report. M.J. contibuted to the interpretation of IHC results and reviewed the report. N.H. designed the study, analyzed data, and reviewed the report.

The authors have no financial conflicts of interest to declare.

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Figure 1. Overview of experimental design.

Figure 2. Outcome of transfusions as a function of the stage of disease incubation in the donor. A. BSE-infected donors. B. Scrapie-infected donors. For each stage of infection in the donor sheep, the number of uninfected (open bars), clinically positive/IHC positive (solid bars) and clinically negative/IHC positive (cross-hatched bars) recipients are shown.

Table 1. Outcome of transfusions from BSE-exposed donor sheep.

Donor sheep details							Recipient sheep details				
Donor sheep ID	Donor genotype	Clinical status at donation	% actual or average incubation period at donation[a]	Clinical outcome	IHC result	Incubation period (days)	Component transfused	Recipient sheep ID	Recipient PrP 168 codon genotype	Clinical outcome	IHC result	Incubation period (days)
58x51	ARQ/ARQ	Preclinical	12	+	+	2131	WB	D529	PP	+[c]	-	-
60x49	ARQ/ARQ	Preclinical	22	-	+/- (DRG)[b]	-	WB	D433	PL	-	-	-
			44				WB	F14	PL	-	-	-
J2747	ARQ/AHQ	Preclinical	42	-	-	-	BC	F182	PP	-	-	-
			44				WB	F181	PP	-	-	-
61x24	ARQ/AHQ	Preclinical	42	-	-	-	BC	F238	PP	+[c]	-	-
		Preclinical	43				WB	F234	PP	-	-	-
J2746	AHQ/AHQ	Preclinical	45	-	-	-	WB	F19	PP	+	+	536
J2559	AHQ/AHQ	Preclinical	51	+	+	629	WB	D505	PP	+	+	610
58x81	ARQ/AHQ	Preclinical	61	-	+/- (IPP)[b]	-	BC	D358	PP	-	-	-
58x28	ARQ/AHQ	Preclinical	61	-	-	-	WB	D421	PP	-	-	-
			61				BC	D384	PP	-	-	-
58x27	AHQ/AHQ	Preclinical	61	-	-	-	WB	D452	PP	-	+[d]	-
			61				BC	D318	PP	-	-	-
58x39	ARQ/AHQ	Preclinical	62	-	-	-	WB	D337	PP	-	+[c]	-
			62				WB	D386	PP	-	-	-
J2499	AHQ/AHQ	Preclinical	86	+	+	761	WB	D341	PP	-	-	-
J2771	AHQ/AHQ	Clinical	100	+	+	561	BC	G61	PL	-	+	-
J2770	AHQ/AHQ	Clinical	100	+	+	589	WB	G74	PP	+	+	594
60x69	AHQ/AHQ	Clinical	100	+	+	660	WB	G78	PP	+	+	556
							BC	G49	PP	+	+	531
D383	ARQ/ARQ	Clinical	100	+	+	671	WB	G92	PL	-	+	-

Key: WB = whole blood, BC = buffy coat, DRG = dorsal root ganglion, IPP = ileal Peyer's patch

[a] Calculated from the days post-infection at the time of donation, as a percentage either of the final incubation period (in sheep kept alive until the development of clinical signs), or of the average incubation period in orally-infected donors (640 days), excluding the out-lying incubation period of 2131 days for 58x51.

[b] These tissues were initially scored weakly positive by IHC, but the results were not reproducible in two laboratories and can therefore be considered as inconclusive.

[c] No evidence of infection was found on post mortem examination of tissues from these clinical suspects; therefore it is most likely they were clinically misdiagnosed.

[d] This sheep died of unrelated causes (i.e. without showing clinical signs of BSE) at 1139 days post transfusion, but was positive by IHC.

[a] This apparently healthy sheep was culled 3018 days post transfusion and found to be positive by IHC; however further analysis suggested this was a case of "atypical" scrapie, and therefore unlikely to be transfusion related (see text for details).

