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
			2010年2月22日	該当なし	
一般的名称	別紙のとおり	研究報告の公表状況	CDC／Travelers' Health (Updated: February 18, 2010)	公表国 インドネシア タイ マレーシア	
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：2009年から2010年初頭にかけてインドネシア、タイ、マレーシアにおいてチクングニヤウイルス熱のアウトブレイクが発生している。 2006年以降、アジア及びインド洋領域でチクングニヤ熱の活動が報告されているが、2009年にインドネシアでは12の県に渡って43,206例（死亡例は無し）が、タイではPhuketを含む南部を中心に49,069例超が、マレーシアではSarawak Kedah県北部を中心に4,430例超（死亡例は無し）の当該症例が報告された。また、インドネシアではSumatra島のlampung県南部において2009年12月後半から2010年1月初頭に6,700例のチクングニヤ感染例が、マレーシアでは2010年最初の5週間で325例のチクングニヤ熱症例が報告されている。	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。

MedDRA12.1

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人免役グロブリン、⑤人免役グロブリン、⑥人免役グロブリン、⑦乾燥ペプシン処理人免疫グロブリン、⑧乾燥ペプシン処理人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン、⑫乾燥スルホ化人免疫グロブリン、⑬乾燥スルホ化人免疫グロブリン、⑭乾燥スルホ化人免疫グロブリン*、⑮乾燥濃縮人活性化プロテインC、⑯乾燥濃縮人血液凝固第Ⅷ因子、⑰乾燥濃縮人血液凝固第Ⅷ因子、⑱乾燥濃縮人血液凝固第Ⅷ因子、⑲乾燥濃縮人血液凝固第Ⅷ因子、⑳乾燥濃縮人血液凝固第Ⅸ因子、㉑乾燥濃縮人血液凝固第Ⅸ因子、㉒乾燥濃縮人血液凝固第Ⅸ因子、㉓乾燥濃縮人血液凝固第Ⅸ因子、㉔乾燥抗破傷風人免疫グロブリン、㉕乾燥抗破傷風人免疫グロブリン、㉖抗HBs人免疫グロブリン、㉗抗HBs人免疫グロブリン、㉘トロンビン、㉙フィブリノゲン加第XIII因子、㉚フィブリノゲン加第XIII因子、㉛乾燥濃縮人アンチトロンビンIII、㉜乾燥濃縮人アンチトロンビンIII、㉝ヒスタミン加人免疫グロブリン製剤、㉞タミン加人免疫グロブリン製剤、㉟人血清アルブミン*、㊱人血清アルブミン*、㊲乾燥ペプシン処理人免役グロブリン*、㊳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ガンマーグロブリン筋注450mg/3mL「化血研」、⑥ガンマーグロブリン筋注1500mg/10mL「化血研」、⑦献血静注グロブリン"化血研"、⑧献血グロブリン注射用2500mg「化血研」、⑨献血ベニロン－I、⑩献血ベニロン－I静注用500mg、⑪献血ベニロン－I静注用1000mg、⑫献血ベニロン－I静注用2500mg、⑬献血ベニロン－I静注用5000mg、⑭ベニロン*、⑮注射用アナクトC2,500単位、⑯コンファクトF、⑰コンファクトF注射用250、⑱コンファクトF注射用500、⑲コンファクトF注射用1000、⑳ノバクトM、㉑ノバクトM注射用250、㉒ノバクトM注射用500、㉓ノバクトM注射用1000、㉔テタノセーラ、㉕テタノセーラ筋注用250単位、㉖ヘパトセーラ、㉗ヘパトセーラ筋注200単位/mL、㉘トロンビン"化血研"、㉙ボルヒール、㉚ボルヒール組織接着用、㉛アンスロビンP、㉜アンスロビンP500注射用、㉝ヒスタグロビン、㉞ヒスタグロビン皮下注用、㉟アルブミン20%化血研*、㊱アルブミン5%化血研*、㊲静注グロブリン*、㊳アンスロビンP1500注射用
報告企業の意見	チクングニヤウイルス（Chikungunya virus）は、トガウイルス科（Togaviridae）のアルファウイルス属（Alphavirus）に分類される1本鎖のRNAを核酸として持つ直径70nmのエンベロープを有する球状粒子であり、これまでに国内での発生、流行は報告されていないが、2010年12月までに海外からの輸入症例として15例の報告がある。チクングニヤウイルスは蚊によって媒介されるが、感染後ウイルス血症を起こすことから、血液を介してウイルス感染する可能性を完全に否定できないため本報告を行った。 上記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・不活化工程が存在しているので、仮にウイルスが原料血漿に混入していたとしても、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。今回報告したチクングニヤウイルス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、これまでに上記製剤によるチクングニヤウイルス感染の報告例は無い。 以上の点から、上記製剤はチクングニヤ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

Centers for Disease Control and Prevention
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Outbreak Notice

Chikungunya Fever in Asia and the Indian Ocean

This information is current as of today, April 07, 2010 at 21:25 EDT

Updated: February 18, 2010

Situation Information

Since 2006, parts of Asia and the Indian Ocean region have reported chikungunya fever activity. Several countries have increased surveillance for this disease, and cases continue to be reported throughout this region.

Chikungunya fever is a disease caused by a virus that is spread to people through the bite of infected mosquitoes. Symptoms can include sudden fever, joint pain with or without swelling, chills, headache, nausea, vomiting, lower back pain, and a rash. Chikungunya mainly occurs in areas of Africa and Asia. In 2007, limited transmission of *chikungunya virus occurred in Italy.*

The following examples highlight some recent chikungunya activity in Asia and the Indian Ocean region:

Indonesia

A chikungunya outbreak has been reported in the southern province of Lampung on the island of Sumatra. From the second half of December 2009 through the beginning of January 2010, 6,700 chikungunya cases were reported. In 2009, no deaths due to chikungunya fever were reported, although a total of 43,206 cases were reported across the country from 12 provinces.

Thailand

In 2009, a large outbreak of chikungunya fever affected the country, particularly the southern region, including some tourist destinations, such as Phuket. According to the Ministry of Public Health in Thailand, over 49,069 cases were documented in more than 50 provinces. Reports from Thailand show that chikungunya virus continues to circulate throughout the country.

Malaysia

In 2009, the Ministry of Health in Malaysia reported over 4,430 cases of chikungunya fever. No deaths were reported. The most affected areas are the northern provinces of Sarawak Kedah, followed by Kelantan, Selangor, and Perak. Chikungunya activity has continued in 2010, with an additional 325 cases reported in the first 5 weeks. The cases occurred predominately in Sarawak.

Advice for Clinicians

Clinicians should be aware of the ongoing global chikungunya activity. Chikungunya may present in a similar fashion to malaria and dengue, with fever, chills, and generalized myalgias. However, after the acute illness, patients with chikungunya may have a prolonged course of arthralgias or arthritis, which may lead health-care providers to consider and begin testing for rheumatic diseases. These signs and symptoms can persist for several months.

For more information, please see Chikungunya Fever section of *CDC Health information for International Travel 2010.*

Advice for Travelers

No medications or vaccines are available to prevent a person from getting sick with chikungunya fever. CDC recommends that people traveling to areas where chikungunya fever has been reported take the following steps to protect themselves from mosquito bites.

- When outdoors during the day and at night, use insect repellent on exposed skin.
  - Look for a repellent that contains one of the following active ingredients: DEET, picaridin (KBR 3023), Oil of Lemon Eucalyptus/PMD, or IR3535. Always follow the instructions on the label when you use the repellent.
  - In general, repellents protect longer against mosquito bites when they have a higher concentration (%) of any of these active ingredients. However, concentrations above 50% do not *offer a distinct increase in* protection time. Products with less than 10% of an active ingredient may offer only limited protection, often only 1–2 hours.
  - The American Academy of Pediatrics approves the use of repellents with up to 30% DEET on children over 2 months of age.

If you get sick with a fever and think you may have chikungunya fever, you should seek medical care. Although there is no specific treatment for the disease, a doctor may be able to help treat your symptoms. Avoid getting any other mosquito bites, *because if you are sick and a mosquito bites you, it* can spread the disease to other people.

For more travel health information, see the destinations section and search for the country you are planning to visit.

More Information

The incubation period for chikungunya (time from infection to illness) is usually 3–7 days, but it can range from 2–12 days. Chikungunya fever typically lasts a few days to 2 weeks, but some

patients feel fatigue lasting several weeks. Most patients report severe joint pain or arthritis, which may last for weeks or months. The symptoms are similar to those of dengue fever, but, unlike some types of dengue, people who have chikungunya fever do not experience hemorrhage (bleeding) or go into shock. People with chikungunya fever generally get better on their own and rarely die from the disease.

Medical care for chikungunya fever is usually focused on treating the symptoms of the disease. Bed rest, fluids, and mild pain medications such as ibuprofen, naproxen, or acetaminophen (paracetamol) may relieve symptoms of fever and aching, provided there are no medical contraindications for using these medications. Most people are not sick enough to need to stay in the hospital. All people who become sick with chikungunya fever should be protected against additional mosquito bites to reduce the risk of further transmission of the virus.

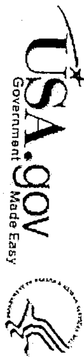
For more information, see—

- Chikungunya (CDC Fact Sheet)
- Traveling with Children: Resources (CDC Travelers' Health website)

Other Mosquito-Related Diseases

In many of the areas where chikungunya is present, mosquito bites spread other diseases, such as dengue, malaria, Japanese encephalitis, and yellow fever. If you are traveling to any tropical and subtropical areas of the world, you should take steps to avoid mosquito bites.

- Page last reviewed: November 19, 2009
- Page last updated: February 18, 2010
- Page created: August 21, 2008
- Content source:
  Division of Global Migration and Quarantine
  National Center for Preparedness, Detection, and Control of Infectious Diseases



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別紙様式第2-1 No. 15

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
				2009. 11. 19	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Stramer S L, Linnen J M, Carrick J M, Krysztof D, McMilin K D, De Vera A, Hunsperger E A, Muñor J L, Dodd R Y. AABB Annual Meeting and TXPO; 2009 Oct. 24-27; New Orleans.	公表国 米国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○2007年のデング熱アウトブレイク時におけるプエルトリコからの供血のデング熱ウイルス血症

背景:デング熱ウイルスは世界で最も重要なアルボウイルスであり、流行範囲を拡大させている。WNV同様、デング熱は蚊によって自然感染するが、輸血によっても伝播する。デング熱流行地域であるプエルトリコの2005年流行期後半のウイルス血症発現率は1:1300を示した。2007年に非常に大規模なデング熱アウトブレイクが発現し、流行期間中の供血者検体が保管された。

方法:ウイルス血症検査のために検体を2セットに分類した(アメリカ本土/プエルトリコで輸血された血液)。研究用transcription mediated amplification assay (TMA)により個別に検体を検査した。初回陽性(IR)検体に再検査を行い、反復陽性(RR)検体は確定とみなされた。プエルトリコのCDCデング熱部門にて、血清型およびウイルス量を明らかにするためPCR、蚊細胞培養、IgM検査などを実施した。RR血液供給先の病院に連絡を取り、受血者の調査を行った。

結果:合計15,350検体を検査し、28がIR、25がRRとなった。有病率は1:614であった。陽性血液のうち12(1:533)が米国本土に輸出され、13がプエルトリコに残った。特異性は99.98%であった。1:16希釈で14/25(56%)のRR供血が検出された。CDCの追加検査では、プエルトリコで循環している血清型1、2、3が示され、11/25(44%)の検体は、RNA力価10^5-10^9copies/mLであり、11検体すべてが細胞培養で感染性があった。9/11(82%)のPCR陽性検体が1:16希釈で検出された。IgM検査を行った6/22(27%)検体のうち2検体のみウイルスの定量が可能(10^6、10^8)であり、うち1つは1:16の希釈で検出された。残り4つのIgM陽性検体では1つのみが1:16希釈で陽性であり、合計2つのIgM陽性検体が希釈時に陽性であった。米国本土とプエルトリコで受血者調査を実施中である。

結論:流行期間中のウイルス血症頻度が高いことが示された。RNA陽性血液の半数近くがIgM陰性で高力価ウイルス血症があり、細胞培養で感染性が確認された。デング熱流行時には供血者のスクリーニングを検討すべきである。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
プエルトリコにおけるデング熱流行期間中の供血者のウイルス血症頻度が高いことが示され、RNA陽性血液の半数近くがIgM陰性で高力価ウイルス血症があり、細胞培養で感染性が確認されたとの報告である。 デングウイルスは脂質膜を持つ中型RNAウイルスである。これまで、本製剤によるデングウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。	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、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

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MedDRA/J Ver.12.0J

JRC2009T-064

S64-030F

HOD RBCs Stored For 14 Days Are Significantly More Immunogenic Than Fresh HOD RBCs

J E Hendrickson[1] *(jeanne.hendrickson@choa.org)*, E A Hod[2], S L Spitalnik[2], C D Hillyer[3], J C Zimring[3]. [1]*Department of Pediatrics, Emory University, AFLAC Cancer Center and Blood Disorders Service, Atlanta, GA, USA;* [2]*Department of Pathology and Cell Biology, Columbia University Medical Center, New York, NY, USA;* [3]*Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA, USA*

Background: Within FDA limits, red blood cells (RBCs) are generally transfused without regard to length of in vitro storage. However, recent studies have raised concerns that transfusion of older stored (i.e. aged) RBCs may lead to adverse events in certain patients. We hypothesized that aged RBC transfusions would lead to higher rates of RBC alloimmunization, and developed a murine model to test this hypothesis. **Materials and Methods:** RBCs from HOD donors (expressing transgenic RBC specific hen egg lysozyme (HEL) fused to human Fyb) were collected in 12.3% CPDA, leukoreduced (LR) with a Pall neonatal LR filter, volume reduced to a Hct of 75%, and stored at 4° C for 14 days. C57BL/6 recipients were transfused intravenously with 500 µL of a 20% solution of fresh or aged (stored 14 days) LR or non-LR RBCs. Flow cytometric testing of HEL and Fyb expression on pre and post-transfusion RBCs was done, with 24 hour post-transfusion survival determined by extrapolation to time 0. Blood cultures were performed on representative samples prior to transfusion. Alloimmunization was tested 2 weeks post-transfusion by anti-HEL IgG ELISA using titrated sera. **Results:** In 5 of 6 independent experiments (n = 62 mice), transfused aged RBCs were 10-100 fold more immunogenic than fresh RBCs as determined by HEL specific ELISA (p < 0.05 by 2 way ANOVA with Bonferroni posttest). This increase in immunogenicity was also seen with LR RBCs: in 3 of 4 experiments (n = 42 mice), aged LR RBCs were more immunogenic than fresh LR RBCs (p < 0.001). In 2 of 2 experiments (n = 20 mice), aged RBCs washed 3 times in saline led to similar levels of alloimmunization as did unwashed aged RBCs. Gram's stain and culture of 7 of 9 representative units was negative. The calculated 24 hour post-transfusion survival for fresh, aged, and aged LR blood was 100%, 38.7% (95% CI 31.8-45.6), and 43.9% (95% CI 35.9-51.9). In 4 of 5 experiments, HEL and Fyb expression on aged RBCs was identical to that of fresh RBCs. **Conclusions:** Transfusion of LR and non-LR transgenic HOD RBCs, stored for 14 days in conditions similar to those used in human blood banking, induce higher levels of alloimmunization than freshly collected and transfused RBCs. This cannot be explained solely by the presence of contaminating WBCs or bacteria. In addition, because washed RBCs are as immunogenic as unwashed RBCs, the RBCs themselves may be responsible for the increased immunogenicity. Although the 24 hour post-transfusion survival is below the average for human RBCs, this study is a proof of principle testing of the effect of aging on RBC alloimmunization. The reproducibility of these findings in other RBC antigen systems, as well as the potential translational applicability, remains to be determined.

Disclosure of Commercial Conflict of Interest

J. E. Hendrickson: Nothing to disclose; C. D. Hillyer: Nothing to disclose; E. A. Hod: Nothing to disclose; S. L. Spitalnik: Nothing to disclose; J. C. Zimring: Nothing to disclose

Disclosure of Grants Conflict of Interest

J. E. Hendrickson: Nothing to disclose; C. D. Hillyer: Nothing to disclose; E. A. Hod: Nothing to disclose; S. L. Spitalnik: Nothing to disclose; J. C. Zimring: Immucor Inc., Grants or Research Support

S65-030F

Crossmatch Incompatible RBCs Have an Intrinsic Range of Susceptibility to Hemolysis

J S Liepkalns[1] *(jsliepk@emory.edu)*, J C Zimring[1]. [1]*Center for Transfusion and Cellular Therapies, Emory University School of Medicine, Atlanta, GA, USA*

Background: During crossmatch incompatible transfusions, clinically significant antibodies can lead to brisk hemolysis. However, for some blood group antigens, antibodies are hemolytic in certain patients but not in others. The reason for this variability is poorly understood. Using a mouse model of crossmatch incompatible transfusion involving human glycophorin A (hGPA) as an RBC antigen, we have previously observed that some hGPA RBCs clear but others continue to circulate despite being coated with IgG. We have also reported that the mechanism of resistance was neither antibody depletion nor saturation of the reticuloendothelial system. To further characterize hemolysis resistance, we tested whether resistance is an acquired or intrinsic property of the RBC. **Methods:** Incompatible hGPA RBCs and compatible wild-type RBCs were labeled with fluorescent dyes DiI and DiO, respectively. Mixtures of the labeled RBCs were transfused into wild-type recipients (transfusion 1) that had been passively immunized with a monoclonal antibody against hGPA (6A7). Two days post transfusion, RBCs were collected and were mixed with freshly isolated hGPA RBCs labeled in a third color (DiD). This mixture was then transfused into naive mice (transfusion 2), which were likewise passively immunized with 6A7. In all cases, RBC survival was determined through enumerating each population by flow cytometry. Clearance in transfusion 1 was determined by calculating survival of hGPA RBCs as a function of compatible wild-type RBCs. Hemolysis resistance was defined during transfusion 2 as decreased clearance of hGPA RBCs from transfusion 1 compared to clearance of fresh hGPA RBCs. Titrations of 6A7 were performed in transfusions 1 and 2. **Results:** In transfusion 1, hGPA RBCs showed initial rapid clearance proportional to the amount of 6A7 injected. In all cases, the surviving hGPA RBCs were 90-100% resistant to clearance in transfusion 2 when exposed to the same concentration of 6A7 as in transfusion 1. However, if an increased concentration of 6A7 was used in transfusion 2, then resistance to clearance was less (range 20-60%). **Conclusion:** The observation that hGPA RBCs are resistant to clearance by the same concentration of 6A7 in transfusion 2 as in transfusion 1, but are less resistant to increased amounts of 6A7 in transfusion 2, suggest that RBCs have a range of susceptibility to clearance as a function of antibody concentration. The mechanism of differential susceptibility to clearance is uncertain, but may include RBC age or antigen density.

Disclosure of Commercial Conflict of Interest

J. S. Liepkalns: Nothing to disclose; J. C. Zimring: Nothing to disclose

Disclosure of Grants Conflict of Interest

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Transfusion-Transmitted Diseases: Arboviruses

S66-030G

Dengue Viremia in Donations from Puerto Rico During the 2007 Dengue Outbreak

S L Stramer[1] *(stramers@usa.redcross.org)*, J M Linnen[2], J M Carrick[2], D Krysztof[1], K D McMilin[3], A De Vera[4], E A Hunsperger[5], J L Muñor[5], R Y Dodd[6]. [1]*Scientific Support Office, American Red Cross, Gaithersburg, MD, USA;* [2]*Gen-Probe, Inc., San Diego, CA, USA;* [3]*Biomedical Services - Alabama, American Red Cross, Birmingham, AL, USA;* [4]*Biomedical Services - Puerto Rico, American Red Cross, San Juan, PR, USA;* [5]*Centers for Disease Control (CDC), San Jaun, PR, USA;* [6]*Holland Laboratory, American Red Cross, Rockville, MD, USA*

Background: Dengue virus is the most important arbovirus in the world; its range is expanding. Like WNV, dengue is transmitted naturally by the bite of an infected mosquito but also is transfusion transmitted. Data from 2005 in Puerto Rico (PR), a dengue-endemic area, demonstrated a rate of donor viremia of 1:1300 during the latter half of the 2005 epidemic season. In 2007, a much larger dengue outbreak occurred in PR from which samples from donors during the epidemic period were retained for testing to further confirm donor viremia rates and for recipient tracing of components from positive donations. **Methods:** Samples were retained in a repository and split into two sets for viremia studies: those units exported and transfused in the continental US and those transfused in PR. Samples were tested individually by a research transcription mediated amplification assay (TMA, Gen-Probe). Initially reactive (IR) samples were retested by the original TMA and an alternate TMA (alt TMA used for the units transfused in PR only) without dilution and at a 1:16 dilution to model pooling. All TMA-repeat reactive (RR) samples were considered confirmed. Additional virologic/infectivity and serologic testing was performed at the CDC dengue branch in PR including PCR to define the dengue serotype and viral load, mosquito cell culture and IgM testing. Hospitals receiving components from RR donations were contacted to initiate recipient tracing including a detailed questionnaire about symptoms and risk factors. The study was IRB approved. **Results:** A total of 15,350 samples were tested with 28 IR and 25 RR samples considered confirmed positive (pos) for a prevalence of 1:614 consisting of 12 dengue-pos donations exported from PR into the continental US (1:533) and 13 pos donations that remained in PR (1:689). Specificity was 99.98%. A 1:16 dilution detected 14/25 (56%) RR donations. Further supplemental testing (CDC) demonstrated dengue virus serotypes 1, 2 and 3 (corresponding to those circulating in PR); 11/25 (44%) samples had RNA titers of 10^5-10^9 copies/mL of which all 11 also infected C636 mosquito cell cultures. 9/11 (82%) PCR-pos samples were detected at a 1:16 dilution. 6/22 (27%) samples tested for IgM were pos, only 2 of which had quantifiable virus (10^6 and 10^8) with 1 detected at a 1:16 dilution. Of the 4 remaining IgM pos samples, only 1 was pos at a 1:16 dilution (low level pos) for a total of 2 IgM-pos samples detected when diluted. Recipient tracing in the continental US and PR is underway. **Conclusions:** Like the prior study identifying dengue viremic donations in PR, this study demonstrates a high frequency of viremia during dengue-epidemic periods with nearly half of the RNA-pos donations lacking IgM, having high-titer viremia and infectious in cell culture. Screening of donors should be considered during dengue-epidemic periods.

Disclosure of Commercial Conflict of Interest

J. M. Carrick: Gen-Probe Incorporated, Ownership or Partnership; A. De Vera: Nothing to disclose; R. Y. Dodd: Nothing to disclose; E. A. Hunsperger: Nothing to disclose; D. Krysztof: Nothing to disclose; J. M. Linnen: Gen-Probe Incorporated, Stocks or Bonds; K. D. McMilin: Gen-Probe Incorporated, Other; J. L. Muñor: Nothing to disclose; S. L. Stramer: Nothing to disclose

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S67-030G

Highly Sensitive and Equivalent Detection of Dengue Virus Serotypes 1, 2, 3, and 4 with an Enhanced Transcription-Mediated Amplification Assay

J M Carrick[1] *(jamesca@gen-probe.com)*, J Knight[1], C Lontoc-Bugay[1], C Motta[1], J B Wellbaum[1], C Fleischer[1], J L Muñor[3], S L Stramer[2], J M Linnen[1]. [1]*Gen-Probe Incorporated, San Diego, CA, USA;* [2]*American Red Cross, Gaithersburg, MD, USA;* [3]*Centers for Disease Control and Prevention, San Juan, PR, USA*

Background: Based on WHO estimates, the incidence of dengue has grown dramatically around the world in recent decades and is now considered to be a major international public health concern. To investigate the risk of dengue virus (DENV) transfusion transmission, we developed a prototype nucleic acid test (NAT) based on Transcription-Mediated Amplication (TMA) that was used to show the feasibility of detecting DENV RNA in asymptomatic blood donors from Honduras, Brazil, and Puerto Rico and in clinically ill patients from Puerto Rico. Our previous results demonstrated the importance of detecting all 4 DENV serotypes at low copy levels with equivalent sensitivity. Recently, we developed an improved TMA Assay with increased sensitivity for each of the 4 serotypes. **Methods:** The enhanced TMA assay uses the same technology as other PROCLEIX® assays, consisting of lysis and target capture of viral RNA followed by TMA and chemiluminescent detection by Hybridization Protection Assay (HPA). Analytical sensitivity for serotypes 1, 2, 3, and 4 were determined by probit analysis of results from testing serially diluted live DENV and DENV RNA transcripts. Live DENV was obtained from the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO. Assay specificity was determined by testing 988 US blood donor specimens and 8,680 donor specimens from Puerto Rico that were screened previously with the earlier version of the TMA assay. Previous screening of these specimens yielded 14 positive results. Samples were tested on the fully automated PROCLEIX® TIGRIS® System. **Results:** The enhanced dengue assay showed 95% detection at 14.9, 18.3, 13.0, and 16.4 copies/mL of DENV 1, DENV 2, DENV 3, and DENV 4, respectively. Analytical sensitivities for each of the four serotypes were determined to be not statistically different. There were no reactive samples among the US donations. The improved assay was able to detect all 14 positive donations identified by the original assay in the Puerto Rican donations; an additional 7 reactive samples were identified with the improved assay, of which 4 were repeat reactive. The overall assay specificity from testing the US and Puerto Rican donations was 99.97% (95% CI: 99.91-99.99). **Conclusions:** Using the improved dengue TMA assay we demonstrated reliable detection of all 4 serotypes of DENV below 20 copies/mL while maintaing high clinical specificity. The analytical and clinical sensitivity results from this study indicate that the improved dengue assay has the potential to identify a larger number of low viral load DENV infections in both blood screening and diagnostic applications.

Disclosure of Commercial Conflict of Interest

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S68-030G

Correlation between Yield of WNV NAT Screening of North Dakota Donors Over 6 Epidemic Seasons with WNV Seroprevalence at the End of 2008

M P Busch[1] *(mbusch@bloodsystems.org)*, V Winkelman[2], J Dunn Williams[2], H E Prince[3], C Yeh[3], B Custer[1], L R Petersen[4]. [1]*Blood Systems Research Institute; & Univ California, San Francisco, San Francisco, CA, USA;* [2]*Blood Systems Laboratory, Tempe, AZ, USA;* [3]*Focus Diagnostics, Cypress, CA, USA;* [4]*DVBID, Centers for Disease Control & Prevention, Ft Collins, CO, USA*

Background: MP-NAT for WNV was implemented in 2003, with progressive enhancement in screening sensitivity over the next 6 years by using targeted ID-NAT in epidemic areas with increasingly stringent trigger criteria. In our system, North Dakota (ND) has had the highest overall rate of WNV+ donations. Seasonal yield has fluctuated, but remained below the 2003 peak yield. This lower yield may be partly attributable to prior WNV infections in the population, leading to population immunity. This cross-sectional study determined WNV antibody seroprevalence after the 2008 transmission season, and correlated this seroprevalence with annual NAT yield rates in the state. **Methods:** 5000 samples from ND blood donations were archived from late Oct-Dec 2008, >1 month after the last NAT yield donation and last WNV case report in ND. Samples from donors resident in ND were selected and tested for WNV IgG; IgG-positive donations were further tested for WNV IgM to identify recent infections (Focus Diagnostics). NAT yield cases (confirmed by replicate NAT/serology on index donation and/or follow-up samples) from ND donors were compiled by year, and further sorted into those detectable by MP-NAT (based on MP-NAT detection, or reactivity at 1:16 dilution if detected by ID-NAT) vs those detectable only by ID-NAT. Annual incidence was projected based on annual MP-NAT yield and a 6.9-day MP-NAT yield window period (Busch et al, EID, 2005). **Results:** Of 3594 donations by ND donors from Oct-Dec 2008 tested for IgG, 296 (8.2%; 95%CI 7.3-9.1) were positive for WNV IgG; of these 26 (8.8%) confirmed positive for WNV IgM. The yield of WNV MP-detectable (MP-NAT+) and ID-only detectable (ID-NAT+) donations, and the projected WNV incidence/year, are shown in the table. **Conclusions:** The proportion of ND residents previously exposed to WNV, based on donor IgG seropositivity in late 2008, is currently 8.2%. Thus the general decline in WNV NAT yield in the past 6 years is not attributable to human population immunity, but rather likely due to ecological factors influencing WNV transmission to humans. The 8.8% rate of IgM detection among IgG+ donations is consistent with the proportionate yield of infections in 2008 (7/124, 5.6%), with some contribution of persistent IgM from 2007 infections. Cumulative annual incidence projected from annual MP-NAT yield cases correlated reasonably well with observed IgG seroprevalence, suggesting that cumulative MP-NAT yield data from other areas can be used to project WNV infection rates throughout the US.

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Year	Donations	Total NAT+	MP-NAT+	ID-NAT+	Incidence
2003	66,109	62	42	20	3.3%
2004	67,117	1	1	0	0.1%
2005	68,150	10	5	5	0.4%
2006	68,652	16	6	10	0.5%
2007	73,640	28	12	16	0.9%
2008	78,306	7	5	2	0.4%

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
			2009. 11. 24	該当なし	
一般的名称	新鮮凍結人血漿	研究報告の公表状況	ABC Newsletter #41. 2009 Nov 13; 4-6.	公表国 米国	
販売名（企業名）	新鮮凍結血漿「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社）				

研究報告の概要

○バベシア症拡大を示す研究を受け、米国赤十字は7州での供血検査の実施を提案

Transfusion誌で近く発表される最近の3つの研究で、供血血液におけるバベシア症の原因となる寄生虫の陽性率増加および輸血伝播バベシア症(TTB)の増加が認められた。1つはコネチカット州とマサチューセッツ州において供血血液のどの程度の範囲に拡大しているかを示した。2つ目の研究ではロードアイランド州において、輸血を介した伝播範囲を確認した。3つ目では、米国赤十字のヘモビジランス・プログラムを通して報告された症例を用いて、感染した供血者と受血者の特徴を調べている。これらの研究から、バベシア症とTTBの危険が増大しているという懸念が明らかになった。

これらのデータに基づき、米国赤十字は疾患が流行する北東部および中西部北部の7州で供血血液のIFA検査を開始する2つの計画を作成した。

バベシア症は、Ixodes属のマダニによって伝播し、米国ではほとんどがBabesia microti(マラリアと類似した赤血球を汚染する寄生虫)に起因する。大部分の感染者は無症候か軽症であるが、特定の健康要因を有する人では、重症、致命的となる場合もある。無症候感染は何ヶ月も続く可能性がある。現在、米国食品医薬品局(FDA)が認可したバベシア症の検査はなく、血液センターは供血者にバベシア症の既往を尋ねるのみである。しかし、大半の罹患者は自分の感染を知らないため、問診の効果には疑問がある。寄生虫保有者が供血した場合、受血者に輸血を介して感染させる可能性がある。これまでに赤血球と血小板で輸血による伝播が報告されている。合併症と死亡者数の急増により、TTBに対する懸念が浮上した。1997～2004年にFDAに報告されたTTB関連死亡例は1例のみだったが、2005年11月～2008年9月では9例以上となった。FDAは2008年9月にワークショップを開催し、AABBは70以上の症例の報告を受けて2009年8月に公報を発行した。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
米国東部と中西部におけるバベシア症拡大を示す研究を受け、米国赤十字が7州での供血検査の実施を提案したとの報告である。	今後も引き続き情報の収集に努める。

MedDRA/J Ver.12.1J

(14)

Red Cross Lobbying (continued from page 3)

In 2008, Congress appropriated $100 million in emergency funding to the American Red Cross to replenish its disaster relief reserves, which were depleted when the charity provided shelter, food and other services during a string of hurricanes earlier in the year. The Red Cross appropriation was set out in two sections 10502-03 of the Homeland Security bill, HR 2638 (Consolidated Security, Disaster Assistance, and Continuing Appropriations Act, 2009) and is explicitly for disaster relief purposes.

So far this year, the organization reports spending $154,890 on lobbying. Lobbyists for 2009 are listed as Cherae Bishop, Marc Decourcey, Neal Denton, Dawn Latham and Marin Reynes. (Sources: Senate Lobbying Disclosure Database; Implu Corp., an online business intelligence database) ♦

With Studies Showing Spread of Babesiosis, ARC Proposing to Test Donated Blood in Seven States

Three recent studies have discovered increases in the incidence of the parasite that causes babesiosis in donated blood and of transfusion-transmitted babesiosis (TTB). On the strength of that data, the American Red Cross (ARC) has developed two proposals to begin testing donated blood in states in the Northeast and the upper Midwest where the disease is endemic.

The picture emerging from the studies – each of which is forthcoming in *Transfusion* – shows babesiosis to be a growing threat. Each focuses on a different aspect of the problem. One study shows how widespread it is among blood donations in Connecticut and Massachusetts, another identifies the extent of its transmission through transfusions in Rhode Island, and the third determines the characteristics of infected donors and recipients, using cases reported through ARC's Hemovigilance Program.

> **Individually and collectively, the studies emphasize that concerns over the dangers of babesiosis and TTB are increasing. The ARC proposals involve setting up testing in affected areas, starting with Connecticut and potentially expanding to seven states – 16 percent of the nation's population.**

Babesiosis is carried by *Ixodes* ticks; in the US, it is mostly caused by *Babesia microti*, a parasite that is similar to malaria and that infects red blood cells. Most people infected with it do not experience any symptoms or experience only mild symptoms that can be mistaken for the flu; however, the disease can be severe and even fatal, particularly for people with certain complicating health factors. Asymptomatic infection may last for months. Currently, there is no Food and Drug Administration-approved test for the disease, and blood centers merely ask potential donors whether they have a history of babesiosis. But the fact that most people with the disease do not know they have it casts doubt on the effectiveness of the question.

If a person who carries the parasites donates blood, the disease can be transmitted through transfusion to a susceptible recipient. To date, transmission has been reported only with red blood cells (both fresh and frozen) and platelets.

Concerns about TTB have risen as the number of complications and deaths related to it has jumped. The Food and Drug Administration received only one report of a TTB-related death from 1997 to 2004; however, from November 2005 to September 2008, it received at least nine (see *ABC Newsletter*, 12/5/08). In September 2008, FDA held a workshop on TTB in the US. In August 2009, AABB issued a bulletin on it, prompted by reports of more than 70 cases of it (see *ABC Newsletter*, 8/14/09).

(continued on page 5)

Babesiosis (continued from page 4)

The three forthcoming studies aim at shedding light on the epidemiology of the babesiosis. In one study, led by Stephanie T. Johnson, MT (ASCP), MPH, who is with the ARC branch in Farmington, Conn., scientists tested blood donated at selected drives in Connecticut and Massachusetts from 2000 to 2007 for the presence of immunoglobulin (Ig)G antibodies to *Babesia microti*. Using an immunofluorescence assay (IFA), they found the antibodies in blood donated in all eight counties in Connecticut and three counties in Massachusetts. They also found it in blood donated not just during the season peak for the tick that causes the virus – from July through September – but also during the rest of the year.

Although the results of this study helped them identify particular areas and times of the year when the likelihood of *Babesia microti* in blood is highest, they also made clear that the threat extended beyond certain areas and months, which led the scientists to conclude that year-round, regional testing may be necessary to fully safeguard the blood supply from the transmission of the disease.

Scientists in Rhode Island reached a similar conclusion when they carried out a retrospective study in which they analyzed babesiosis cases that were reported to the Department of Health in that state from 1999 to 2007. Led by Leonard Mermel, DO, an infectious disease specialist and the director of infection control for the Rhode Island Hospital, this team identified 21 cases of TTB in the nine years they studied.

Their analysis of information about where donors lived and when they donated reinforced the finding in Johnson's study that some people with babesiosis lived in areas without high tick populations and had merely traveled to an area where babesiosis is more common. Drawing also on other studies that show that the virus can survive for extended periods in blood bank conditions, including refrigeration up to 35 days, these researchers conclude that TTB is possible any time of year and in any location. Their study also revealed a troubling rise in cases of TTB: from 1999 to 2007, 326,081 units of red blood cells were transfused, according to the Rhode Island Blood Center. The 21 cases of TTB during that period give an incidence rate for TTB of just more than 1 in 15,000 transfusions. However, by the last three years studied, that rate had risen to 1 in 9,000 units transfused.

To determine the characteristics of infected donors and recipients, the third team of researchers – led by Laura Tonnetti, PhD, a scientist with the ARC's Transmissible Diseases Department, Jerome H. Holland Laboratory, in Rockville, Md. – analyzed cases of suspected TTB that were reported to ARC's Hemovigilance Program from 2005 to 2007.

They carried out follow-up testing of previously collected blood donations, by IFA, Western blot, and/or real-time polymerase chain reaction (PCR) analysis. They found 18 definite or probable *Babesia microti* infections among transfusion recipients. Five of those recipients died. Of the 18 cases, two recipients had sickle cell disease and four were asplenic; 13 were between the ages of 61 and 84 and two were 2 years old or younger. The researchers concluded that TTB "can be a significant cause of transfusion-related morbidity and mortality," particularly when transfusion recipients were elderly, very young, or asplenic. Like the researchers in Rhode Island, these scientists also found that TTB stemmed both from donors who lived in areas where the disease is endemic as well as those who had merely traveled to those areas. They also found that IFA testing was more effective than PCR analysis: the formed identified all 18 donors, while the latter identified only one.

What Should Be Done? The conclusions of these studies – that babesiosis can occur anywhere at any time, that the number of TTB cases is rising, and that TTB can lead to serious complications from transfusions, including death – gave new data to support ARC proposals for testing donated blood for evidence of infection, which Dr. Tonnetti discussed in a presentation at the recent AABB Meeting.

(continued on page 6)

Babesiosis (continued from page 5)

The first proposal is to establish testing donated blood in Connecticut by IFA. ARC's recommendations include year-round IFA testing under investigational new drug regulations. Only whole-blood donations would be tested. Donors associated with positive results would be deferred, and their donations would be discarded. Testing could be done throughout the state or only in highly endemic areas. The latter approach would be less expensive, but it may only identify one-third of at-risk donors, so ARC favors testing across the state.

Depending on the results of that project, said Dr. Tonnetti, ARC would like to expand the area to include Rhode Island, Massachusetts, New York, New Jersey, Minnesota, and Wisconsin. Connecticut was chosen as the starting point, she explained in a phone call, because earlier studies had found a number of endemic areas in the state. But she emphasized that expanding the testing to other states would be important, given that babesiosis and TTB can spread so easily. No timeline has been set for testing under either proposal.

Citations. Asad S, *et al.* Transfusion-transmitted babesiosis in Rhode Island. Transfusion. 2009 Sep 16 [epub ahead of print]; Johnson ST, *et al.* Seroprevalence of *Babesia microti* in blood donors from *Babesia*-endemic areas of the northeastern United States: 2000 through 2007. Transfusion 2009 Oct. 10 [epub ahead of print]; Tonnetti L, *et al.* Transfusion-transmitted *Babesia microti* identified through hemovigilance. Transfusion. 2009 Jul 16 [epub ahead of print] ♦

FDA Finalizes Guidance on Testing Donated Blood for West Nile Virus

The Food and Drug Administration has finalized its guidance for blood centers on how they should test donations of whole blood and blood products for West Nile Virus (WNV). This guidance replaces the draft guidance dated April 28, 2008, and it takes into account a number of the comments FDA received from America's Blood Centers (ABC) and other sources.

While the draft guidance included recommendations for screening cells, tissues, and cellular-based products, the final guidance covers only donations of whole blood and blood products. Key recommendations are that blood centers should test whole blood and blood products for WNV year-round; that they may use minipool tests when there is not high WNV activity in their area; that each center may establish its own criteria for high WNV activity; that centers switch to individual testing as soon as possible, but not later than 48 hours, after high WNV activity is found in their area; and that if a minipool tests as reactive for WNV, each unit in that minipool should be tested with an individual test. It also recommended that, for individual units that test positive, additional testing "may be of value in donor counseling."

Background. It has been known since 2002 that donors who were infected with WNV could be viremic but not have any symptoms; it has also been known that the virus could be transmitted through blood transfusions and organ transplantation. FDA began studies the following year aimed at evaluating nucleic acid tests (NAT) for detecting WNV, and it has approved biologics license applications for two NAT since 2005. Both tests are used for individual donor samples, and for minipools of samples taken from either 6 or 16 donations.

Studies have found that the individual test (ID-NAT) has greater sensitivity than the minipool test (MP-NAT), and that, in fact, up to 25 percent of viremic units were not detected by the MP-NAT. However, it is not feasible or practical to test every unit individually, because of limited availability of the tests and personnel and logistical issues. This guidance, then, is meant to clarify when blood centers should use ID-NAT and when they may use MP-NAT.