Table 2: Outcome of transfusions from scrapie-exposed donor sheep

Donor sheep details								Recipient sheep details			
Donor sheep ID	Donor genotype	Clinical status at donation	% actual or average incubation period at donation[a]	Clinical outcome	IHC result	Incubation period (days)	Component transfused	Recipient sheep ID	Clinical outcome	IHC result	Incubation period (days)
67x42	VRQ/VRQ	Preclinical	17	+	+	1274	BC	G247	-	-	-
			19				WB	G230	-	-	-
66x45	VRQ/VRQ	Preclinical	17	-	-	-	WB	G267	-	-	-
			19				BC	G265	-	-	-
67x23	VRQ/VRQ	Preclinical	18	+	+	1207	BC	G241	-	-	-
			20				WB	G228	-	-	-
65x13	VRQ/VRQ	Preclinical	28	+	+	1556	WB	F275	-	-	-
			30				BC	F273	-	-	-
65x02	VRQ/VRQ	Preclinical	34	-	+	-	WB	F310	-	-	-
			37				BC	F309	+	+	1101
65x03	VRQ/VRQ	Preclinical	34	-	+	-	WB	F277	+	+	1138
			37				BC	F276	+[b]	-	-
61x75	VRQ/ARQ	Preclinical	53	+	+	1324	BC	F149	+	+	782
			57				WB	F144	+	+	672
61x68	VRQ/VRQ	Preclinical	64	+	+	1113	BC	F152	+	+	853
			69				WB	F153	+	+	660
61x66	VRQ/VRQ	Preclinical	62	-	ND	-	WB	F286	-	-	-
			64				BC	F284	-	-	-
59x27	VRQ/VRQ	Preclinical	73	+	+	1137	BC	F126	+	+	826
			77				WB	F141	+	+	575
59x28	VRQ/VRQ	Clinical	100	+	+	1081	BC	F143	+	+	737

[a] Calculated from the age at the time of donation, as a percentage either of the final incubation period (for sheep that survived until the development of clinical signs), or of the average incubation period (1296 days) for sheep that died or were culled before developing clinical signs.

[b] No evidence of infection was found on post mortem examination of tissues from this clinical suspect; therefore it is most likely it was clinically misdiagnosed.

From www.bloodjournal.org at Central Blood Institute c/o Japanese Red Cross Society on October 16, 2008. For personal use only.