(continued on page 7)

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2010 年 2 月 17 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion. Steven H. Kleinman et al., BLOOD, 22 OCTOBER 2009 _ VOLUME 114, NUMBER 17	公表国 米国	
販売名（企業名）						

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
ヒトパルボウイルス B19 (B19V) 感染症は，溶血または赤血球生成不全症候群などのある血液病患者にとって重篤な感染症となり得る。血漿製剤とは対照的に，成分輸血による B19V 感染症例報告は稀であるが，いずれの研究においても，B19V DNA 陽性成分輸血の受血者への感染率は体系的に測定されていない。本研究では，供血者および受血者由来の保存血液検体中の B19V DNA 量を高感度のリアルタイム定量 PCR アッセイにより測定し，B19VDNA 陽性成分（赤血球製剤 77%，全血由来血小板製剤 13%，新鮮凍結血漿製剤 10%）の成分輸血による B19V 感受性（輸血前に B19V IgG 抗体陰性）受血者の B19V 感染率を評価した。実際には B19VDNA 陽性であった 105 例の供血者由来の B19VDNA 陽性成分 112 検体が輸血された，輸血前 B19VIgG 抗体保有率 78%の 112 人の患者群（２４名が感受性受血者）について調査を行い，IgG あるいは IgM への抗体陽転，もしくは B19V DNA の新規検出をもって，B19V 感染成立と定義した。その結果，B19V DNA 量が 10^6 IU/mL 以下の成分輸血を受けた感受性受血者 24 例への B19 感染伝播は見られなかった (95%信頼区間，11.7%)。B19V DNA 量が 10^{10} IU/mL 以上の成分輸血を受けた非感受性受血者（輸血前 B19V IgG 抗体陽性）1 例で既往反応が認められた。本研究では，B19V DNA 量 10^6 IU/mL 以下の成分輸血による感染伝播は起こらない，また，もし感染が起こったとしても，感染率が 50%以上を示す多くの輸血感染症（HIV，HCV など）と比較すると，B19V 感染はまれな事象であることが示された。	BYL-2010-0397

報告企業の意見	今後の対応
本研究では，受血者の状態による評価はなされておらず，また調査の規模つまり，評価のターゲットである感受性受血者数が少ないためこれらを加味した研究がという問題が残されてはいるが，ヒトパルボウイルス B19 の DNA 量について，10E6IU/m l という安全域の目安が示された。なお，弊社のコージネイト FS の製造工程培地で使用されている血漿分画成分に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。現在の科学水準では，ヒトパルボウイルス B19 を確実に不活化する方法は存在しないため，感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	現時点で新たな安全対策上の措置を講ずる必要はないと考えるが，今後ともウヒトパルボウイルス B19 の感染に関する情報収集に努める。

(15)

BYL-2010-0397

A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion

Steven H. Kleinman,[1,2] Simone A. Glynn,[3] Tzong-Hae Lee,[4] Leslie H. Tobler,[4] Karen S. Schlumpf,[1] Deborah S. Todd,[1] Hannah Qiao,[1] Mei-ying W. Yu,[5] and Michael P. Busch,[4,6] for the National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study-II (NHLBI REDS-II)

[1]Westat Inc, Rockville, MD; [2]Department of Pathology, University of British Columbia, Vancouver, BC; [3]National Heart, Lung, and Blood Institute, Rockville, MD; [4]Blood Systems Research Institute, San Francisco, CA; [5]Division of Hematology, Center for Biologics Evaluation and Research, US Food and Drug Administration, Bethesda, MD; and [6]Department of Laboratory Medicine, University of California, San Francisco

Parvovirus B19V infection can be a serious infection for hematology patients with underlying hemolysis or compromised erythropoiesis syndromes. Although case reports of B19V transmission by blood component transfusion (as contrasted to manufactured plasma derivatives) are rare, no studies have systematically determined a rate of transmission to recipients transfused with B19V DNA–positive components. We used a linked donor and recipient repository and a sensitive, quantitative B19V DNA polymerase chain reaction (PCR) assay to assess such transmission in B19V-susceptible (ie, anti-B19V immunoglobulin G [IgG] negative) recipients. We assessed 112 B19V DNA–positive components from 105 donors (of 12 529 tested donations) transfused into a population of surgical patients with a pretransfusion B19V IgG seroprevalence of 78%. We found no transmission to 24 susceptible recipients from transfusion of components with B19V DNA at concentrations less than 10^6 IU/mL (upper 95% confidence interval, 11.7%). We found an anamnestic IgG response in one pretransfusion seropositive recipient transfused with a component containing greater than 10^{10} IU/mL B19V DNA. These findings show either that transmission from components with less than 10^6 IU/mL does not occur, or, if it does, it is an uncommon event. These data do not support the need to routinely screen blood donations with a sensitive B19V DNA nucleic acid assay. (Blood. 2009;114:3677-3683)

Introduction

There have been multiple reports of parvovirus B19 (B19V) transmission by pooled plasma products, including factor VIII concentrate and solvent-detergent-treated pooled plasma, documented by recipient seroconversion in asymptomatic cases or, less frequently, by clinical diagnosis of B19V-related disease in association with positive B19V test results.[1-5] These cases, combined with the potential for very high B19V DNA concentrations (up to 10^{12} IU/mL) in plasma donations[4] and the relative resistance of B19V to inactivation methods,[4,6] have led to B19V DNA testing of plasma donations to ensure that manufacturing plasma pools destined for plasma derivatives have a B19V DNA concentration less than or equal to 10^4 IU/mL, a limit proposed by the Food and Drug Administration (FDA).[7-9] The same limit for this so-called "in process testing" is a European regulatory requirement for anti-D immunoglobulin (Ig) preparations and plasma treated for virus inactivation.[10] To achieve this B19V DNA concentration in the final plasma pool, B19V DNA screening of the plasma donations used to make the pool is performed using assays (applied in minipool format) with the ability to detect approximately 10^6 IU/mL in an input unit of plasma.[8]

To date, no B19V transmissions from pooled plasma products have been documented when less than 10^3 to 10^4 IU/mL B19V DNA is present in an infused product.[3,4,11-13] The reason for this lack of infectivity is not completely understood. It may be due to an inadequate amount of infused infectious virions, a neutralization effect from B19V antibody present in other plasma units in the plasma pool, or a combination of these factors. Recipient factors may also play a role because it has been reported that B19V antibody is protective against B19V reinfection, and most of the adult population is B19V seropositive as a result of previous infection.[13]

Although concern for transmission of B19V from pooled plasma products has resulted in B19V DNA screening of input plasma donations, less is known about the potential for B19V transmission by transfusion of individual blood components (eg, red cells, platelets, plasma). There are only 4 published clinical cases of B19V transmissions from blood component transfusion (3 from red cells and 1 from platelets).[14-17] An additional asymptomatic case has been reported from a recent prospective study of transfusion-transmitted viral infections.[18] In contrast, 2 studies have reported a small number of negative results when patients transfused with B19V DNA–positive components were evaluated for laboratory markers of B19V infection.[19,20] Nevertheless, given the tropism for[21] and potential pathophysiologic effects of B19V infection on erythroid precursor cells,[22] concern remains for potential deleterious outcomes in frequently transfused hematology patients with underlying hemolysis or compromised erythropoiesis syndromes.[13]

Because the sensitivity of B19V DNA assays has improved, B19V DNA prevalence in blood donors has been shown to be

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higher than initially documented. B19V DNA is detectable in 0.5% to 0.9% of blood donations, with most showing relatively low DNA concentrations (< 100-1000 IU/mL).[23-25] In addition, it has become established that B19V infection is often persistent.[25-27] Thus, some donors may continue to donate for many years with B19V DNA (and potentially infectious virions) in their blood. These observations suggest that the potential for recipients to be exposed to low levels of B19V DNA from blood component transfusion is greater than previously thought.

To our knowledge, there have been no large-scale donor/recipient-linked transfusion-transmission studies to evaluate the rate of B19V transfusion transmission. Although it has been assumed by extrapolation from pooled plasma transfusions that single unit blood components with low-level B19V DNA should be noninfectious, this remains speculative because the mechanism of protection in the pooled plasma setting has not been established and may not apply to single unit transfusions.[12,13]

We undertook this present study to systematically evaluate whether transfusion of blood components with low or moderate levels of B19V DNA (defined as < 10^6 IU/mL) transmits infection to B19V-seronegative susceptible recipients.

Methods

Source of donor and recipient samples

Tested specimens were from the National Heart, Lung, and Blood Institute (NHLBI) Retrovirus Epidemiology Donor Study Allogeneic Donor and Recipient (RADAR) repository, which was established to investigate possible transfusion-transmitted infections and which has been described in detail in a previous publication.[28] Repository specimens were collected from 2000 through 2003 by blood centers and selected hospitals at 7 geographically dispersed US locations. Repository specimens consisted of 2 frozen 1.8-mL plasma aliquots and a 1.5-mL sample of frozen whole blood.

All enrolled donors and recipients gave informed consent for frozen specimen storage and for subsequent specimen testing for possible transfusion-transmissible infections, in accordance with the Declaration of Helsinki. The study protocol was approved by the institutional review board of each participating institution.

The linked portion of this donor-recipient repository contains pretransfusion and/or peritransfusion specimens and follow-up specimens, collected at a 6- to 12-month interval, from 3575 enrolled recipients. It also contains 13 201 donation specimens given by 12 408 distinct donors that were transfused to these recipients. The RADAR enrollment procedure targeted recipients with expected high 1-year survival rates; 88% were cardiac or vascular surgical patients, and the median recipient age was 68 years (range, 59-74 years). Recipients were not evaluated for coexisting immunosuppression, but this is considered unlikely given the primary diagnoses. The mean number of RADAR donation exposures per recipient was 3.9. The distribution of component types transfused was 77% red cells, 13% whole-blood–derived platelet concentrates, and 10% fresh-frozen plasma (FFP). In addition to receiving components with a stored donation specimen in the RADAR repository, these recipients also received a mean of 3.1 components not linked to stored RADAR donations.

The RADAR repository also contains 99 906 specimens from blood donations that were not transfused to enrolled RADAR recipients; this supplementary repository served as a sample source during the assay validation and donor prevalence phase of the study, which has previously been reported.[24]

Selection and testing of donations

All RADAR donations transfused to enrolled recipients were tested for B19V DNA, provided there was adequate specimen volume available.[24] Donations found reactive on the B19V DNA assay were subjected to DNA confirmatory and quantitative testing; confirmed positive donations were also tested for B19V IgG and IgM.

Selection and testing of recipients

Cases were recipients who were transfused with one or more B19V DNA–positive components. Control recipients were selected to measure the background rate of new infection as a result of factors other than transfusion of a B19V DNA–positive RADAR unit (ie, community-acquired infection in the 6- to 12-month follow-up interval or a transfusion-acquired infection from a B19V DNA–negative RADAR unit or a nontested, non-RADAR unit). A 1:2 case-control design was used to select control recipients fulfilling the following criteria: all RADAR units received by the recipient were B19V DNA negative, enrollment occurred at the same participating center in approximately the same time frame (to control for community-acquired infection), and age was within 10 years of the case recipient. Using this control selection algorithm, we established that all controls met preestablished age and center criteria, and 94.4% received their transfusion within 11 days of their matched recipient.

Enrollment specimens from all case and control recipients were tested for B19V IgG. Before knowledge of B19V IgG enrollment results, posttransfusion follow-up specimens from all cases and controls were tested for B19V IgG, IgM, and DNA (see "Assay methods"). A positive B19V DNA or IgM result on the follow-up specimen triggered additional testing of the enrollment specimen for these analytes.

For analysis, case and control recipients with negative B19V IgG results before transfusion were subsequently classified as B19V susceptible, and those with positive results were classified as B19V nonsusceptible.

Protocol for evaluating transfusion-transmission

B19V transmission was defined as seroconversion to IgG or IgM or new detection of B19V DNA. Because our previous experience with B19V antibody testing has shown that specimens near the cutoff could show fluctuating results on different test runs, we required that seroconversion be independently shown by 2 laboratories.

Assay methods

B19V DNA PCR assay. The B19V DNA polymerase chain reaction (PCR) assay was originally developed by Chiron Corporation and subsequently refined through collaboration between Chiron and Blood Systems Research Institute (BSRI). We previously reported data on assay performance on 5020 plasma samples from the unlinked donor portion of the RADAR repository.[24] The assay had a 50% limit of detection (LOD) of 1.6 IU/mL (95% confidence interval [CI], 1.2-2.1 IU/mL) and a 95% LOD of 16.5 IU/mL (95% CI, 10.6-33.9 IU/mL). We determined that the assay could be used as a quantitative as well as a qualitative assay; because quantitation might not be precise at the lower LOD, we categorized all specimens with quantitative DNA values of greater than 0 but less than 20 IU/mL as having a value of less than 20 IU/mL.

The assay, performed at BSRI, included a magnetic-bead B19V DNA capture step followed by a TaqMan real-time PCR assay targeting the VP1 region of the genotype 1 B19V genome. The assay was subsequently validated as detecting genotype 2 but does not detect genotype 3, which has been identified in Africa but which is very rare outside that continent.[20] An internal control sharing homologous primer region sequences but with a different internal probe binding sequence as the viral target was included in each assay tube. All captured target DNA from 0.5 mL input plasma and the spiked internal control was amplified in a single PCR reaction by using the same primer pair. Amplification and detection occurred in a 96-well optical plate by using dual-plexed TaqMan PCR technology. B19V target and internal control DNA were detected and distinguished by fluorophore-tagged sequence-specific probes. Each plate contained 2 known positive, 2 blinded negative, and 2 blinded positive controls and up to 90 study specimens. A more detailed assay description is provided in the previous publication.[24]

Because the chosen assay cutoff of a cycle threshold (C_T) of less than 40 was designed to maximize assay sensitivity, an algorithm was developed for final test interpretation so as to avoid classifying nonspecific reactivity on a single assay run as a confirmed positive result (Figure 1). All initially positive, indeterminate, and invalid specimens were retested in duplicate on plates that included quantitative run standards by using 2 separate 0.5-mL subaliquots subjected to the full extraction, amplification, and detection procedure. This testing served both as confirmation and quantitation. Final interpretation was based on the results of the 3 assays (ie, the initial screening assay and the duplicate repeat assays). Specimens were classified as B19V DNA positive if at least 2 of 3 tests showed reactivity at a C_T less than 40.

For determining DNA concentration, duplicate quantitative run standards (containing B19V DNA at 10^0 to 10^6 IU/mL) were placed on each plate, and quantitative results were determined by comparing the specimen C_T to the C_T of the known standards on the same test run.[24] The assigned quantitative value for each specimen was the average of the duplicate quantitative assays (including zero for a negative test result). Specimens with low C_T values (< 30) were diluted 1:10 and 1:100 and then run in triplicate at each dilution. The quantitative result was the average of the 3 test results at the most appropriate dilution adjusted by the dilution factor.

Serologic assays. Testing for B19V IgG and IgM was directed against a recombinant VP2 protein and was performed in duplicate by using FDA-cleared test kits (Biotrin) according to the manufacturer's instructions. Testing was conducted at BSRI and, for a large subset of samples, was repeated at a Center for Biologics Evaluation and Research/Food and Drug Administration (FDA) laboratory (Bethesda, MD). If results fell into the equivocal zone, the assay was repeated in duplicate on a new aliquot, and this repeat result was taken as the final result for the specimen.

Quantitative B19V IgG testing was performed by using a standard curve dilutional analysis method with the World Health Organization First International Standard for B19V serum IgG (93/724) obtained from the National Institute for Biological Standards and Control.[29] This testing was applied to enrollment and follow-up specimens of B19V IgG–positive ("nonsusceptible") recipients who had been transfused with the 5 highest titer B19V DNA components identified through donor testing.

Statistical methods

On the basis of a review of donor B19 viremia and recipient B19V serologic data from phase 1 of this study,[24] we determined that testing of the linked donor and recipient RADAR repository specimens would have sufficient statistical power such that a finding of zero documented transmissions to susceptible recipients would indicate with 95% confidence that the true B19 transfusion–transmission rate was between 0% and 25%. In this current study, StatXact (Cytel) was used to generate upper 95% confidence limits based on zero observed infections.[30] The upper confidence limit for transmission was calculated as a one-sided exact 95% confidence interval for the difference between the infection rate among susceptible cases and susceptible controls, using StatXact (Cytel).[30]

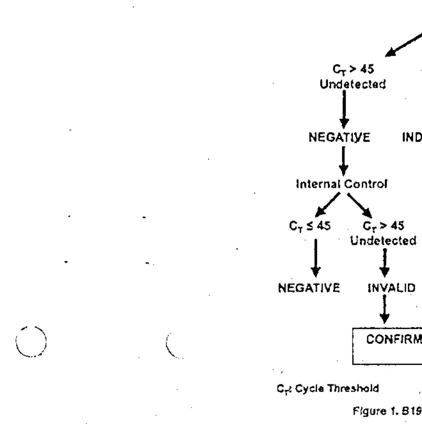

Figure 1. B19V DNA testing algorithm.

Results

Of the 13 201 linked blood donation repository specimens, 12 529 (95%) had adequate volume for testing. B19V DNA was detectable in 105 donations for a prevalence of 0.84% (95% CI, 0.68%-1.00%). As shown in Table 1, 53%, 71%, and 93% of these donations had B19V DNA concentrations below 20, 100, and 1000 IU/mL, respectively. The 2 donations with DNA concentrations greater than 10^6 IU/mL were negative for B19V-specific IgM and IgG, whereas B19V IgG was detectable in 96% and B19V IgM in 28% of the evaluable remaining B19V DNA–positive donations.

These 105 B19V DNA–positive donations came from 103 donors, 2 of whom gave positive donations on 2 occasions. The 105 positive donations resulted in the transfusion of 112 positive components to enrolled recipients. Four recipients received multiple DNA-positive components such that a total of 107 distinct recipients were transfused with one or more DNA-positive components. Table 2 provides a description of the DNA-positive components transfused to recipients, classified by the DNA concentration of the component, by whether the recipients were susceptible to B19V infection (ie, B19V IgG negative on their enrollment

Table 1. Quantitative B19V PCR and antibody results on confirmed positive donations

B19V DNA concentration, IU/mL, in donation	No. of B19V DNA–positive donations	No. (%) B19V IgM and IgG positive	No. (%) B19V IgM negative, IgG positive	No. (%) B19V IgM negative and IgG negative
Less than 20	56	2 (4%)	52 (93%)	2 (4%)
20 to less than 100	19*	5 (28%)	13 (72%)	0
10^2 to less than 10^3	23	18 (78%)†	2 (9%)	1 (4%)
10^3 to less than 10^4	4	4 (100%)	0	0
10^4 to less than 10^5	0	0	0	0
10^5 to less than 10^6	1	0	0	1 (100%)
Subtotal	103*	29 (28%)	67 (66%)	4 (4%)
More than 10^6	2	0	0	2
Total	105‡	29	67	6

The prevalence of B19V DNA–positive donations in 12 529 tested donations was 0.84%.

*One donor was not tested for B19V antibody; percentages have been calculated eliminating that donor from both the numerator and the denominator.

†Two donors were IgM equivocal and IgG positive.

‡The 105 B19V DNA–positive donations came from 103 donors, 2 of whom gave positive donations on 2 occasions.

Table 2. Transfusion of B19V DNA-positive components to recipients

B19V DNA concentration, IU/mL, in donation	No. of B19V DNA-positive donations	No. of B19V DNA-positive components transfused to susceptible recipients*				No. of B19V DNA-positive components transfused to nonsusceptible recipients				Total no. of B19V DNA-positive components transfused
		Red cells	Platelets	Plasma	Subtotal	Red cells	Platelets	Plasma	Subtotal	
Less than 20	56	15	0	1	16	33	6	5	44	60
20 to less than 100	19	3	0	0	3	9	5	3	17	20
10^2 to less than 10^3	23	3	1	0	4	16	3	2	21	25
10^3 to less than 10^4	4	0	0	1	1	2	0	1	3	4
10^4 to less than 10^5	0	0	0	0	0	0	0	0	0	0
10^5 to less than 10^6	1	0	0	0	0	1	0	0	1	1
Subtotal	103	21	1	2	24	61	14	11	86†	110†
More than 10^6	2	0	0	0	0	1	1	0	2	2
Total	105	21	1	2	24	62	15	11	88†	112†

*All B19V DNA-positive units transfused to susceptible recipients contained B19V-specific IgG.

†For 7 B19V DNA-positive donations, more than 1 component was transfused; also 4 nonsusceptible recipients received more than 1 positive component.

specimen), and the type of blood component. As per RADAR repository design, the majority (74%) of transfused DNA-positive components were red cell concentrates. Twenty-four of the 112 components (21%) were transfused into susceptible recipients. Among the 214 control recipients (2 controls selected per case), a very similar percentage (20%) were susceptible. Six of the 7 DNA-positive components with the highest concentrations were transfused to nonsusceptible recipients; these included all 3 components with DNA concentrations greater than 10^5 IU/mL.

The primary analysis of transfusion transmission was restricted to the 24 susceptible (B19V IgG negative) cases (21 transfused with red cells) and the 42 susceptible controls. There were no B19V infections observed in these 66 susceptible recipients based on the absence of B19V IgG, IgM, and DNA in the follow-up specimens. Thus, the transmission rate was 0% in both cases and controls, with an upper 95% CI of 11.7% in cases and 6.9% in controls. The transfusion-transmission rate was therefore estimated at 0.0% [0.0% (cases) − 0.0% (controls)], with an upper 95% CI of 11.7%.

Although IgG seroconversion could not be used as a criterion for establishing transfusion-transmission in nonsusceptible subjects (those with preexisting B19V IgG), the criteria of newly developed B19V DNA or IgM were still applicable. There were no such findings in case recipients. However, one IgM seroconversion was identified in a B19V IgG-positive (nonsusceptible) control recipient who remained DNA negative. Because this recipient was transfused with only 2 DNA-negative red cell units (and no non-RADAR units), it is likely that the IgM seroconversion represents a false-positive result or possibly a new community-acquired infection. Testing also identified B19V DNA in follow-up specimens of 3 other control recipients. However, testing of their enrollment specimens indicated that B19V DNA was present before transfusion at approximately the same concentration in all 3 cases. Furthermore, their enrollment and follow-up specimens were positive for B19V IgG antibodies. Thus, this pattern indicated persistent B19V infection (existing before receiving RADAR transfusions) rather than recent B19V acquisition.

To further evaluate whether transfusion with B19V DNA-containing units elicited an immune response in subjects with preexisting B19V IgG, we performed quantitative B19V IgG testing of enrollment and follow-up specimens of the 5 recipients who were B19V IgG positive at enrollment and who received the highest titer DNA-positive components, reasoning that these would provide the maximal stimulus for such an immune response. Pretransfusion B19V IgG levels were highly variable, ranging from 7 to 165 IU/mL. As seen in Table 3, 1 of the 5 recipients, who received the highest titer component (at a B19V DNA concentration of 2.9×10^{10} IU/mL or a total dose of ~ 5.8×10^{11} IU in the 20 mL plasma contained in the red blood cell component), showed a 4-fold increase in B19V IgG titer. This recipient had a relatively low pretransfusion titer of B19V IgG (15 IU/mL). Of the other 4 recipients, 1 showed a 2-fold increase, 2 had unchanged titers, and 1 showed an almost 2-fold decrease.

Discussion

In this study we identified donations that had a potential marker of B19V infectivity (ie, B19V DNA) through retrospective screening of blood donations and subsequently tested recipients of components from these donations for the development of new B19V infection. Our approach was designed to systematically determine a rate of transmission from all units with this potential infectivity marker and to establish either the presence or absence of transmission when it was known that a susceptible (ie, B19V IgG negative) recipient was transfused with a potentially infectious (ie, B19V DNA positive) unit. This study design is in contrast to most other B19V studies in which investigations were structured to prove that transmission occurred in a particular case.

On the basis of our finding of nontransmission in 24 evaluable susceptible (B19V seronegative) recipients of components with a B19V DNA concentration less than 10^6 IU/mL, we conclude that the rate of transmission from such components ranges from 0% to 11.7% (which is the upper 95% confidence bound); thus, either transmission from such components does not occur, or, if it does, it is a relatively uncommon event in comparison to most other transfusion-transmissible viruses in which infection rates exceed 50% (eg, HIV, HCV).[31]

Table 3. Antibody quantitation studies in recipients transfused with components with the highest B19V DNA concentrations

Transfused component results		Recipient results	
B19V DNA concentration, IU/mL, in donation	B19V IgM/IgG status	Enrollment B19V IgG titer, IU/mL	Follow-up B19V IgG titer, IU/mL
2.9×10^{10}	−/−	14.9	61.1
8.2×10^7	−/−	53.5	33.4
4.3×10^5	−/−	37.5	40.2
8.6×10^3	+/+	7.6	15.2
1.8×10^3	+/+	165.1	157.9

*One recipient who received a component with a DNA concentration of 3.1×10^3 IU/mL (which was also positive for B19V IgM and IgG) was not included in this table because the enrollment and follow-up specimens were both B19V IgG negative.

Our study is the first to evaluate transmission in multiple recipients who do not have preexisting B19V IgG and hence do not have this mechanism for potential protection against acquiring B19V infection. In a study from Africa, there was a single documented case of lack of B19V transmission to a susceptible pediatric recipient transfused with a red cell unit that had a B19V DNA concentration of 6×10^2 IU/mL in the presence of B19V IgG.[20] There are somewhat more data about the lack of transmission to recipients with preexisting B19V IgG. In a study conducted in an adult hematology service, 6 adult recipients with hematologic malignancies (5 of whom underwent stem cell transplantation) were identified as transfused with blood components that were retrospectively found to contain B19V DNA at less than 10^6 geq/mL; in 4 of 5 evaluated cases, the DNA-positive component also contained B19V IgG. Each recipient was B19V DNA negative when tested 3 to 18 days after transfusion,[19] and none showed clinical symptoms of B19V infection on retrospective chart review.[19]

The mechanism to explain lack of transmission to susceptible recipients by B19V DNA-containing units is unknown but could be related to the lack of a large enough inoculating dose of B19 virions to establish infection. This could be due to the ratio between infectious dose and virion number (which is not known), the low levels of transfused intact and/or replication competent virions in units with low DNA concentrations, or neutralization of otherwise infectious virions either by antibody in the transfused unit or by passively transfused antibody from other units.[12] In support of the latter explanations, we note that all DNA-positive units transfused to susceptible recipients in our study contained B19V-specific IgG. In addition, it is highly probable that all recipients of B19V DNA-containing components received some additional blood components with B19V IgG; this is based on our previous findings that 73% of donors who contributed to the RADAR repository had B19V IgG[24] and that RADAR recipients were transfused with an average of 7 blood components.[28]

Our negative transmission findings are consistent with previous publications that have shown that high plasma concentrations of B19V DNA are required for transmission in the setting of transfused pooled plasma products. The minimal infectious dose of B19V DNA documented to cause a symptomatic B19V infection in a recipient of factor VIII concentrate devoid of B19V IgG was 2×10^4 IU based on the infusion of 3 vials of a product with a DNA concentration of 6.5×10^3 IU/vial (ie, 1.3×10^3 IU/mL when each vial was reconstituted in a 5-mL volume).[3] Furthermore, we are aware of only one comprehensive quantitative transmission study of pooled plasma products manufactured from multiple donations.[11,32] That study, conducted approximately 10 years ago, was an open-label phase 4 trial of pooled plasma, solvent detergent-treated (PLAS + SD produced by Vitex, now defunct). One hundred B19V-seronegative volunteers were infused with product from 17 different manufacturing lots. Of 19 subjects who received the product from 3 lots that contained at least 2×10^9 geq B19V DNA (ie, 200 mL product infused at > 10^7 B19V DNA geq/mL), 18 seroconverted and 17 showed B19 viremia. Although the investigators expressed their results in geq/mL, it has subsequently become established that for B19V, an IU and a geq are approximately equivalent. In contrast, there were no seroconversions in 81 subjects who received product from 1 of 14 lots containing less than 10^4 geq/mL B19V DNA; however, the investigators did not more precisely quantitate the amount of B19V DNA in these nontransmitting lots.

In our study, which was designed to systematically study transmissibility from B19V DNA-positive units with less than 10^6 IU/mL, we transfused only 2 components with high B19V DNA concentrations (> 10^7 IU/mL) but were unable to directly evaluate their transmissibility in susceptible recipients, because both were transfused to recipients with preexisting B19V IgG. We used quantitative B19V antibody testing to investigate whether exposure to this very high B19V DNA concentration could stimulate the recipient's immune system to respond. Although not definitive, a 4-fold boost in B19V IgG in the follow-up specimen from one of these recipients suggests that a component with very high B19V DNA concentration (~ 5.8×10^{11} IU B19V DNA infused) can result in an anamnestic response (implying transient active viral replication) in a previously exposed recipient when the pretransfusion antibody titer is relatively low (15 IU/mL in this recipient). Our results are consistent with similar 4-fold B19V IgG increases which were reported 1 month after transfusion in 2 of 2 B19V IgG-positive volunteers who remained asymptomatic after transfusion of 200 mL PLAS + SD at a B19V DNA concentration of 1.6×10^8 IU/mL.[32] In addition, in the previously described study of adult hematology patients, there was also one B19V IgG-positive recipient of a red blood cell unit containing 2.2×10^6 geq/mL of B19V DNA; this recipient was positive for B19V DNA at posttransfusion day 5, negative when retested on day 35, and asymptomatic for B19V infection on chart review; B19V IgG titer was not reported.[19]

Despite the large size of our linked donor-recipient repository, the use of a very sensitive B19V DNA assay, and a rigorous testing algorithm, this study was subject to several limitations. The collection of recipient follow-up specimens 6 to 12 months after transfusion limited the laboratory techniques that we could use to diagnose new B19V infection. In addition to our primary assessment of the development of new B19V IgG formation, we also tested for new appearance of B19V IgM and B19V DNA. However, the natural history of acute B19V infection predicts that both of these markers would probably no longer be detectable at the time our follow-up specimens were collected, unless the recipient had developed a persistent infection.[13,33] Our study was also limited because most recipients (78%) of B19V DNA-positive units were B19V IgG positive before transfusion and thus presumably were partially or totally protected against B19V reinfection. This limited the statistical power of our negative result such that the upper 95% CI could not rule out a transmission rate as high as 11.7%. Furthermore, most of the 24 susceptible recipients received components with very low B19V DNA concentrations (< 20 IU/mL). We identified only 5 transfused components with DNA concentrations between 10^3 and 10^6 IU/mL; 4 of these were B19V IgM and IgG positive, and one of these (DNA level of 4.3×10^5 IU/mL) lacked B19V antibody. Furthermore, only one of these components, a plasma unit containing a total infused dose of approximately 7×10^5 IU in the presence of B19V IgG, was transfused to a susceptible recipient. Similarly, although we identified 45 transfused components with B19V DNA concentrations between 20 and 1000 IU/mL, only 7 were transfused to susceptible recipients. Finally, although we obtained questionnaires from recipients at the time of follow-up (6-12 months after transfusion) and none of the recipients had been diagnosed with B19V disease, we were unable to definitively assess nonspecific symptoms that can occur with B19V infection at such a long interval after transfusion.

We expressed our findings as the rate of transmission in susceptible recipients because this allowed us to extrapolate our findings to other transfused recipient populations; ie, it allowed us to calculate a per unit risk. This per unit risk in our older surgical recipients can then be applied to populations with a higher susceptibility rate (eg, fetuses undergoing intrauterine transfusion, young patients with sickle cell anemia or thalassemia, patients with

congenital or acquired hypogammaglobulinemia), based on the assumption that the equivalent dose of B19V transfused into a B19V IgG–negative hematology or surgical patient will result in productive infection (ie, viral replication) at the same rate. In our opinion, it is unlikely that the infectivity of a B19V DNA–positive transfused unit will be related either to the underlying disease or to the overall immune status of a B19V seronegative recipient, even though it is well accepted that the clinical manifestations of a B19V infection will be influenced by such host factors (ie, if infected with B19V, an immunosuppressed patient or one with an underlying hemolytic syndrome might have a worse clinical outcome).[7]

We can also analyze our data on a population-wide basis; looked at in this way, we did not detect any cases of definite B19V transmission (with the exception of the one possible case of an anamnestic immune response) after the transfusion of blood components from 12 529 B19V DNA–tested donations into a recipient population with a pretransfusion B19V IgG prevalence of 78%.

As part of this study, we also generated a large body of blood donor data. We found that B19V DNA prevalence in 12 529 tested donations was 0.84%, consistent with our previous report of 0.88% in 5020 donation samples from the same RADAR repository and with higher end estimates in literature.[23-25] The large majority of our DNA-positive donations had low or very low DNA concentrations (53%, 71%, and 93% below 20, 100, and 1000 IU/mL, respectively), consistent with the interpretation that the increased DNA prevalence found in recent donor studies is due to the use of more sensitive nucleic acid testing assays. In contrast to the high rate of overall DNA detection, our rate of detection of high-titer DNA positives (> 10^6 IU/mL) was approximately 1 in 6000, consistent with both the newer and older literature.[7,34,35] These high-titer units are known to occur in the acute phase of B19V infection; thus, they lack both B19V IgG and IgM antibody as was the case in this study.[31] In contrast, 96% of the remaining DNA-positive donations were B19V IgG positive, which is the expected result in resolved or persistent infection.[35,36]

Current practices for blood donor screening for B19V in developed countries are almost exclusively confined to testing plasma designated for fractionation for the presence of high B19V DNA concentrations.[8,13] There has been recent debate about whether such screening should also be applied to transfused blood components; this is currently not done because of the lack of demonstrated adverse clinical outcomes from B19V infection in blood component recipients and the considerable expense of such testing. We are aware of only one country, Germany (which also performs blood testing for Austria), in which some blood banks currently conduct B19V DNA screening of blood donations and use the results to release blood components for transfusion.[35] Their testing is conducted in pools of 96 samples with an assay that can reliably detect units with B19V DNA greater than 10^5 IU/mL. Other German blood banks conduct B19V DNA testing retrospectively after the red cell component has been transfused.[35] In a recent abstract, preliminary data indicate that B19V transmission (documented by a positive B19V DNA test in the transfused recipient) from retrospectively tested red cell components occurred when the B19V DNA concentration was greater than 10^5 IU/mL but not when the concentration was below this threshold.[37]

Our study results confirm that, if prospective, real-time B19V DNA blood donor screening were to be performed, the assay sensitivity used in Germany (ie, detection limit < 10^5 IU/mL) is reasonable in that it ensures recipient safety while preventing unnecessary discard of a much larger number of blood components. Our findings do not support the need to use more-sensitive B19V DNA nucleic acid screening assays. In conclusion, our data indicate that blood components with B19V DNA less than 10^6 IU/mL (almost all of which contain B19V-specific antibody) are unlikely to transmit B19V infection.

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The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any agency determination or policy.

Authorship

Contribution: S.H.K., S.A.G., and M.P.B. designed the study; T.-H.L., L.H.T., D.S.T., and M.-y.W.Y. supervised laboratory testing; S.H.K., S.A.G., M.P.B., K.S.S., D.S.T., and H.Q. analyzed data; and S.H.K., S.A.G., M.P.B., and M.-y.W.Y. wrote the manuscript.

Conflict of interest disclosure: The authors declare no competing financial interests.

A complete list of the members of the NHLBI REDS-II appears in the supplemental Appendix (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

Correspondence: Steven Kleinman, 1281 Rockcrest Ave, Victoria, BC, Canada, V9A 4W4; e-mail: skleinman@shaw.ca.

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研究報告の概要

○慢性疲労症候群において新規レトロウイルスXMRVは検出されなかった

背景：2009年10月、米国の慢性疲労症候群(CFS)患者101名のうち68名が、異種指向性ネズミ白血病ウイルス関連ウイルス(XMRV；以前に前立腺がんとの関連性が示された新規ガンマレトロウイルス)に感染していることが報告された。本知見が確認された場合、世界中で数百万人が罹患し、身体機能を奪う当該疾患の理解と治療に多大な影響を及ぼすであろう。我々は、英国のCFS患者がXMRVキャリアであるかどうかを調べた。

方法：本試験のCFSコホート患者は、検査により他の器質性疾患を除外されており、CFSのCDC基準を満たしていた。CFS患者186名の血液検体から抽出したDNAについて、特異的オリゴヌクレオチド・プライマーを用いたnested PCRによる、XMRVプロウイルスおよび関連性の高いネズミ白血病ウイルス(MLV)のスクリーニングを行った。DNAの内部コントロールのため、細胞βグロビン遺伝子を増幅した。陰性対照(水)と陽性対照(XMRV感染分子クローンDNA)を含めた。βグロビン遺伝子を186名全員の検体で増幅したが、XMRVもMLV配列も検出されなかった。

結論：英国のCFS患者由来DNAからは、XMRVまたはMLV配列は増幅されなかった。本試験では英国のXMRVがCFSに関連する証拠を見つけなかったが、北アメリカとヨーロッパ間でのXMRV感染の一般有病率に集団差がある可能性があり、米国の2グループが前立腺がん組織にXMRVを発見したにもかかわらずヨーロッパの2試験で発見されなかったのは、このためであるかもしれない。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
英国の慢性疲労症候群患者186名の血液検体から、新規レトロウイルスXMRVのDNAは検出されなかったとの報告である。 XMRVはマウス白血病ウイルスと類縁な脂質膜を持つ大型RNAウイルスである。この性状からは本製剤の製造工程でウイルス不活化・除去されると期待されることから、本製剤の安全性は確保されていると考える。	注目すべきウイルスとして今後も引き続き、新たなウイルス等に関する情報の収集に努める。

(16)

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Failure to Detect the Novel Retrovirus XMRV in Chronic Fatigue Syndrome

Otto Erlwein[1], Steve Kaye[1], Myra O. McClure[1*], Jonathan Weber[1], Gillian Wills[1], David Collier[2], Simon Wessely[3], Anthony Cleare[3]

1 Jefferiss Research Trust Laboratories, Section of Infectious Diseases, Wright-Fleming Institute, Faculty of Medicine, Imperial College London, St Mary's Campus, Norfolk Place, London, United Kingdom, 2 Social Genetic and Developmental Psychiatry Centre, Institute of Psychiatry (King's College London) De Crespigny Park, Denmark Hill, London, United Kingdom, 3 Department of Psychological Medicine, Institute of Psychiatry, King's College London, Camberwell, London, United Kingdom

Abstract

Background: In October 2009 it was reported that 68 of 101 patients with chronic fatigue syndrome (CFS) in the US were infected with a novel gamma retrovirus, xenotropic murine leukaemia virus-related virus (XMRV), a virus previously linked to prostate cancer. This finding, if confirmed, would have a profound effect on the understanding and treatment of an incapacitating disease affecting millions worldwide. We have investigated CFS sufferers in the UK to determine if they are carriers of XMRV.

Methodology: Patients in our CFS cohort had undergone medical screening to exclude detectable organic illness and met the CDC criteria for CFS. DNA extracted from blood samples of 186 CFS patients were screened for XMRV provirus and for the closely related murine leukaemia virus by nested PCR using specific oligonucleotide primers. To control for the integrity of the DNA, the cellular beta-globin gene was amplified. Negative controls (water) and a positive control (XMRV infectious molecular clone DNA) were included. While the beta-globin gene was amplified in all 186 samples, neither XMRV nor MLV sequences were detected.

Conclusion: XMRV or MLV sequences were not amplified from DNA originating from CFS patients in the UK. Although we found no evidence that XMRV is associated with CFS in the UK, this may be a result of population differences between North America and Europe regarding the general prevalence of XMRV infection, and might also explain the fact that two US groups found XMRV in prostate cancer tissue, while two European studies did not.

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Competing Interests: The authors have declared that no competing interests exist.

* E-mail: m.mcclure@imperial.ac.uk

Introduction

A recent study by Lombardi *et al.* [1] describing a gamma-retrovirus infection in 68 of 101 chronic fatigue syndrome (CFS) patients was notable not only for its claim of a new viral aetiology of a hitherto controversial disease, but also for the fact that proviral DNA could be amplified from the peripheral blood mononuclear cells (PBMC) of 3.75% (8/218) of the healthy controls. This follows an earlier claim that 1.7% (5/300) of healthy Japanese blood donors carried antibodies to the same virus [2]. The virus in question is a recently discovered retrovirus, Xenotropic Murine Leukaemia Virus (MLV)-Related Virus (XMRV).

In the original identification of XMRV in prostate cancer stromal cells, Urisman *et al.* [3] confirmed by sequence analysis that XMRV is not a laboratory contaminant, as is often the case with claims of new retroviral associations with disease. It shares >90% sequence identity in *gag* and *env* (two of the three viral structural genes) with other xenotropic MLVs.

An association between XMRV and prostate cancer was strengthened with the demonstration of XMRV protein expression in malignant epithelial cells [4]. However, these results have not been duplicated in studies conducted in Europe [5–7]. Both prostate cancer and CFS have been linked to an Arg to Gln mutation at codon 462 (R462Q) in the RNaseL gene, an interferon-induced ribonuclease [8]. On activation, RNaseL destroys single stranded cellular and viral RNA, thereby preventing viral replication, blocking protein synthesis, triggering cellular apoptosis and providing an innate anti-viral response. The two US studies are of interest, not only because this would be a further example of a virus association with cancer, but because they represent the first demonstration of a gamma-retrovirus able to infect human cells, over-riding the intrinsic immune mechanisms that were believed to protect humans from MLV infection.

The XMRV sequences derived from prostate cancer tissue are identical to those from CFS patients, but differ from xenotropic MLV sequences, endorsing a genuine cross-species transmission. However, the claim that XMRV is preferentially found in prostate tumours from patients homozygous for the R462Q variant [3] is not borne out by the second prostate cancer study to find XMRV in patients [4], nor was the genetic variant detected in CFS patients carrying XMRV [5].

The finding of Lombardi *et al.* of a 67% XMRV infection rate among CFS patients, if confirmed, would have a serious impact on understanding the pathogenesis of this complex and debilitating disease and its treatment. Therefore, it was important to determine if CFS sufferers in the UK were carriers of XMRV. We have screened DNA extracts from the blood of CFS sufferers by PCRs targeted at an XMRV-specific sequence and at a sequence conserved amongst most murine retroviruses (MRV).