Figure 1

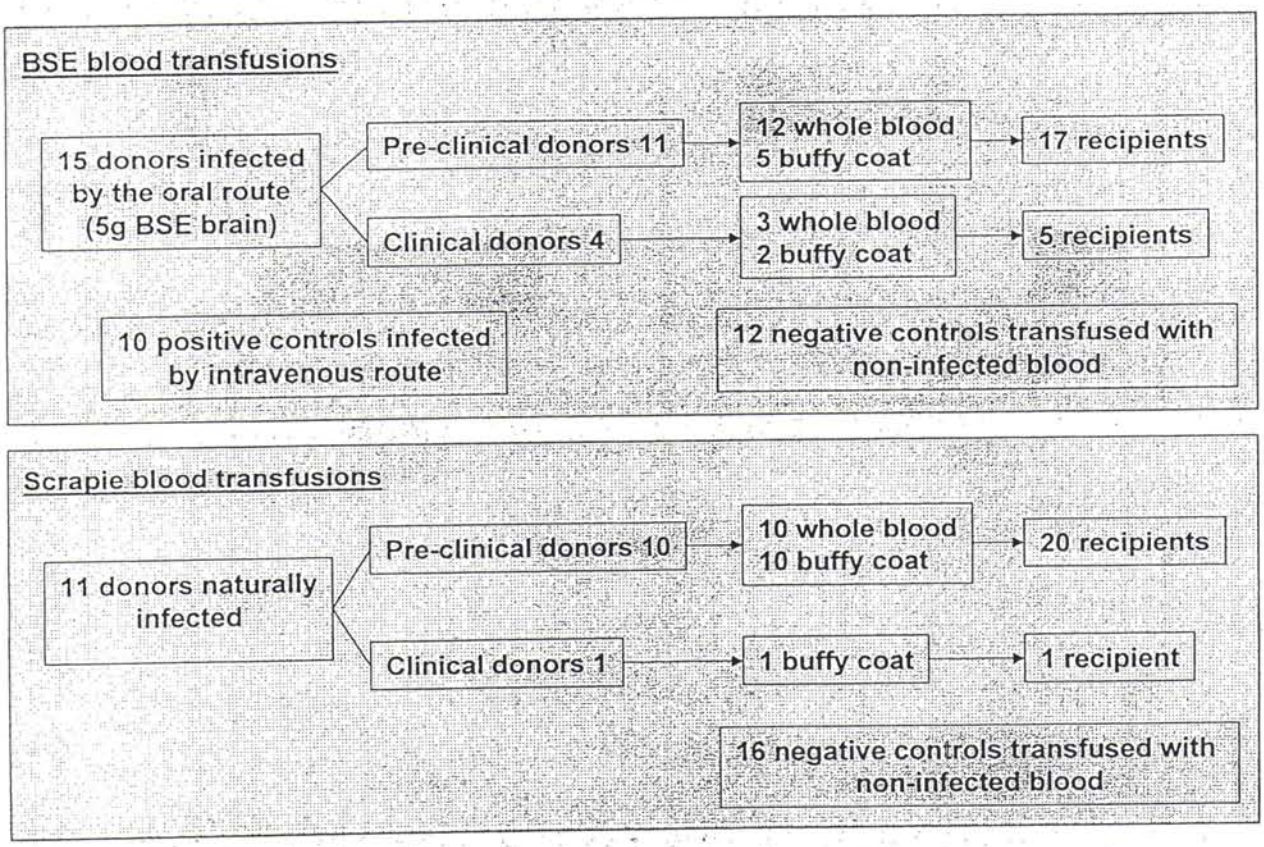
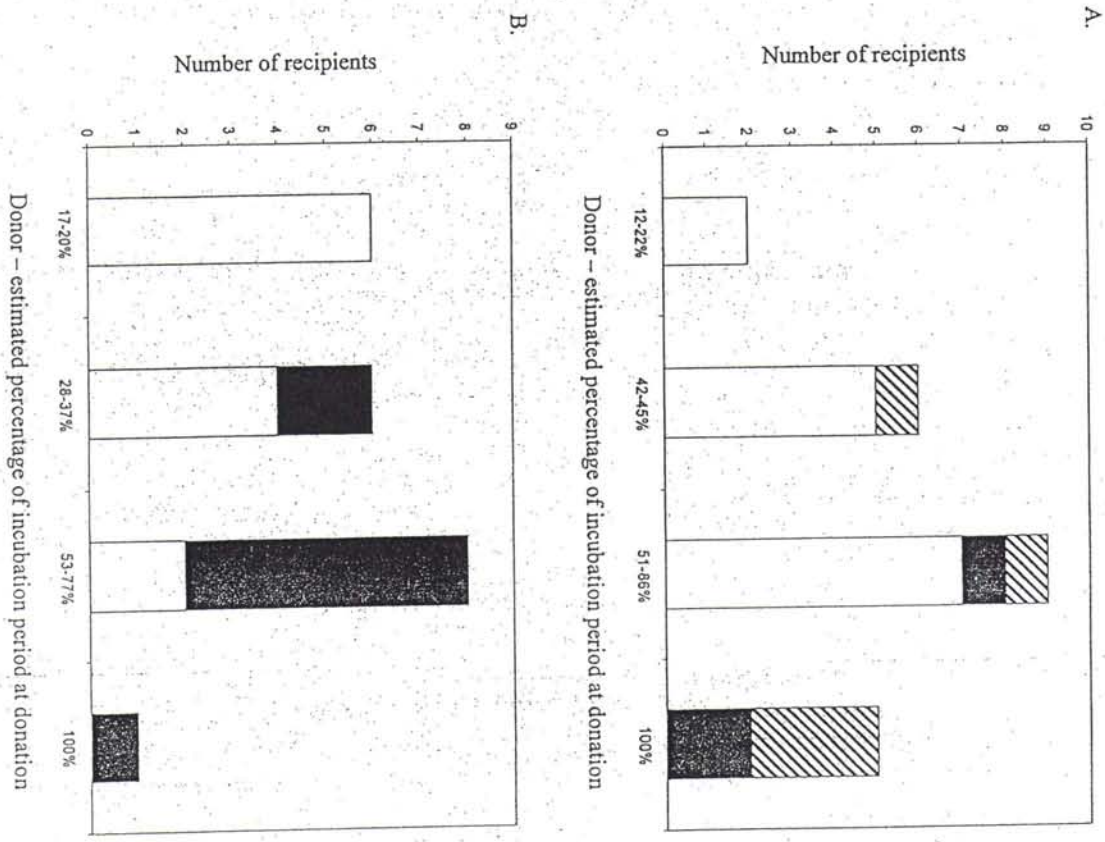


Figure 2.



医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 10. 17	該当なし	
一般的名称	人全血液		研究報告の公表状況	Morgan AE. Am J Infect Control. 2008 Oct;36(8): 602.	公表国 米国	
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)					