Methods

Patients

All patients gave written informed consent for the use of their DNA to test aetiological theories of CFS, and the study was approved by the South London and Maudsley NHS Trust Ethics Committee. The study recruited 186 patients (62% female, age range 19–70, mean 39.6±11.3years) from consecutive referrals to the CFS clinic at King's College Hospital, London. All patients had undergone medical screening to exclude detectable organic illness, including a minimum of physical examination, urinalysis, full blood count, urea and electrolytes, thyroid function tests, liver function tests, 9 a.m. cortisol and ESR. Patients were interviewed using a semi-structured interview for CFS [9] to determine whether they met international consensus criteria for CFS. All subjects met the *CDC criteria* [10]; patients with the Fukuda-specified exclusionary psychiatric disorders, or somatisation disorder (as per DSM-IV), were not included. The patient set studied is a well-characterised and representative sample of CFS patients who have been described previously: all were routine clinic attendees, referred within the UK National Health Service, who had taken part in prior studies of neuroendocrine functioning [11] and/or of cognitive behaviour therapy [12]. As is typical of the patients seen in this tertiary care centre, they were markedly unwell. Few were working, and 19% were members of patient support groups for CFS/ME [12–14]. The levels of fatigue in this sample were high (mean Chalder Fatigue Scale, 26.3±5.4) [15], as were levels of disability (mean Work and Social Adjustment Scale, total score 28.2±7.2) [16]. The mean GHQ-12 score [17] was 19.7±8.1. Patients had been unwell for a median of 4.0 y (range 1–28 y). Of note was that 45% said their illness definitely related to a viral illness and 45% said it might relate to a viral illness. Overall, we conclude that this sample is typical of CFS patients seen in specialist clinical services in the UK. We also know from collaborative studies that our patients resemble those seen in other specialist CFS services in the United States and Australia [18].

PCR detection of XMRV and MLV sequences. DNA was extracted from EDTA whole blood using a standard phenol-based organic deproteinisation procedure [19]. DNA concentrations were determined by absorbance at 260 nm (A_{260}). Each sample was amplified in three nested PCRs using primers targeted to an XMRV-specific sequence, to a sequence conserved amongst most MLV and, as a control for sample addition and PCR-inhibition, to a human beta-globin (hBG) sequence (Table 1). Each first-round reaction was performed in a 25 µl volume containing 0.5 units TaqGold (Applied BioSystems, Warrington, UK), 1 x TaqGold reaction buffer (Applied BioSystems), 1.5 mM Mg^{2+}, 200 mM each dNTP, 2.5 pmol each primer to which 5 µl DNA extract or control was added. Reaction conditions were one cycle of 94°C, 8 minutes, 35 cycles of 94°C 30 seconds, 55°C 30 seconds, 72°C 30 seconds and one cycle 0f 72°C, 7 minutes. Second round reaction mixes were identical to the first round and the sample was a 1 µl transfer from the first round reactions. Second round reaction conditions were as for the first round over 30 cycles. PCR amplicons were visualised on a 1% agarose gel stained with ethidium bromide. Each PCR run consisted of test samples, six negative (water) and two positive controls. The positive control was a dilution of a plasmid with a full-length XMRV (isolate VP62) insert, generously gifted by Dr R. Silverman. To validate the sensitivity of the PCR, an end-point dilution of the plasmid was performed. To determine specificity of the PCR, a sample of human DNA from the LNCaP prostate cancer cell line (American Type Culture Collection, code CRL-1740) was amplified with the XMRV and MLV primer sets. To ensure integrity of the DNA extracts, three randomly selected samples were titrated to end-point using the hBG PCR to determine if the PCR copy number equated with the A_{260}. To determine if the DNA extracts exhibited low level non-specific inhibition of PCR, 10 samples were subjected to 30 cycles of the first round hBG PCR (reaction mix and conditions as above) followed by 40 cycles of a nested real-time SYBR-green PCR using the SYBR-green Fast PCR kit (Roche, Lewes UK) according to the manufacturer's instructions.

Table 1. Oligonucleotide Primers.

Target	Sequence	Location
XMRV	Forward outer 5'CATTCTGTATCAGTTAACCTAC 3'	411–432[1]
	Reverse outer 5' ATGATCTCGAGAACACTTAAAG 3'	606–588[1]
	Forward inner 5' GACTTTTTGGAGTGGCTTTGT 3'	441–461[1]
	Reverse inner 5' ACAGAAGAACAACAAAACAAATC 3'	566–544[1]
MLV	Forward outer 5' GGATCAAGCCCCACATACAG 3'	2796–2847[1]
	Reverse outer 5' CATCAAACAGGGTGGGACTG 3'	3179–3160[1]
	Forward inner 5' AGAAGTCAACAAGCGGGTGG 3'	2926–2945[1]
	Reverse inner 5' GGTGGAGTCTCAGGCAGAAA 3'	3062–3043[1]
hBG	Forward outer 5' TGGTGGTCTACCCTTGGACC 3'	148–162[2]
	Reverse outer 5' GAGGTTGTCCAGGTGAGCCA 3'	296–277[2]
	Forward inner 5' GAGGTTCTTTGAGTCCTTTGG 3'	170–190[2]
	Reverse inner 5' CATCACTAAAGGCACCGAGCA 3'	273–253[2]

Locations in GenBank accessions [1]EF185282, [2]NM000518.4.
doi:10.1371/journal.pone.0008519.t001

Results

Nested PCR Validation

Based on A_{260} of the purified plasmid, both primer sets (XMRV, MLV) were able to amplify a single target copy added to the reaction. Amplification of 600 ng of LNCaP cellular DNA added to XMRV and MLV PCRs yielded no non-specific bands when viewed on an ethidium bromide-stained agarose gel. Quantification of DNA samples from three randomly selected test samples by end-point dilution PCR with the hBG primer set showed concurrence of the PCR-determined copy number with A_{260}, thus indicating integrity of the DNA preparations. Nested real-time amplification of 10 samples showed no evidence of non-specific inhibition as determined by the slope of the amplification curves and the height of the signal plateau.

PCR Analysis of Test Samples

Input DNA ranged from 10 to 600 ng (1.6×10^3 to 1.1×10^5 cell equivalents) as determined by A_{260} of which 149 samples had an input of >100 ng and 106 samples >200 ng. None of the 186 test samples analysed yielded a specific PCR product with either the XMRV or MLV primer sets and no non-specific PCR products were observed. A specific hBG product was amplified from all 186 test samples. The positive control was amplified in each run by the

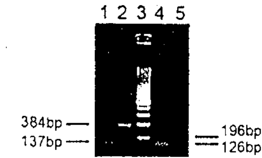

Figure 1. PCR products of the XMRV VP62 clone. Primers are generic to MLV (lanes 1 and 2) or specific to XMRV (lanes 4 and 5). The sizes of the respective fragments are shown. Lane 3-200 bp molecular size ladder.
doi:10.1371/journal.pone.0008519.g001

XMRV and MLV primer sets. A stained gel of the XMRV and MLV PCR products is shown in figure 1 and a representative sample of our results with CFS DNA and MLV primers is shown in figure 2.

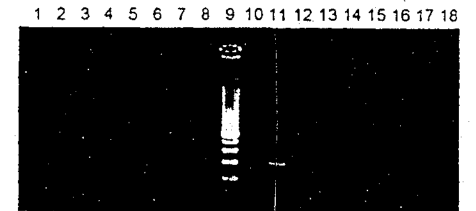

Figure 2. Nested PCR from the DNA of 8 CFS patients. Products of generic MLV primers (including XMRV) are shown. Lanes 1–8, CFS patient DNA (2nd round); lanes 9 and 10, XMRV 2nd round and 1st round positive controls; lanes 11 and 12, DNA of uninfected cell line LNCaP; lanes 13–18, water controls.
doi:10.1371/journal.pone.0008519.g002

Discussion

Unlike the study of Lombardi *et al.*, we have failed to detect XMRV or closely related MRV proviral DNA sequences in any sample from CFS cases. There have been numerous claims for an infective aetiology to CFS over the years, not least because, as in this sample, many patients report that their symptoms were triggered by an infective episode. Prospective epidemiological studies have confirmed that certain infective agents, for example Epstein Barr virus, are unequivocally associated with subsequent CFS [20], even if the mechanisms are unclear and almost certainly multi factorial. Nearly two decades ago, sequences from another retrovirus, the human T-lymphotropic virus type II, were amplified from the PBMCs of 10/12 (83%) adult and 13/18 paediatric CFS patients, but not from healthy control subjects [21]. However, subsequent studies carried out on small numbers (20–30) of CFS patients, failed to confirm evidence for HTLV (type 1 or 11) [22–25] or other retroviruses, including the closely-related simian T lymphotropic virus type I, the prototype foamy virus, simian retrovirus, bovine and feline leukaemia viruses [26] and HIV-1 [23].

The Lombardi paper is the first to study a significantly larger number of people than that in any previous study and to detect a virus only recently discovered. Our study resembles that of Lombardi *et al.* in certain respects. Both studies use the widely accepted 1994 clinical case definition of CFS[10]. Lombardi *et al.* reported that their cases "presented with severe disability" and we provide quantifiable evidence confirming high levels of disability in our subjects. Our subjects were also typical of those seen in secondary and tertiary care in other centres.

Our own study also differs from that of Lombardi in other respects. Firstly, the PCR operator was blinded to the provenance of the DNA samples. In fact, with the exception of the PCR controls, all 186 DNA test samples originated from CFS patients. Care was taken to grow the XMRV plasmid in a laboratory in which no MLV had been cultured and no MLV vectors used and the PCR was carried out in a CPA-accredited Molecular Diagnostics Unit which processes only human tissue. Multiple (six) water (negative) controls were included in every run to detect low level contamination and a PCR to amplify a sequence that is conserved in most murine leukaemia viruses was included in order to expose any circulating MLV contamination and to detect any variant of XMRV that might be circulating in the UK CFS population.

Based on our molecular data, we do not share the conviction that XMRV may be a contributory factor in the pathogenesis of CFS, at least in the U.K.

Acknowledgments

The assistance of Sarah Bull in data collection and processing is gratefully acknowledged.

Author Contributions

Conceived and designed the experiments: SK MM. Performed the experiments: OWE SK. Analyzed the data: SK MM. Contributed reagents/materials/analysis tools: SK GW DC SW AC. Wrote the paper: SK MM. Facilitated the study by setting up the collaboration: JW. Responsible for providing samples and associated data from a well characterised and valuable cohort of subjects: SW.

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別紙様式第２－１

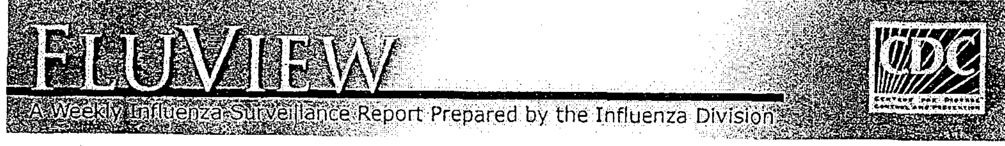
医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2010年1月25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	2009-2010 Influenza Season Week 1 ending January 9, 2010 : 3	公表国 米国	
販売名（企業名）	別紙のとおり					
研究報告の概要	問題点：the Iowa Department of Public Healthから、ヒトにおける初のブタインフルエンザA(H3N2)感染事例が報告された。 ヒトにおける新規インフルエンザAウイルスの感染事例1例がthe Iowa Department of Public Healthから報告された。患者は2009年9月に発症したが、入院の必要は無く、回復した。同ウイルスはブタインフルエンザA（H3N2）と同定され、2009年11月に精査された。ブタからの暴露は不明である一方、同ウイルスのヒト－ヒト感染の証拠は認められていない。新規インフルエンザA感染事例の速やかな同定及び精査は流行の拡大規模及びヒト－ヒト感染の可能性の評価に重要である。新規インフルエンザAウイルスのヒト感染における調査は通年で実施されている。					使用上の注意記載状況・ その他参考事項等 記載なし。
報告企業の意見			今後の対応			
別紙のとおり			今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。			

MedDRA/J ver.12.0

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人免役グロブリン、⑥人免役グロブリン、⑦乾燥ペプシン処理人免疫グロブリン、⑧乾燥ペプシン処理人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン、⑫乾燥スルホ化人免疫グロブリン、⑬乾燥スルホ化人免疫グロブリン、⑭乾燥スルホ化人免疫グロブリン*、⑮乾燥濃縮人活性化プロテインC、⑯乾燥濃縮人血液凝固第Ⅷ因子、⑰乾燥濃縮人血液凝固第Ⅷ因子、⑱乾燥濃縮人血液凝固第Ⅷ因子、⑲乾燥濃縮人血液凝固第Ⅷ因子、⑳乾燥濃縮人血液凝固第Ⅸ因子、㉑乾燥濃縮人血液凝固第Ⅸ因子、㉒乾燥濃縮人血液凝固第Ⅸ因子、㉓乾燥濃縮人血液凝固第Ⅸ因子、㉔乾燥抗破傷風人免疫グロブリン、㉕乾燥抗破傷風人免疫グロブリン、㉖抗 HBs 人免疫グロブリン、㉗抗 HBs 人免疫グロブリン、㉘トロンビン、㉙フィブリノゲン加第XⅢ因子、㉚フィブリノゲン加第XⅢ因子、㉛乾燥濃縮人アンチトロンビンⅢ、㉜乾燥濃縮人アンチトロンビンⅢ、㉝ヒスタミン加人免疫グロブリン製剤、㉞ヒスタミン加人免疫グロブリン製剤、㉟人血清アルブミン*、㊱人血清アルブミン*、㊲乾燥ペプシン処理人免役グロブリン*、㊳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ガンマーグロブリン筋注 450mg/3mL「化血研」、⑥ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑦献血静注グロブリン"化血研"、⑧献血グロブリン注射用 2500mg「化血研」、⑨献血ベニロンーⅠ、⑩献血ベニロンーⅠ静注用 500mg、⑪献血ベニロンーⅠ静注用 1000mg、⑫献血ベニロンーⅠ静注用 2500mg、⑬献血ベニロンーⅠ静注用 5000mg、⑭ベニロン*、⑮注射用アナクトC2,500単位、⑯コンファクトF、⑰コンファクトF注射用 250、⑱コンファクトF注射用 500、⑲コンファクトF注射用 1000、⑳ノバクトM、㉑ノバクトM注射用 250、㉒ノバクトM注射用 500、㉓ノバクトM注射用 1000、㉔テタノセーラ、㉕テタノセーラ筋注用 250 単位、㉖ヘパトセーラ、㉗ヘパトセーラ筋注 200 単位/mL、㉘トロンビン"化血研"、㉙ボルヒール、㉚ボルヒール組織接着用、㉛アンスロビンP、㉜アンスロビンP500 注射用、㉝ヒスタグロビン、㉞ヒスタグロビン皮下注用、㉟アルブミン 20%化血研*、㊱アルブミン 5%化血研*、㊲静注グロブリン*、㊳アンスロビンP1500 注射用
報告企業の意見	インフルエンザウイルス粒子は 70～120nm の球形または多形性で、8本の分節状マイナス一本鎖 RNA を核酸として有する。エンベロープの表面に赤血球凝集素(HA)とノイラミダーゼ(NA)のスパイクを持ち、その抗原性により 16 種類の HA 亜型および 9 種類の NA 亜型に分類される。 今回の報告はヒトにおける初めてのブタインフルエンザA（H3N2）感染事例報告であるが、感染経路は明らかになっていない。また、ヒトに対し高病原性であるような情報も示されていない。 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。今回報告したインフルエンザウイルスは、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、弊所の血漿分画製剤の製造工程が 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インフルエンザの報告例は無い。以上の点から、当該製剤はインフルエンザ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



2009-2010 Influenza Season
Week 1 ending January 9, 2010

All data are preliminary and may change as more reports are received.

Synopsis: During week 1 (January 3-9, 2010), influenza activity continued to decrease in the U.S.

- 139 (3.6%) specimens tested by U.S. World Health Organization (WHO) and National Respiratory and Enteric Virus Surveillance System (NREVSS) collaborating laboratories and reported to CDC/Influenza Division were positive for influenza.
- All subtyped influenza A viruses reported to CDC were 2009 influenza A (H1N1) viruses.
- One human infection with a novel influenza A virus was reported.
- The proportion of deaths attributed to pneumonia and influenza (P&I) was below the epidemic threshold.
- Seven influenza-associated pediatric deaths were reported. Six deaths were associated with 2009 influenza A (H1N1) virus infection and one was associated with an influenza A virus for which the subtype was undetermined.
- The proportion of outpatient visits for influenza-like illness (ILI) was 1.9% which is below the national baseline of 2.3%. One of the 10 regions (region 9) reported ILI above their region-specific baseline.
- No states reported widespread influenza activity, nine states reported regional influenza activity, the District of Columbia, Puerto Rico, and 15 states reported local influenza activity, Guam and 24 states reported sporadic influenza activity, and the U.S. Virgin Islands and two states reported no influenza activity.

National and Regional Summary of Select Surveillance Components

	Data for current week			Data cumulative since August 30, 2009 (Week 35)*						
HHS Surveillance Regions**	Out-patient ILI†	% positive for flu‡	Number of jurisdictions reporting regional or widespread activity§	A (H1)	A (H3)	2009 A (H1N1)	A (unable to sub-type)¥	A (Subty-ping not perfor-med)	B	Pediatric Deaths
Nation	Normal	3.6%	9 of 54	29	52	61,332	313	19,225	228	236
Region 1	Normal	3.0%	1 of 6	4	2	3,320	14	469	10	6
Region 2	Normal	4.9%	2 of 4	4	4	1,484	0	1,098	9	11
Region 3	Normal	2.8%	1 of 6	3	7	10,554	48	1,456	16	13
Region 4	Normal	6.5%	2 of 8	0	5	7,326	90	4,123	63	45
Region 5	Normal	3.1%	0 of 6	7	23	9,356	52	1,333	15	33
Region 6	Normal	2.2%	1 of 5	0	3	3,475	45	4,722	41	66
Region 7	Normal	3.0%	0 of 4	3	1	3,299	3	769	3	8
Region 8	Normal	3.0%	0 of 6	6	2	9,766	0	3,770	59	13
Region 9	Elevated	4.8%	2 of 5	0	4	8,175	47	1,167	10	31
Region 10	Normal	7.3%	0 of 4	2	1	4,567	14	318	2	10

*Influenza season officially begins each year at week 40. This season data from week 35 will be included to show the trend of influenza activity before the official start of the 2009-10 influenza season.
**HHS regions (Region 1 CT, ME, MA, NH, RI, VT; Region 2: NJ, NY, Puerto Rico, US Virgin Islands; Region 3: DE, DC, MD, PA, VA, WV; Region 4: AL, FL, GA, KY, MS, NC, SC, TN; Region 5: IL, IN, MI, MN, OH, WI; Region 6: AR, LA, NM, OK, TX; Region 7: IA, KS, MO, NE; Region 8: CO, MT, ND, SD, UT, WY; Region 9: AZ, CA, Guam, HI, NV; and Region 10: AK, ID, OR, WA). Use of the national baseline for regional data or regional baselines for state data is not appropriate.
† Elevated means the % of visits for ILI is at or above the national or region-specific baseline.
‡ National data are for current week; regional data are for the most recent three weeks.
§ Includes all 50 states, the District of Columbia, Guam, Puerto Rico, and U.S. Virgin Islands.
¥ Subtyping results for the majority of specimens in this category were inconclusive because of low virus titers.

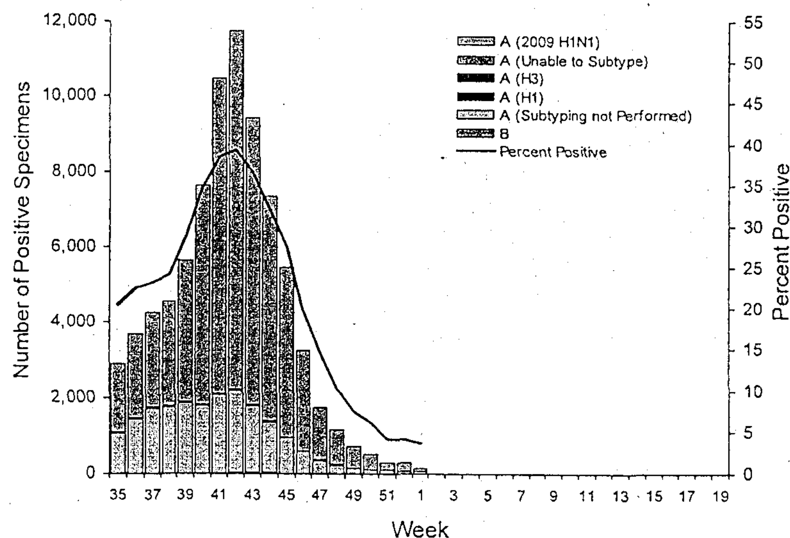
U.S. Virologic Surveillance: WHO and NREVSS collaborating laboratories located in all 50 states and Washington D.C., report to CDC the number of respiratory specimens tested for influenza and the number positive by influenza type and subtype. The results of tests performed during the current week are summarized in the table below.

	Week 1
No. of specimens tested	3,886
No. of positive specimens (%)	139 (3.6%)
Positive specimens by type/subtype	
Influenza A	137 (98.6%)
A (2009 H1N1)	78 (56.9%)
A (subtyping not performed)	58 (42.3%)
A (unable to subtype)*	1 (0.7%)
A (H3)	0 (0.0%)
A (H1)	0 (0.0%)
Influenza B	2 (1.4%)

*Subtyping results for the specimen in this category was inconclusive because of low levels of viral RNA.

During week 1, influenza B viruses co-circulated at low levels with 2009 influenza A (H1N1) viruses. All subtyped influenza A viruses reported to CDC this week were 2009 influenza A (H1N1) viruses.

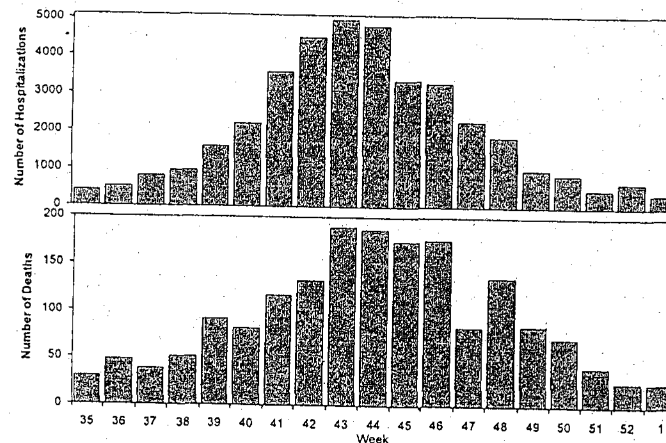
Influenza Positive Tests Reported to CDC by U.S. WHO/NREVSS Collaborating Laboratories, National Summary, August 30, 2009-January 9, 2010



Novel Influenza A Virus: One case of human infection with a novel influenza A virus was reported by the Iowa Department of Public Health. The case patient had onset of symptoms in September 2009, but did not require hospitalization and has fully recovered. The virus was identified as swine influenza A (H3N2) and investigated in November 2009. No clear exposure to swine was identified, but no evidence of sustained human-to-human transmission with this virus was found. Early identification and investigation of novel influenza A cases is critical to evaluate the extent of the outbreak and possible human-to-human transmission. Surveillance for human infections with novel influenza A viruses is conducted year-round.

Pneumonia and Influenza Hospitalization and Death Tracking: The Aggregate Hospitalization and Death Reporting Activity (AHDRA) system was implemented on August 30, 2009, and replaces the weekly report of laboratory confirmed 2009 H1N1-related hospitalizations and deaths that began in April 2009. Jurisdictions can now report to CDC counts of hospitalizations and deaths resulting from all types or subtypes of influenza, not just those from 2009 H1N1 influenza virus. To allow jurisdictions to implement the new case definition, counts were reset to zero on August 30, 2009. From August 30, 2009 – January 9, 2010, 38,454 laboratory-confirmed influenza-associated hospitalizations and 1,779 laboratory-confirmed influenza-associated deaths were reported to CDC. CDC will continue to use its traditional surveillance systems to track the progress of the 2009-10 influenza season.

Weekly Laboratory-Confirmed Influenza-Associated Hospitalizations and Deaths Reported to AHDRA, National Summary, August 30, 2009 – January 9, 2010



Antigenic Characterization: CDC has antigenically characterized one seasonal influenza A (H1N1), seven influenza A (H3N2), six influenza B, and 944 2009 influenza A (H1N1) viruses collected since September 1, 2009.

One seasonal influenza A (H1N1) virus was tested and is related to the influenza A (H1N1) component of the 2009-10 Northern Hemisphere influenza vaccine (A/Brisbane/59/2007).

The seven influenza A (H3N2) viruses tested showed reduced titers with antisera produced against A/Brisbane/10/2007, the 2009-2010 Northern Hemisphere influenza A (H3N2) vaccine component, and were antigenically related to A/Perth/16/2009, the WHO recommended influenza A (H3N2) component of the 2010 Southern Hemisphere vaccine formulation.

Influenza B viruses currently circulating globally can be divided into two distinct lineages represented by the B/Yamagata/16/88 and B/Victoria/02/87 viruses. The influenza B component of the 2009-10 vaccine belongs to the B/Victoria lineage. The six influenza B viruses tested belong to the B/Victoria lineage and are related to the influenza vaccine component for the 2009-10 Northern Hemisphere influenza vaccine (B/Brisbane/60/2008).

Nine hundred forty-two (99.8%) of 944 2009 influenza A (H1N1) viruses tested are related to the A/California/07/2009 (H1N1) reference virus selected by WHO as the 2009 H1N1 vaccine virus. Two viruses (0.3%) tested showed reduced titers with antiserum produced against A/California/07/2009.

Annual influenza vaccination is expected to provide the best protection against those virus strains that are related to the vaccine strains, but limited to no protection may be expected when the vaccine and circulating virus strains are so different as to be from different lineages. Antigenic characterization of 2009 influenza A (H1N1) viruses indicates that these viruses are only distantly related antigenically and genetically to seasonal influenza A (H1N1) viruses, suggesting that little to no protection would be expected from vaccination with seasonal influenza vaccine. It is too early in the influenza season to determine if seasonal influenza viruses will circulate widely or how well the seasonal vaccine and circulating strains will match.

Antiviral Resistance: Since September 1, 2009, one seasonal influenza A (H1N1), eight influenza A (H3N2), one influenza B, and 830 2009 influenza A (H1N1) virus isolates have been tested for resistance to the neuraminidase inhibitors (oseltamivir and zanamivir), and 2,096 2009 influenza A (H1N1) original clinical samples were tested for a single known mutation in the virus that confers oseltamivir resistance. In addition, one seasonal influenza A (H1N1), 11 influenza A (H3N2), and 837 2009 influenza A (H1N1) virus isolates have been tested for resistance to the adamantanes (amantadine and rimantadine). The results of antiviral resistance testing performed on these viruses are summarized in the table below. Additional laboratories perform antiviral testing and report their results to CDC and positive results from that testing are included in the footnote.

Antiviral Resistance Testing Results on Samples Collected Since September 1, 2009.

	Viruses tested (n)	Resistant Viruses, Number (%)	Viruses tested (n)	Resistant Viruses, Number (%)	Isolates tested (n)	Resistant Viruses, Number (%)
		Oseltamivir		Zanamivir		Adamantanes
Seasonal Influenza A (H1N1)	1	1 (100.0)	0	0 (0)	1	0 (0)
Influenza A (H3N2)	8	0 (0)	0	0 (0)	11	9 (81.8)
Influenza B	1	0 (0)	0	0 (0)	N/A*	N/A*
2009 Influenza A (H1N1)	2,926	39†‡ (1.3)	830	0 (0)	837	834 (99.6)

*The adamantanes (amantadine and rimantadine) are not effective against influenza B viruses.
†Two screening tools were used to determine oseltamivir resistance: sequence analysis of viral genes and a neuraminidase inhibition assay.
‡ Additional laboratories perform antiviral resistance testing and report their results to CDC. Three additional oseltamivir resistant 2009 influenza A (H1N1) virus has been identified by these laboratories since September 1, 2009, bringing the total number to 42.

All of the subtyped influenza A viruses reported during week 1 were 2009 influenza A (H1N1) viruses, and nearly all of 2009 H1N1 viruses tested since April 2009 have been resistant to the adamantanes (amantadine and rimantadine).

Antiviral treatment with oseltamivir or zanamivir is recommended for all patients with confirmed or suspected influenza virus infection who are hospitalized, are at higher risk for influenza complications, or who have lower respiratory tract or progressive disease. Additional information on antiviral recommendations for treatment and chemoprophylaxis of influenza virus infection is available at http://www.cdc.gov/H1N1flu/recommendations.htm.

2009 influenza A (H1N1) viruses were tested for oseltamivir resistance by a neuraminidase inhibition assay and/or detection of genetic sequence mutation, depending on the type of specimen tested. Original clinical samples were examined for a single known mutation in the virus that confers oseltamivir resistance in currently circulating seasonal influenza A (H1N1) viruses, while influenza virus isolates were tested using a neuraminidase inhibition assay that determines the presence or absence of neuraminidase inhibitor resistance, followed by neuraminidase gene sequence analysis of resistant viruses.

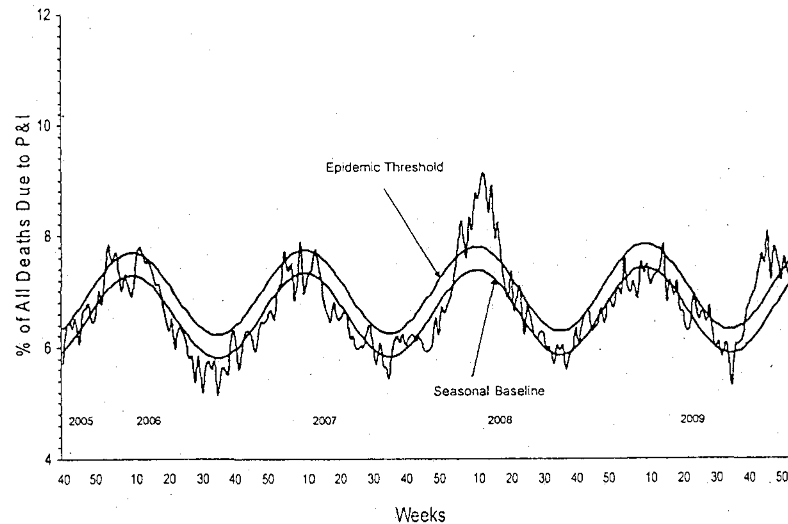
The majority of 2009 influenza A (H1N1) viruses are susceptible to the neuraminidase inhibitor antiviral medication oseltamivir; however, rare sporadic cases of oseltamivir resistant 2009 influenza A (H1N1) viruses have been detected worldwide. A total of 52 cases of oseltamivir resistant 2009 influenza A (H1N1) viruses have been identified in the United States since April 2009. While the total number of cases has not increased over the previous week, one previously reported case was reclassified and one new case was identified. Forty-two of these specimens

were collected after September 1, 2009. The proportion of oseltamivir-resistant 2009 H1N1 viruses does not represent the prevalence of oseltamivir-resistant 2009 H1N1 in the U.S. Most cases were tested because drug resistance was suspected. All tested viruses retain their sensitivity to the neuraminidase inhibitor zanamivir. Of the 52 total cases identified since April 2009, 40 patients had documented exposure to oseltamivir through either treatment or chemoprophylaxis, nine patients are under investigation to determine exposure to oseltamivir, and three patients had no documented oseltamivir exposure. Occasional development of oseltamivir resistance during treatment or prophylaxis is not unexpected. Enhanced surveillance, an increased availability of testing performed at CDC, and an increasing number of public health and other clinical laboratories performing antiviral resistance testing increase the number of cases of oseltamivir resistant 2009 influenza A (H1N1) viruses detected. All cases are investigated to assess the spread of resistant strains in the community.

To prevent the spread of antiviral resistant virus strains, CDC reminds clinicians and the public of the need to continue hand and cough hygiene measures for the duration of any symptoms of influenza, *even while taking antiviral medications* (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5832a3.htm).

Pneumonia and Influenza (P&I) Mortality Surveillance: During week 1, 7.3% of all deaths reported through the 122-Cities Mortality Reporting System were due to P&I. This percentage was below the epidemic threshold of 7.6% for week 1.

Pneumonia and Influenza Mortality for 122 U.S. Cities
Week ending 1/9/2010



Influenza-Associated Pediatric Mortality: Seven influenza-associated pediatric deaths were reported to CDC during week 1 (Illinois, Michigan, New York [2], Oregon, and Texas [2]). Six deaths were associated with 2009 influenza A (H1N1) virus infection and one was associated with an influenza A virus for which the subtype was undetermined.. The deaths reported during week 1 occurred between October 11 and December 19, 2009.

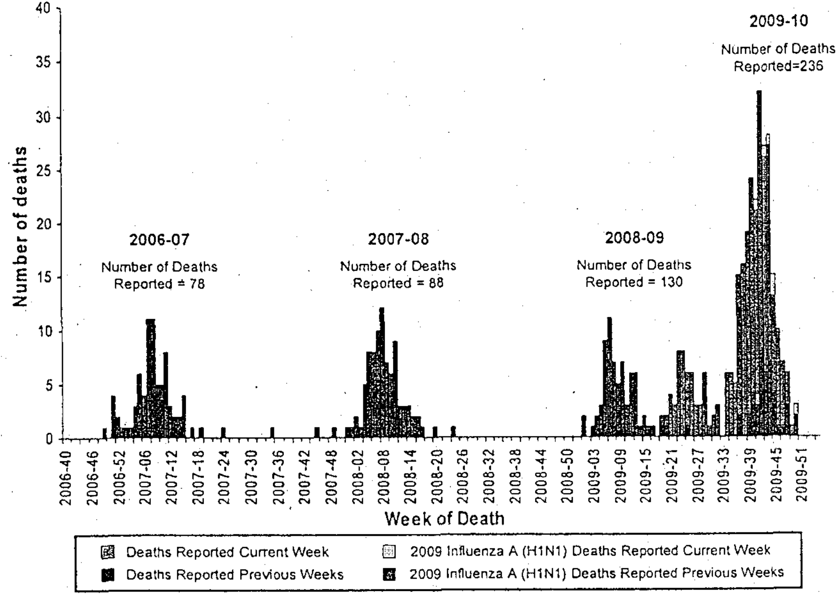
Since August 30, 2009, CDC has received 236 reports of influenza-associated pediatric deaths that occurred during the current influenza season (43 deaths in children less than 2 years old, 26 deaths in children 2-4 years old, 87 deaths in children 5-11 years old, and 80 deaths in children 12-17 years old). One hundred ninety-five (83%) of the 236 deaths were due to 2009 influenza A (H1N1) virus infections, 40 were associated with an influenza A virus for which the subtype is undetermined, and one was associated with an influenza B virus infection. A total of 255 deaths in children associated with 2009 influenza A (H1N1) virus infection have been reported to CDC.

Among the 236 deaths in children, 121 children had specimens collected for bacterial culture from normally sterile sites and 39 (32.2%) of the 121 were positive; *Streptococcus pneumoniae* was identified in 10 (25.6%) of the 39 children and *Staphylococcus aureus* was identified in 11 (28.2%) of the 39 children. Two *S. aureus* isolates were sensitive to methicillin, eight were methicillin resistant, and one did not have sensitivity testing performed. Twenty-six (66.7%) of the 39 children with bacterial coinfections were five years of age or older, and 14 (35.9%) of the 39 children were 12 years of age or older.

Laboratory-Confirmed Influenza-Associated Pediatric Deaths by Date and Type/Subtype of Influenza.

Date	2009 H1N1 Influenza	Influenza A-Subtype Unknown	Seasonal Influenza	Total
Number of Deaths REPORTED for Current Week – Week 1 (Week ending January 9, 2010)	6	1	0	7
Number of Deaths OCCURRED since August 30, 2009	195	40	1	236
Number of Deaths OCCURRED since April 26, 2009	255	43	2	300

Number of Influenza-Associated Pediatric Deaths by Week of Death: 2006-07 season to present

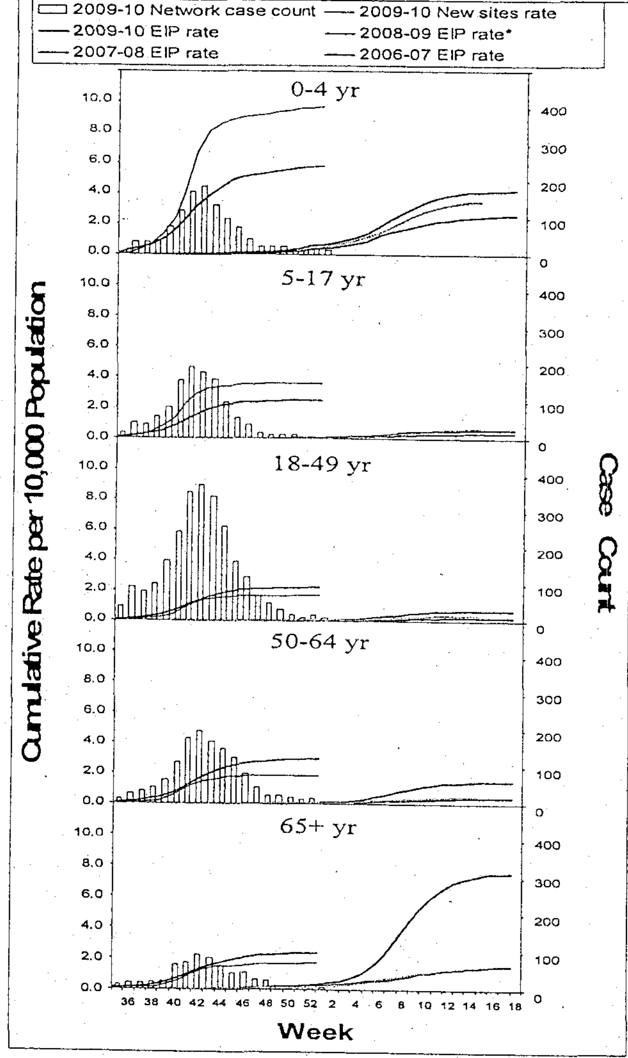


Influenza-Associated Hospitalizations: Laboratory-confirmed influenza-associated hospitalizations are monitored using a population-based surveillance network that includes the 10 Emerging Infections Program (EIP) sites (CA, CO, CT, GA, MD, MN, NM, NY, OR and TN) and 6 new sites (IA, ID, MI, ND, OK and SD).

During September 1, 2009 – January 9, 2010, the following preliminary laboratory-confirmed overall influenza associated hospitalization rates were reported by EIP and the new sites *(rates include influenza A, influenza B, and 2009 influenza A (H1N1)):*

Rates [EIP (new sites)] for children aged 0-4 years and 5-17 years were 5.9 (9.7) and 2.5 (3.6) per 10,000, respectively. Rates [EIP (new sites)] for adults aged 18-49 years, 50-64 years, and ≥ 65 years were 2.2 (1.7), 2.9 (1.8) and 2.4 (1.7) per 10,000, respectively.

EIP Influenza Laboratory-Confirmed Cumulative Hospitalization Rates, 2009-10 and Previous Three Seasons*

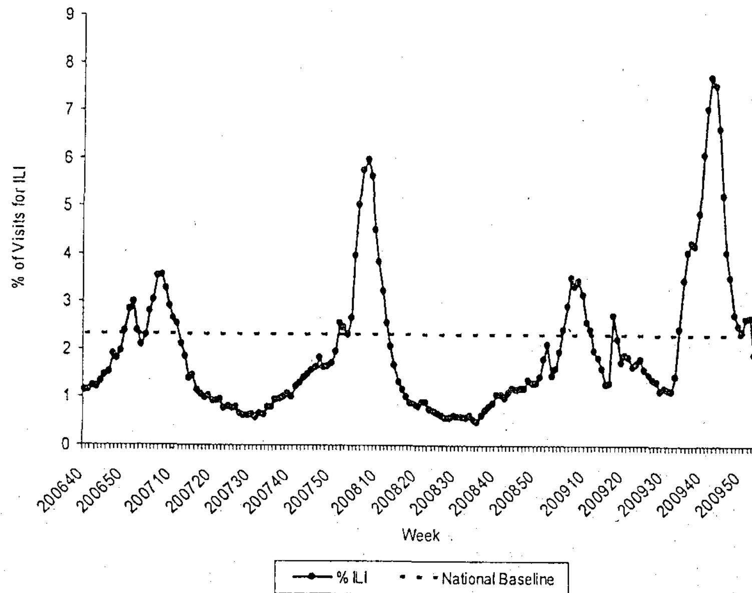


* The 2008-09 EIP rate ended as of April 14, 2009 due to the onset of the 2009 H1N1 season.