研究報告の概要

○耳鍼による緑膿菌感染
両耳用置き鍼治療(Stapling)は、効果的な減量法としてメディアで大きく取上げられている。鍼師は食欲抑制を目的として耳介軟骨の「つぼ」に鍼を留置する。現在多くの保険会社が鍼治療を保険適用にしている。
2週前に鍼治療院を訪れ両耳軟骨の置き鍼治療を受けた病歴のない16歳の女性は、左耳の鍼周囲の紅斑および圧痛がみられた。鍼を除去し、アモキシリン・クラブラン酸の経口投与を行ったが、1週間後、紅斑および圧痛が進行し膿瘍が現れた。ドレナージ検体を培養と感受性試験に供した。もう片耳の鍼も除去し排膿を認め検体を採取した。試験の結果が得られるまで、トリメトプリム・スルファメトキサゾール(TMP/SMX)の経口投与を行った。両耳で著しい緑膿菌の生育が認められたため、シプロフロキサシンの経口投与を行い、治療21日目に完全消失となった。
外耳軟骨は、血流に乏しく特に感染しやすい。さらに、鍼刺による周囲軟骨膜の破損は、耳軟骨の完全性に損傷を与える可能性がある。耳介軟骨炎で最も一般的な感染は、黄色ブドウ球菌と緑膿菌によるものである。緑膿菌は治療が困難であり、長期入院や再建手術を要する重度感染を引き起こす場合がある。
減量のための耳鍼は非常に人気のある方法になりつつあるが、患者はプラセボ効果の可能性と感染のリスクを考慮すべきである。もっとも重要なことは、耳鍼が危険な緑膿菌感染を起こす可能性があることを医師が認識することである。

報告企業の意見	今後の対応
減量法として両耳用置き鍼治療(Stapling)を受けた女性の鍼周辺に緑膿菌が感染したとの報告である。	日本赤十字社は、細菌・ウイルス等の血液を介する感染防止の目的から、献血時にピアスについて確認し施術後1ヵ月ないし1年間献血延期としている。鍼治療についても申告があった場合は「鍼治療における感染防止の指針」に準拠していることを確認し、そうでない場合は1年間献血延期としている。今後も細菌感染に関する新たな知見及び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

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(17)

MedDRA/J Ver.11.0J

AJIC Letters to the Editor

Pseudomonas aeruginosa infection due to acupunctural ear stapling

To the Editor:

Bilateral ear stapling is widely advertised in the media (including the Internet) as a popular and successful weight reduction strategy. Acupuncture providers performing the technique place staples into ear cartilage "reflex points" to decrease craving.[1] Many insurance carriers now provide coverage for most acupuncture treatments.

A 16-year-old female with no medical history presented with a complaint of external ear pain. Two weeks earlier, she visited an acupuncture parlor, where she underwent bilateral ear stapling of her upper ear cartilage to induce weight loss. Examination revealed erythema and tenderness around the left ear staple. The staple was removed, and the patient was placed on oral amoxicillin/clavulanic acid. One week later, the erythema and tenderness had progressed, and an abscess was present. The lesion was drained, and a specimen of the drainage was sent for culture and sensitivity testing. At this time, the staple on the other ear was removed, and pus drainage was identified and collected. The patient was placed on oral trimethoprim/sulfamethoxazole (TMP/SMX) pending culture and sensitivity results.

Laboratory evaluation subsequently revealed heavy growth of *Pseudomonas aeruginosa* on both ears. The patient was placed on oral ciprofloxacin. Complete resolution occurred after 21 days of treatment.

The cartilage of the external ear is particularly vulnerable to infection due to its limited blood supply. In addition, disruption of the surrounding perichondrium due to stapling can damage ear cartilage integrity. The most common infectious agents in auricular chondritis are *Staphylococcus aureus* and *P aeruginosa*.[2] In this case, the patient failed a 1-week course of amoxicillin/clavulanic acid, which is highly effective against methicillin-sensitive *S aureus*. Due to the high prevalence of methicillin-resistant *S aureus* skin infections,[3] the patient was started on TMP/SMX before laboratory testing confirmed the *P aeruginosa* infection. *P aeruginosa* can be particularly difficult to treat because of its high resistance to oral antibiotic regimens.[4] In addition, auricular chondritis due to this organism can cause severe infection, necessitating prolonged hospitalization and reconstructive surgery.[4]

Studies on ear stapling have demonstrated that patients who strictly monitor their daily food consumption experienced comparable weight loss to those who undergo ear stapling.[5] Another study requiring patients to wear a simple wrist device to remind them of their dietary restrictions found comparable weight loss to ear stapling.[6] These studies indicate that the presence of an ear staple may have a placebo effect and that the increased attention to daily food consumption, possibly through daily logging, is actually responsible for the enhanced weight loss.

Ear stapling for weight loss is becoming an increasingly popular modality. The possibility of a placebo effect and the risk of infection should be considered in a patient's decision to receive the treatment. Most importantly, physicians should be aware that acupunctural ear stapling can cause dangerous *P aeruginosa* infection.