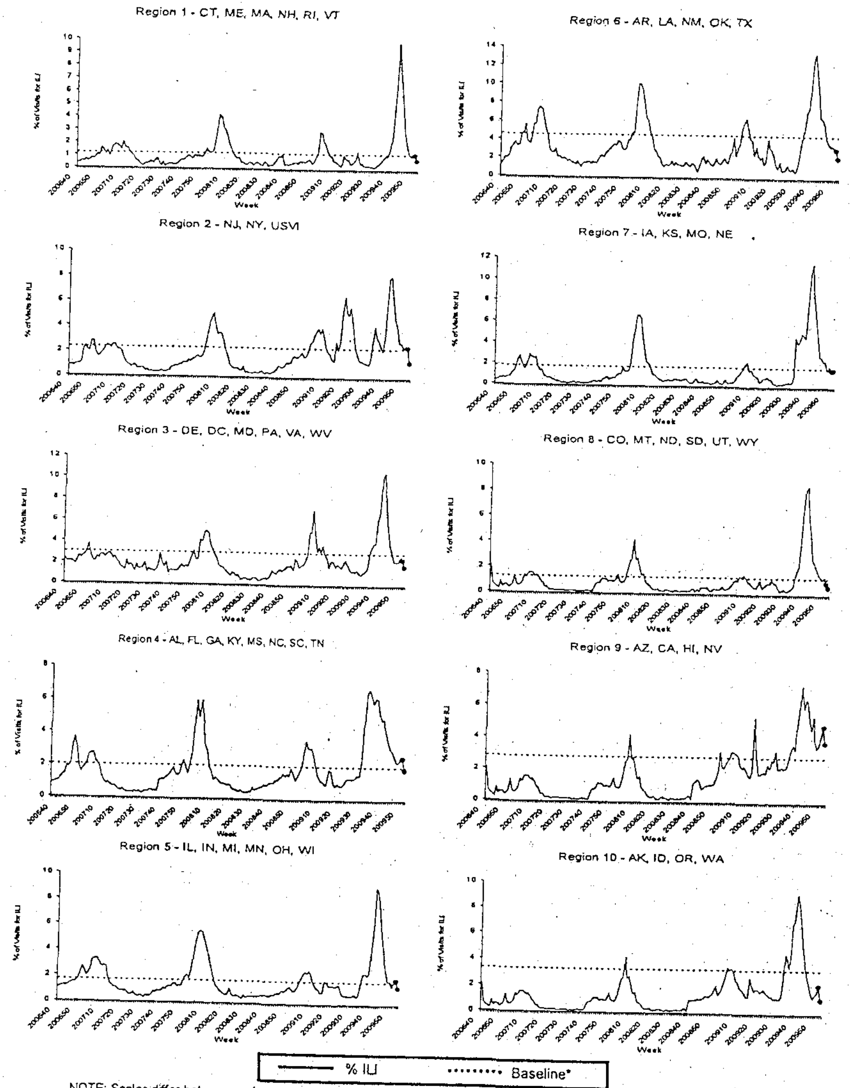
Outpatient Illness Surveillance: Nationwide during week 1, 1.9% of patient visits reported through the U.S. *Outpatient Influenza-like Illness Surveillance Network (ILINet) were due to influenza-like* illness (ILI). This percentage is below the national baseline of 2.3%.

The increase in the percentage of outpatient visits for ILI during weeks 51 and 52 is likely influenced by a reduction in routine health care visits during the holiday season, as has occurred during previous seasons.

Percentage of Visits for Influenza-like Illness (ILI) Reported by the U.S. Outpatient Influenza-like Illness Surveillance Network (ILINet), Weekly National Summary, October 1, 2006 – January 9, 2010



On a regional level, the percentage of outpatient visits for ILI ranged from 0.6% to 3.8% during week 1. *One of the 10 regions (Region 9) reported a proportion of outpatient visits for ILI above its* region-specific baseline levels. Regions 1, 2, 3, 4, 5, 6, 7, 8, and 10 reported ILI below their region-specific baselines. (Note: Use of the national baseline for regional ILI data or regional baselines for state-level data is not appropriate.)



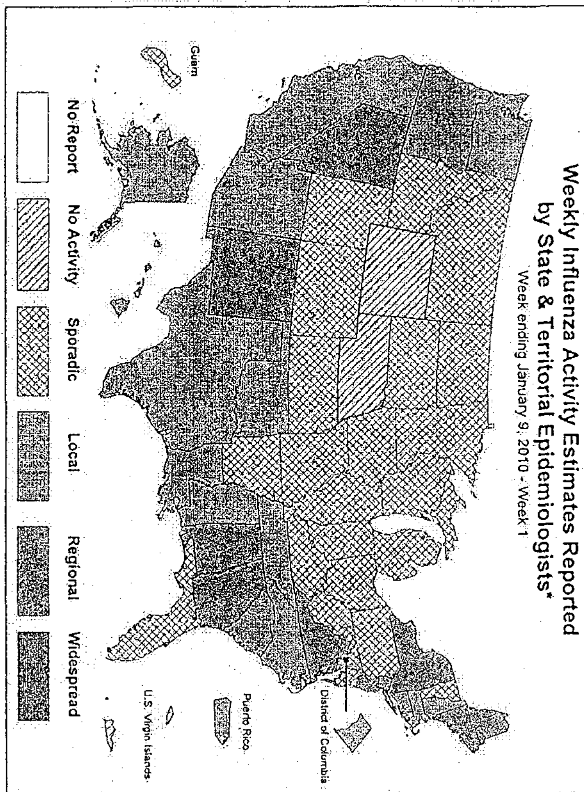
NOTE: Scales differ between regions

*Use of the regional baselines for state data is not appropriate.

Geographic Spread of Influenza as Assessed by State and Territorial Epidemiologists: The influenza activity reported by state and territorial epidemiologists indicates geographic spread of both seasonal influenza and 2009 influenza A (H1N1) viruses and does not measure the severity of influenza activity.

During week 1, the following influenza activity was reported:

- No states reported widespread influenza activity.
- Regional influenza activity was reported by nine states (Alabama, Georgia, Hawaii, Maine, Nevada, New Jersey, New Mexico, New York, and Virginia).
- Local influenza activity was reported by the District of Columbia, Puerto Rico, and 15 states (Alaska, Arizona, California, Connecticut, Louisiana, Massachusetts, Mississippi, New Hampshire, North Carolina, Oklahoma, Oregon, South Carolina, Tennessee, Texas, and Washington).
- Sporadic influenza activity was reported by Guam and 24 states (Arkansas, Colorado, Delaware, Florida, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maryland, Michigan, Minnesota, Missouri, Montana, North Dakota, Ohio, Pennsylvania, Rhode Island, South Dakota, Utah, Vermont, West Virginia, and Wisconsin).
- The U.S. Virgin Islands and two states (Nebraska and Wyoming) reported no influenza activity.



* This map indicates geographic spread & does not measure the severity of influenza activity

A description of surveillance methods is available at: http://www.cdc.gov/flu/weekly/fluactivity.htm
Report prepared: January 15, 2010.

別紙様式第2-1

No. 6

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 2009. 11. 12	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	人血清アルブミン		研究報告の公表状況	ABC Newsletter #38. 2009 Oct 23; 13-14.	公表国 ヨーロッパ	
販売名（企業名）	赤十字アルブミン20（日本赤十字社） 赤十字アルブミン25（日本赤十字社） 赤十字アルブミン20%静注4g/20mL（日本赤十字社） 赤十字アルブミン20%静注10g/50mL（日本赤十字社） 赤十字アルブミン25%静注12.5g/50mL（日本赤十字社）					

研究報告の概要	使用上の注意記載状況・その他参考事項等
○EU規制当局はインフルエンザパンデミック時の献血条件緩和を検討 欧州連合の血液規制委員会(Blood Regulatory Committee)は、H1N1インフルエンザ・パンデミック時の供給確保のため2つの緩和策を検討していると報告した。ヨーロッパ各国の代表は、パンデミックが深刻化した場合、輸血用血液が10-15%不足するのではと懸念している。血液規制委員会は、ヨーロッパ血液連盟(EBA)や各国の監督官庁に9月末開催の会議への出席を依頼し、血液の安定供給のためにどの基準を緩和するかを検討した。 この結果、インフルエンザ様症状回復後の献血延期期間はEU指令では14日間だが、これを7日間に短縮することがドナー確保に大きな効果があると多くの国が評価した。また、ヘモグロビン値を女性12.5g/dL、男性13.5g/dLから女性12g/dL、男性13g/dLにすることについて合意した。	赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/50mL 血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
欧州連合の血液規制委員会は、H1N1インフルエンザ・パンデミック時の供給確保のため、インフルエンザ様症状回復後の献血延期期間の短縮とヘモグロビン値の基準の緩和を検討しているとの報告である。 インフルエンザは毎年流行をみる最もポピュラーな疾患であるが、本剤によるいかなるインフルエンザウイルス感染の報告はない。本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれているため、本剤の安全性は確保されていると考える。	日本赤十字社では、問診で発熱などの体調不良者を献血不適としている。更に、平成21年5月18日付薬食血発第0518001号「新型インフルエンザの国内発生に係る血液製剤の安全性確保について」に基づき、新型インフルエンザの患者又は罹患の疑いのある患者と7日以内に濃厚な接触があった人の献血を制限するほか、献血後に新型インフルエンザと診断された場合には当該血漿の使用を禁止している。新型インフルエンザが流行した場合、献血者減少につながることも予想されることから、今後も引き続き情報の収集に努める。

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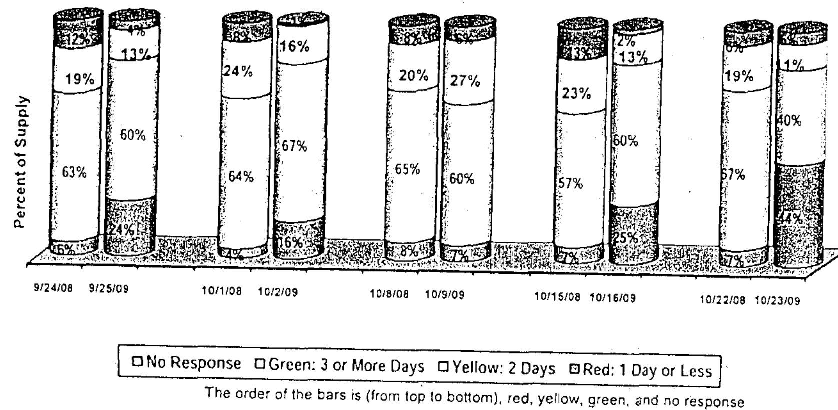
(18)

JRC2009T-060

STOPLIGHT: Status of the ABC Blood Supply, 2008 vs. 2009



The order of the bars is (from top to bottom), red, yellow, green, and no response

EU Regulator Considers Relaxing Blood Donor Requirements for Flu Pandemic

The Blood Regulatory Committee of DG SANCO, the European regulator for blood requirements, is considering relaxing two of its rules to help assure sufficient blood supplies should an H1N1 flu pandemic create shortages, according to a summary report issued by the committee. Representatives from various European countries and member states are concerned that a severe pandemic could result in a shortage of blood components of up to 10 or 15 percent.

To address this possibility, the committee asked the European Blood Alliance (EBA), the association of national suppliers and regional alliances in Europe, and the national regulators (the so-called "competent authorities" for each European Union [EU] member state or country) to attend a meeting at the end of September to discuss the potential impact of the flu on supply, to consider which rules might be relaxed to maintain an adequate supply, and to gather information from the member states on the measures and contingency plans they are considering in case the blood supply is at risk because an NHIN1 influenza pandemic affects both donors and the staffs of national blood services.

The Blood Regulatory Committee sets standards of quality and safety for the collection, testing, processing, storage, and distribution of human blood and blood components. In advance of the meeting, it prepared a working paper providing background information on the following points to be addressed. The paper included:

1. An overview of the potential impact of a pandemic on the blood supply in the EU;
2. Identification of the best ways to correct a potential impact and maintain supply; and
3. An analysis of the potential conflicts between these strategies and the minimum standards for blood and blood components set by the European legislation.

During the meeting, participants were provided with several supporting documents, originating from either member states or the EBA.

(continued on page 14)

EBA Standards (continued from page 13)

Two EU standards were identified as being levers to increase the blood supply on an exceptional and temporary basis in case of a severe shortage. The first involves the deferral period after a potential donor's recovery from a flu-like illness. The EU directive requires that 14 days must elapse between the end of flu-like symptoms in a prospective donor and the donation. Most member states said that reducing this deferral to seven days would have a major effect on accepting donors during a pandemic.

The member states and the committee agreed to request a risk assessment from the European Centre for Disease Control and Prevention on the impact of reducing this deferral period from 14 days to seven or even five days.

In terms of acceptable hemoglobin levels in donors prior to donation, current EU rules state thresholds of 12.5 grams per deciliter (g/dL) for women and 13.5 g/dL for men. There was a consensus among the delegates to the meeting that for a pandemic, these levels could be reduced to 12 and 13 g/dL, respectively, without putting the health of the donors at risk.

FDA prefers to defer decisions. When a similar meeting was held earlier this year with officials from the FDA Centers for Biologics Research and Review and representations of various blood organizations, FDA said it prefered not to address "theoretical" questions on donor criteria. It said it would consider such issues as needed. (Source: Blood Regulatory Committee, Summary Report, 9/29/09) ♦

PEOPLE

Elizabeth G. Nabel will be leaving her current position as director of the National Heart Lung and Blood Institute (NHLBI) at the National Institutes of Health to become the next president of Brigham and Women's Hospital and Faulkner Hospital in Boston, the two medical centers announced on Thursday. She will start the new job on January 1, 2010, when the hospitals' current president, **Gary Gottlieb**, becomes president and chief executive of Boston's Partners HealthCare, the parent organization of the two medical centers and Massachusetts General Hospital. He is replacing **James Mongan**, who will be retiring at the end of the year. Nabel, a cardiologist who graduated from Cornell University Medical College, has served at Brigham and Women's before: she completed her internship and residency in internal medicine there, as well as a clinical and research fellowship in cardiovascular medicine. She served on the faculty at the University of Michigan in the 1990s, and she joined NHLBI in 1999.

CORRECTION: An article in the Oct. 16, 2009, *ABC Newsletter* misstated the relationship between Tom and Sue Zuck. She is his wife. We apologize for the mistake. ♦

Save the Date: FDA Workshop on Emerging Arboviruses

The blood banking community has learned that the Food and Drug Administration will be holding a workshop on emerging arboviruses and recipient safety on Dec. 14-15, 2009 at the National Institutes of Health in Bethesda, Md. The official announcement will be made in the next few weeks. Pre-registration for this free workshop will be required, and forms will be available at the time of the announcement.

別紙様式第 2-1
番号 11

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2010 年 3 月 8 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	乾燥濃縮人アンチトロンビンIII		研究報告の公表状況	Clinical Infectious Diseases 2010; 50(5): 672-678	公表国 オーストラリア	
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研究報告の概要

背景
パンデミック 2009 インフルエンザ A 型ウイルス(H1N1)の重症感染は、妊娠、肥満、および免疫抑制を含むリスクファクターと関連している。重症の 1 例で免疫グロブリン G2(IgG2)欠損が同定されたことを受けて、我々は H1N1 感染患者のコホートでの IgG サブクラスのレベルを調べた。

方法
H1N1 の急性で重症の感染患者(集中治療室での呼吸のサポートを必要とする感染と定義した)、中等度の H1N1 感染患者(入院患者だが集中治療室へは収容されていない患者と定義した)、および健康な妊娠女性からランダムにサンプリングした被験者を対照として、患者および対照の血清 IgG および IgG サブクラスのレベルを含む特性を調べた。

結果
H1N1 感染した 39 例の患者(重症感染が 19 例、そのうち 7 例が妊娠中；中等度感染が 20 例でそのうち 2 例が妊娠中)のうちで、低アルブミン血症(P<0.001)、貧血(P<0.001)、および総 IgG(P=0.01)、IgG1(P=0.022)、IgG2(19 例中 15 例 vs. 20 例中 5 例；P=0.001；平均値±標準偏差[SD]，1.8±1.7 g/L vs. 3.4±1.4 g/L；P=0.003)が低レベルであったことは、統計学的に有意に重症 H1N1 感染と関連していたが、多変量解析で統計学的に有意であったのは低アルブミン血症(P=0.02)と平均の IgG2 レベルが低値であったこと(P=0.043)のみであった。IgG2 欠損患者で生存していた 15 例(79%)のフォローアップを急性期の最初の検体採取後、平均(±SD)で 90±23 日目(範囲は 38-126 日目)に行ったところ、低アルブミン血症は大多数の症例で解消していたが、15 例中 11 例(73%)の患者では IgG2 欠損はそのままであった。対照の健康な妊娠女性 17 例では、10 例で軽度の IgG1 および/または IgG2 レベルの低値が認められたが、H1N1 感染のあった妊娠患者では IgG2 レベルが有意に低かった(P=0.001)。

結論
重症 H1N1 感染は IgG2 の欠損と関連し、それは患者の多くで持続性となるものと考えられる。IgG2 レベルの妊娠に関連した低下が、妊娠女性の全てとは言えないまでもいくらかの比率で H1N1 感染の重症度が増加することを説明するものかもしれない。H1N1 感染の発症機序における IgG2 欠損の役割を知るにはさらに研究が必要であるが、それはこのことが治療上意義を有する可能性があるからである。

使用上の注意記載状況・その他参考事項等

代表としてノイアート静注用 500 単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アンチトロンビン III を濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
パンデミック 2009 インフルエンザ A 型ウイルス(H1N1)重症感染と血清中の IgG2 低値は関連しているとの報告である。 インフルエンザA(H1N1)はオルソミクソウイルス科に属し、ビリオンは球形で、直径80～120nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、インフルエンザA(H1N1)が原料血漿に混入したとしてもBVDをモデルウイルスとしたウイルスバリデーション試験成績から、製造工程にて十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。

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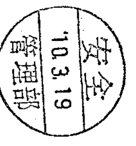

Association between Severe Pandemic 2009 Influenza A (H1N1) Virus Infection and Immunoglobulin G_2 Subclass Deficiency

C. L. Gordon,[1] P. D. R. Johnson,[1,10] M. Permezel,[5] N. E. Holmes,[1] G. Gutteridge,[2] C. F. McDonald,[3] D. P. Eisen,[6,10] A. J. Stewardson,[6] J. Edington,[7] P. G. P. Charles,[1] N. Crinis,[4] M. J. Black,[8] J. Torresi,[1,10] and M. L. Grayson,[1,4,9,10]

[1]Infectious Diseases, [2]Intensive Care, [3]Respiratory, and [4]Pathology Departments, Austin Health, [5]Department of Obstetrics and Gynaecology, Mercy Hospital for Women, [6]Victorian Infectious Diseases Service, Royal Melbourne Hospital, University of Melbourne, Melbourne, [7]Intensive Care Unit, Bendigo Health, [8]Pathology Department, Alfred Health, [9]Department of Epidemiology and Preventive Medicine, Monash University, and [10]Department of Medicine, University of Melbourne, Melbourne, Australia

Background. Severe pandemic 2009 influenza A virus (H1N1) infection is associated with risk factors that include pregnancy, obesity, and immunosuppression. After identification of immunoglobulin G_2 (IgG_2) deficiency in 1 severe case, we assessed IgG subclass levels in a cohort of patients with H1N1 infection.

Methods. Patient features, including levels of serum IgG and IgG subclasses, were assessed in patients with acute severe H1N1 infection (defined as infection requiring respiratory support in an intensive care unit), patients with moderate H1N1 infection (defined as inpatients not hospitalized in an intensive care unit), and a random sample of healthy pregnant women.

Results. Among the 39 patients with H1N1 infection (19 with severe infection, 7 of whom were pregnant; 20 with moderate infection, 2 of whom were pregnant), hypoalbuminemia ($P < .001$), anemia ($P < .001$), and low levels of total IgG ($P = .01$), IgG_1 ($P = .022$), and IgG_2 (15 of 19 vs 5 of 20; $P = .001$; mean value ± standard deviation [SD], 1.8 ± 1.7 g/L vs 3.4 ± 1.4 g/L; $P = .003$) were all statistically significantly associated with severe H1N1 infection, but only hypoalbuminemia ($P = .02$) and low mean IgG_2 levels ($P = .043$) remained significant after multivariate analysis. Follow-up of 15 (79%) surviving IgG_2-deficient patients at a mean (±SD) of 90 ± 23 days (R, 38–126) after the initial acute specimen was obtained found that hypoalbuminemia had resolved in most cases, but 11 (73%) of 15 patients remained IgG_2 deficient. Among 17 healthy pregnant control subjects, mildly low IgG_1 and/or IgG_2 levels were noted in 10, but pregnant patients with H1N1 infection had significantly lower levels of IgG_2 ($P = .001$).

Conclusions. Severe H1N1 infection is associated with IgG_2 deficiency, which appears to persist in a majority of patients. Pregnancy-related reductions in IgG_2 level may explain the increased severity of H1N1 infection in some but not all pregnant patients. The role of IgG_2 deficiency in the pathogenesis of H1N1 infection requires further investigation, because it may have therapeutic implications.

Since the onset of the current novel influenza A (H1N1) virus pandemic, it has been recognized that certain risk factors, such as pregnancy, obesity, and immunosuppression, are associated with severe disease [1, 2]. In Victoria, Australia, which was one of the key regions for the H1N1 pandemic in the Southern Hemisphere [3, 4], such risk factors have been frequently observed in our sickest patients, but the explanation for this association has remained elusive [5].

We identified immunoglobulin G_2 (IgG_2) subclass deficiency in 1 young pregnant patient who had an unusual presentation with severe H1N1 infection that required intensive care unit (ICU) admission. Because of this observation, we systematically assessed total IgG and IgG subclasses in all patients with H1N1 infection requiring ICU care (many of whom were pregnant) and

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Reprints or correspondence: Prof M. Lindsay Grayson, Infectious Diseases Dept, Austin Hospital, Austin Health, PO Box 5555, Studley Rd, Heidelberg, VIC, Australia 3084 (Lindsay.Grayson@austin.org.au).