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doi:10.1016/j.ajic.2007.07.014

0196-6553/$34.00

Hand hygiene in Iranian health care workers

To the Editor:

Hand hygiene (HH) remains the single most important measure to prevent nosocomial infections.[1] Despite universal awareness of HH role in reducing nosocomial infection, compliance among health care

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2008年10月24日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	MMWR. 2008;57:1145-1148	公表国 米国	
販売名（企業名）	別紙のとおり					

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
問題点：輸血によるアナプラズマ症感染事例。過去に輸血によるアナプラズマ症の報告はあったが、本症例は血液ドナーに感染源が確認された初の事例。 2007年11月、入院中のミネソタ州住民が *Anaplasma phagocytophilum* に感染しているとの報告を受けた。患者は68歳男性、慢性腎不全、乾癬性関節炎、強直性脊椎炎の既往があり、ステロイド投与を受けていた。入院する3週間前にマダニのいる地域へ旅行したが、咬まれたかどうかは不明である。2007年10月12日、膝関節形成術および滑膜切除術が行われたが、数時間後に手術部位から出血、INRおよびPPT上昇を伴う凝固障害を来たし、フィブリノーゲンおよび血小板数が減少、外科処置と輸血が行われた。10月12～21日、赤血球34単位、血小板4単位、新鮮凍結血漿14単位、寒冷沈降物7単位が輸血され、19日、敗血症および多臓器不全をきたし、セファゾリン、ピペラシリン/タゾバクタム、バンコマイシン、レボルフロキサシンが投与された。10月18、20、31日の血液培養、19、25日の尿培養検査はいずれも陰性であった。31日、血小板減少が進行(31日:178,000/mm³、11月5日:54,000/mm³)、翌11月1日には低血圧、尿路感染症による発熱を来たし、レボフロキサシンとST合剤が投与された。入院22日目(11月3日)、末梢血塗抹検体から *A. phagocytophilum* の桑実胚が認められ、11月3～5日のPCRによるDNAアッセイにて *A. phagocytophilum* が確認され、CDCによりIgG抗体陽性も確認された。11月5日よりドキシサイクリンが投与され、血小板数は回復、10日には163,000/mm³となり、13日にリハビリ病棟へ移動、12月3日に退院した。この患者に輸血された血液ドナー(59名)の調査を行ったところ、64歳女性の血液がPCR、IFA検査により *A. phagocytophilum* 陽性と確認されたが、この女性は献血の前後1ヵ月間、発熱などの症状は認めていなかった。輸血後の発熱を伴う急性血小板減少症は、アナプラズマ症の可能性を考慮し、輸血による感染の疑いを州や地方の保険局に報告すべきと考える。	記載なし

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

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(18)

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗HBs人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名（企業名）	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ベバトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用
報告企業の意見	アナプラズマ症はマダニにより媒介される発熱性疾患で、その病原体は顆粒球に特異的に感染する0.2～2μmの大きさの球状もしくは楕円状の偏性寄生性のグラム陰性桿菌である。1994年、米国で発熱性疾患患者の好中球の中にエーリキア様細菌の感染が認められ、ヒト顆粒球エーリキア症病原体［Human Granulocytic Ehrlichiosis (HGE) agent］と呼ばれるようになった。その後、1996年にはその病原体が分離報告され、さらに2001年にはEhrlichia属からAnaplasma属へと配置換えされて、*Anaplasma phagocytophilum* という学名が付された。それに伴って、昨今ではその病名もヒト顆粒球アナプラズマ症［Human Granulocytic Anaplasmosis (HGA)］と呼ばれている。*A. phagocytophilum* は、ヒトの他、ウマやヒツジなどにも感染し、アナプラズマ症を引き起こすことから「人獣共通感染症」病原体としても知られている。(http://idsc.nih.go.jp/iasr/27/312/dj312d.html) *A. phagocytophilum* によるアナプラズマ症の発生は欧米が中心であるが、2006年に日本においても *A. phagocytophilum* がマダニから検出されたことが初めて報告された。 弊所で製造している全ての血漿分画製剤の製造工程には、約0.2μmの「無菌ろ過工程」および、*A. phagocytophilum* よりも小さいウイルスの除去を目的とした平均孔径19nm以下の「ウイルス除去膜ろ過工程」が導入されているので、仮に製造原料に *A. phagocytophilum* が混入していたとしても、これらの工程により除去されるものと考えられる。更に、これまでに本剤によるアナプラズマ症感染の報告例は無い。 以上の点から、本剤はアナプラズマ症感染に対して一定の安全性を確保していると考えるが、今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

*現在製造を行っていない