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compared these results with those obtained from all inpatients with less severe H1N1 infection (ie, those patients who did not require ICU admission), as well as a random sample of healthy pregnant women who presented for routine antenatal care.

METHODS

The study was initially undertaken at Austin Health (AH), a tertiary university teaching hospital in Melbourne, Australia. After the observation of IgG_2 deficiency in a patient with H1N1 infection, all patients with polymerase chain reaction (PCR)–proven H1N1 infection who were sufficiently unwell to require admission to AH underwent routine hematological and biochemical assessment, had their serum immunoglobulin levels and subclasses determined, and were reviewed for their clinical features, demographic characteristics, and treatment outcome. Acute-phase serum samples were either assessed prospectively or were retrieved from storage for analysis; patients for whom there were no appropriate stored serum samples were noted but not included in the study. Because of the potential therapeutic implications of our initial findings, and after discussions with the Department of Human Health Victoria, we subsequently broadened recruitment to 2 other hospitals in Victoria (Royal Melbourne Hospital [RMH] and Bendigo Health [BH]), which were actively managing patients with severe H1N1 infection and had ICU admission criteria that were similar to those at AH, to obtain similar acute-phase serum specimens and clinical details.

The following definitions were used for the study: patients with severe H1N1 infection were defined as those with confirmed H1N1 infection who required admission to the ICU for respiratory (invasive or noninvasive mechanical ventilation) and/or vasopressor support, whereas patients with moderate H1N1 infection were defined as those who required hospital inpatient (but not ICU) care. Community-acquired pneumonia was defined according to the Infectious Diseases Society of America guidelines [6].

The clinical and laboratory features of patients with severe H1N1 infection at the 3 recruitment sites (AH, RMH, and BH) were compared with those of patients with moderate H1N1 infection (AH). All patients who were found to be IgG subclass deficient during their acute illness were followed up to obtain convalescent immunoglobulin and IgG subclass levels to assess whether the identified deficiency was transitory or persistent.

Because a large number of our patients with severe H1N1 infection were pregnant, we investigated the immunological status of a random sample of healthy pregnant women to compare these results with those observed among pregnant women with moderate and severe H1N1 infection. Thus, we obtained serum samples from 15–20 healthy pregnant women who had antenatal outpatient visits at the Mercy Hospital for Women (Melbourne, Australia) on 19 or 20 July 2009.

All data were summarized and analyzed according to H1N1 infection severity (severe vs moderate), presence of pregnancy, and, if the patient was pregnant, presence of H1N1 illness (patients with H1N1 infection vs healthy control subjects). Ethics committee approval was obtained at all 4 participating centers that undertook the study.

Laboratory assays. The presence of H1N1 infection was confirmed by strain-specific PCR at the Victorian Infectious Diseases Reference Laboratory and World Health Organization Influenza Reference Laboratory (Melbourne, Australia) using standard H1N1 assays.

Serum immunoglobulins (IgG, IgM, and IgA) were assessed using both a Beckman IMMAGE 800 analyzer (Beckman Coulter) and an Abbott Architect *ci*8200 analyzer (Abbott Laboratories, Abbott Park) in accordance with the manufacturers' instructions. Similarly, immunoglobulin subclasses (IgG_1, IgG_2, IgG_3, and IgG_4) were measured using Binding Site Human IgG Subclass kits on a Beckman IMMAGE 800 analyzer in accordance with the manufacturer's instructions. The reference ranges for normal adults according to the manufacturer were as follows: total IgG, 7.0–16.5 g/L; IgG_1, 3.8–9.3 g/L; IgG_2, 2.4–7.0 g/L; IgG_3, 0.22–1.76 g/L; IgG_4, 0.04–0.86 g/L. Routine hematological and biochemical analyses were performed in the Pathology Departments at contributing hospitals.

Statistical analysis. Univariate analysis was undertaken using Fisher's exact test, Student's *t* test, or the Wilcoxon rank-sum test (as appropriate) with Stata software, version 8.2 (Stata Corporation), to identify features associated with H1N1 infection severity. Variables that were potentially associated ($P < .2$) on univariate analysis were included in a multivariate analysis to identify features statistically associated with severe H1N1 infection. Similarly, a univariate analysis of the clinical and laboratory features of healthy vs H1N1-infected pregnant participants was undertaken to assess for any associations with the presence of H1N1 infection. A *P* value of $\leq .05$ was considered to be statistically significant.

RESULTS

Severe versus moderate H1N1 infection. A total of 47 patients with acute H1N1 infection (19 with severe infection and 28 with moderate infection) were assessed from 30 May through 16 August 2009. Appropriate serum specimens were available for 39 patients (19 with severe infection and 20 with moderate infection), and results are shown in Table 1. Among the 8 patients for whom no serum samples were available, no special features were noted to explain the lack of stored serum samples.

Patient demographic data and comorbidities for the 39 participants were similar between the severe and moderate H1N1

Table 1. Comparison of Results for Immunoglobulin (Ig) Levels for Patients with Severe versus Moderate H1N1 Infection

Variable	Severe H1N1 infection (n = 19)	Moderate H1N1 infection (n = 20)	*P*
Age, mean years ± SD (range)	36 ± 19 (16–79)	41 ± 16 (19–76)	.32
Male sex	7	11	.34
Pregnant[a]	7	2	.065
Comorbidity			
Hematological malignancy[b]	1	2	>.99
Solid-organ transplantation	0	2	.49
Asthma (requiring inhaled corticosteroids only)	3[c]	6[d]	.45
Obesity	1[c]	3[d]	.60
Diabetes mellitus	3[c]	5[d]	.70
Influenza-related myocarditis	1	0	...
Pneumonia present[e]	16	4	<.001
ICU management[f]			
Endotracheal intubation/ventilation alone	12	...	...
Endotracheal intubation/ventilation plus ECMO	2	...	...
Noninvasive ventilation/high-flow oxygen	5	...	...
Mortality	2	0	.23
Laboratory results			
Hemoglobin level, mean g/L (±SD)	104 ± 23	133 ± 21	<.001
Leukocyte count, mean cells × 10^9/L (±SD)	10.4 ± 10.5	8.7 ± 8.3	.56
Lymphocyte count, mean cells × 10^9/L (±SD)	0.94 ± 0.5	3.0 ± 8.8	.31
Renal impairment (creatine level >110 μmol/L)	4	3	.70
Abnormal liver function	16	11	.08
Serum albumin level, mean g/L ± SD (range)[g]	23 ± 5 (16–34)	35 ± 5 (23–42)	<.001
Immunoglobulin data			
Mean day (±SD) of H1N1 illness when serum immunoglobulins assessed (range)	6.2 ± 2.4 (3–11)	6.9 ± 6.1 (1–23)	.67
Low IgA	3[h]	2[h]	.66
Low IgM	2[h]	4[h]	.66
Low total IgG	12[i]	4	.01
Total IgG levels, mean g/L (±SD)	7.2 ± 5.5	9.7 ± 2.4	.069
Patients with low IgG_1	11	4	.022
IgG_1 levels, mean g/L (±SD)	4.2 ± 3.9	5.2 ± 1.9	.31
Patients with low IgG_2	15[i]	5	.001
IgG_2 levels, mean g/L (±SD)	1.8 ± 1.7	3.4 ± 1.4	.003

NOTE. Data are no. of patients, unless otherwise indicated. Severe H1N1 infection was defined as requiring intensive care unit (ICU) admission and respiratory support. Moderate H1N1 infection was defined as requiring hospital admission but not ICU admission. ECMO, extra-corporeal membrane oxygenation; SD, standard deviation.

[a] Of the 7 pregnant women with severe H1N1 infection, 2 had mild asthma (not using inhaled corticosteroids), whereas 1 pregnant woman with moderate H1N1 infection had both type 2 diabetes mellitus and obesity.

[b] One patient in each group had chronic lymphocytic leukemia.

[c] One patient had obesity and diabetes, and 1 patient had asthma and diabetes. All 3 patients had type 2 diabetes.

[d] One patient had asthma, obesity, and diabetes; 2 patients had obesity and diabetes; 3 patients had asthma and diabetes; 1 patient had obesity and asthma. Two of 5 patients had type 1 diabetes, and 3 of 5 patients had type 2 diabetes.

[e] Community-acquired pneumonia was defined according to Infectious Diseases Society of America guidelines (6).

[f] Among patients who required endotracheal intubation/ventilation alone, ECMO, and noninvasive ventilation/high-flow oxygen, pregnancy was present in 4, 1, and 2 patients, respectively.

[g] Serum albumin level on same day that immunoglobulin levels were measured.

[h] Deficiencies in IgM and IgA were all mild.

[i] An additional patient who was 16 years and 11 months of age was not reported to have deficient immunoglobulin levels, because her immunoglobulin levels were within the pediatric range; however, these values would have been considered to be deficient if the adult (defined as ≥17 years of age) normal range values had been used.

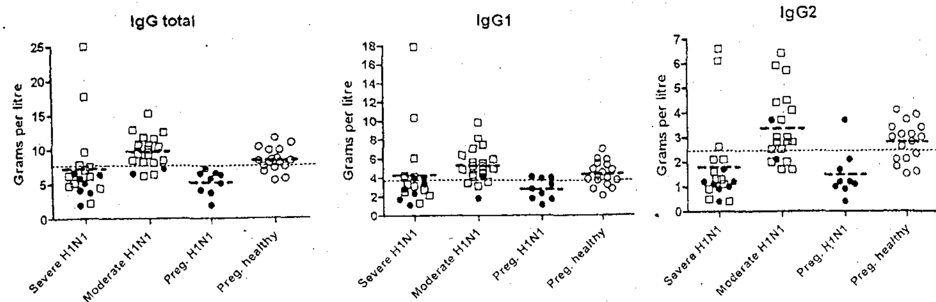

Figure 1. Serum immunoglobulin G (IgG) (total), IgG_1, and IgG_2 levels for patients with acute H1N1 infection stratified according to disease severity (severe vs moderate) and compared with healthy pregnant (Preg) patients. Data are shown for pregnant patients with H1N1 infection (●), nonpregnant patients with H1N1 infection (□), and healthy pregnant control patients (○). *Dashed line,* mean value of each grouping; *dotted line,* lower limit of normal adult range for the relevant immunoglobulin.

infection groups, except that pregnancy was more common among patients in the severe H1N1 infection group (7 of 19 vs 2 of 20); however, this difference did not achieve statistical significance ($P = .065$; Table 1).

Hypoalbuminemia and anemia were more common among patients with severe H1N1 infection ($P < .001$ for both; Table 1). Similarly, the presence of severe H1N1 infection was significantly associated with low levels of total IgG (12 of 19 vs 4 of 20 patients; $P = .01$), IgG_1 (11 of 19 vs 4 of 20 patients; $P = .022$) and IgG_2 (15 of 19 vs 5 of 20 patients; $P = .001$; Table 1 and Figure 1), compared with patients with moderate H1N1 infection. Furthermore, 1 patient with severe H1N1 infection (patient A) was a pregnant woman at 21 weeks gestation (age, 16 years and 11 months) who had an IgG_2 level of 1.1 g/L, which was reported as normal on the basis of the IgG_2 reference ranges used for children (age ≤16 years: 0.6–5.0 g/L) but would have been considered to be deficient if the adult reference ranges (age ≥17 years: 2.4–7.0 g/L) had been applied.

Assessment of the mean (± standard deviation [SD]) concentrations of total IgG and IgG subclasses demonstrated that patients with *severe H1N1 infection* had significantly lower levels of IgG_2 (and therefore lower levels of total IgG) than did patients with moderate H1N1 infection (Table 1). However, the mean (±SD) levels of IgG_1 (4.2 ± 3.9 vs 5.2 ± 1.9 g/L; $P = .31$), IgG_3 (0.50 ± 0.28 vs 0.77 ± 0.55 g/L; $P = .07$) and IgG_4 (0.28 ± 0.43 vs 0.24 ± 0.24; $P = .68$) were not significantly different between patients with severe and patients with moderate H1N1 infection (Figure 1).

The association between pregnancy, hypoalbuminemia, anemia, and low levels of IgG_2 with severe H1N1 infection were assessed in a multivariate model. The results are shown in Table 2. Abnormal liver function test results were not included in this analysis, because they were correlated with hypoalbuminemia ($P = .024$). After this analysis, only low mean serum concentrations of IgG_2 and albumin remained statistically significantly associated with severe H1N1 infection, compared with moderate H1N1 infection ($P = .043$ and $P = .02$, respectively; Table 2).

Among the 21 patients identified as IgG_2 deficient during the acute stage of H1N1 infection (16 with severe infection, including patient A; 5 with moderate infection), convalescent serum samples was obtained from 15 patients (71%; 11 with severe infection, 6 of whom were pregnant; 4 with moderate infection, 1 of whom was pregnant) a mean (±SD) of 90 ± 23 days (range, 38–126 days) after the initial acute-phase specimen was obtained. Convalescent-phase serum samples were not available for 6 patients, because 2 had died, 3 were not contactable, and 1 refused testing. Serum IgG_2 results are shown in Figure 2. Among the 11 patients with previous severe H1N1 infection, serum IgG_2 levels remained in the deficient range for 8 (73%; 3 postpartum, one pregnant, and 4 nonpregnant; Figure 2). Two of the 3 patients with severe H1N1 infection with normal convalescent serum IgG_2 levels were postpartum women; 1 of these 2 women had received intravenous pooled immunoglobulin as a component of her therapy for severe

Table 2. Multivariate Analysis of Features Potentially Associated with Severe versus Moderate H1N1 Infection

Variable	Odds ratio (95% confidence interval)	P
Pregnancy	8.9 (0.32–248.2)	.20
Mean hemoglobin per g/L	1.01 (0.94–1.08)	.80
Mean serum albumin per g/L	1.6 (1.08–2.3)	.02
Mean immunoglobulin G_2 level per g/L	2.25 (1.03–4.92)	.043

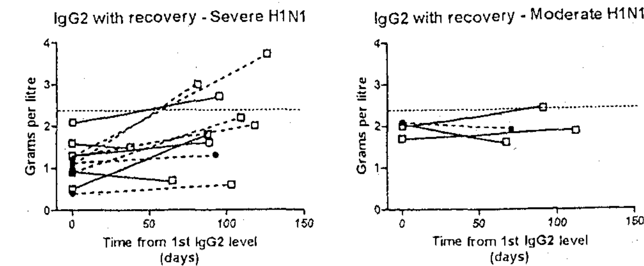

Figure 2. *Comparison of serum immunoglobulin G subclass 2 (IgG_2) levels among patients with IgG_2 deficiency during severe H1N1 infection and with recovery (nonpregnant and pregnant women). Data are shown for pregnant patients with H1N1 infection (●) and nonpregnant patients with H1N1 infection (□). Dashed line,* pregnant patient at time of initial IgG_2 sample; *dotted line,* lower limit of normal adult range for IgG_2.

H1N1 infection, but this was 77 days before testing of convalescent-phase serum samples. Notably, the only patient with severe H1N1 infection with normal convalescent-phase IgG_2 levels who was nonpregnant was only mildly deficient during the acute phase of illness (acute-phase IgG_2 level, 2.1 g/L; convalescent-phase IgG_2 level, 2.6 g/L; normal range, ≥2.4 g/L). Of the 4 patients with moderate H1N1 infection who were assessed at follow-up, 3 remained IgG_2 deficient, including 1 woman who was still pregnant at this time (Figure 2).

Persistence of immunoglobulin deficiency was less prominent for non-IgG_2 subclasses. Among the 8 patients with severe H1N1 infection who were initially deficient in IgG_1, 6 had normal IgG_1 levels on testing of convalescent-phase serum samples (data not shown). Similarly, hypoalbuminemia had resolved in most patients (9 of 14 assessable patients); however, of the other 5 patients, 2 remained pregnant at the time of follow-up.

Immunoglobulin levels and pregnancy. A total of 9 patients with H1N1 infection were pregnant (23%; Table 1). Serum immunoglobulin levels for these patients were compared with levels for 17 healthy pregnant control subjects, and results are shown in Figure 1 and Table 3. The healthy pregnant women were slightly older than those with H1N1 infection, but both groups were similar with regard to mean gestation period (Table 3). Among the 17 healthy patients, 10 had mildly low IgG_1 and/or IgG_2 levels, compared with the standard reference range for nonpregnant women (IgG_1 alone, 4 patients; IgG_2 alone, 4 patients; IgG_1 and IgG_2, 2 patients). However, pregnant women with H1N1 infection had significantly lower mean levels of total IgG ($P < .001$), IgG_1 ($P = .005$), and IgG_2 ($P = .001$) than did the 17 control subjects (Table 3 and Figure 1).

Table 3. Comparison of Results for Pregnant Women with H1N1 Infection versus Healthy Control Subjects

Variable	Patients with H1N1 infection[a] ($n = 9$)	Healthy control subjects[b] ($n = 17$)	P
Age, mean years ± SD (range)	24 ± 6.2 (16–37)	30 ± 3.9 (20–36)	.008
Gestation, mean weeks ± SD (range)	32 ± 6.0 (21–38)	35 ± 2.9 (29–40)	.16
Low total IgG	7[c]	3	.009
Total IgG level, mean g/L (±SD)	5.2 ± 1.7	8.5 ± 1.7	<.001
Low IgG_1	6	6	.22
Mean (±SD) IgG_1 level, mean g/L (±SD)	2.8 ± 1.1	4.4 ± 1.3	.005
Low IgG_2	7[c]	6	.097
IgG_2 level, mean g/L (±SD)	1.5 ± 1.0	2.8 ± 0.8	.001

NOTE. Data are no. of patients, unless otherwise indicated. IgG, immunoglobulin G.
[a] Including 7 patients with severe H1N1 infection and 2 patients with moderate H1N1 infection.
[b] Two healthy pregnant patients had gestational diabetes.
[c] An additional patient who was 16 years and 11 months of age was not reported to have deficient immunoglobulin levels, because her immunoglobulin levels were within the pediatric range; however, these values would have been considered to be deficient if the adult (defined as ≥17 years of age) normal range values had been used.

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DISCUSSION

Although a number of authors have described the clinical features of H1N1 infection [7–9], including those of pregnancy as a risk factor for severe H1N1 infection [10], this is, to our knowledge, the first report to identify a potential association between H1N1 disease severity and the presence of immunoglobulin subclass deficiency. Patients with severe H1N1 infection were significantly more likely to be deficient in IgG$_2$ than were patients with moderate H1N1 infection ($P = .001$); IgG$_2$ deficiency was not necessarily noticeable if only total IgG levels were assessed. Furthermore, our findings suggest that, for the majority of such patients (11 of 15 patients; 73%), IgG$_2$ deficiency persists after recovery from H1N1 infection, regardless of whether the illness was associated with possible risk factors, such as pregnancy. Low IgG$_2$ levels are therefore less likely to be simply related to a severe inflammatory response, as is sometimes noted for acute-phase reactants, such as albumin, creatine kinase, and lactate dehydrogenase [8, 11].

IgG subclass deficiency is usually asymptomatic, and low levels of 1 or more IgG subclasses can be found in 2%–20% of healthy individuals [12, 13]. If symptomatic, patients with IgG subclass deficiency tend to have recurrent sinopulmonary bacterial infections [13]. However, to our knowledge, IgG subclass deficiency has not been studied in detail in humans with influenza infection, although in mouse models, anti-influenza antibody (and specifically IgG) has a key role in virus control in the lower respiratory tract, compared with the upper respiratory tract [14, 15]. In humans, Logtenberg et al [16] described a single patient with severe transitory hypogammaglobulinemia associated with acute influenza A virus infection. However, in this case, all immunoglobulin classes (IgG, IgM, and IgA) were affected. Other than this report, we can find no other association between influenza and immunoglobulin deficiency.

Thus, it is uncertain whether we have simply identified a cohort of patients with H1N1 infection with underlying unrecognized IgG$_2$ deficiency, or whether there is an interaction between the H1N1 virus and the host that leads to such deficiency. Given that the half-life of IgG$_2$ is ~3 weeks [17], a potent and specific interaction between H1N1 virus and host B cells would need to occur to lead to such a precipitous decrease in serum IgG$_2$. Bone marrow apoptosis of B cells by influenza virus has been demonstrated in mice [18], but how this relates to disease in humans remains unclear. However, the fact that the IgG$_2$ deficiency that we identified appears to persist in most cases long after disease resolution (convalescent serum samples were collected a mean (± SD) of 90 ± 23 days after the acute phase of illness) suggests the possibility of potential long-term implications for these patients and that follow-up of moderate and severe cases of H1N1 infection may be warranted.

Because of our findings, we hypothesize that IgG$_2$ deficiency may be associated with an inability to mount an early effective immune response to influenza and may therefore be linked to severe disease. Furthermore, if the IgG$_2$ deficiency that we observed is long-lasting or permanent, will this affect the patients' likely response to influenza vaccination? Response to influenza vaccination is measured by specific neutralization assays, rather than by total immunoglobulin concentrations, and it is not known whether response to influenza vaccination by individuals who are IgG$_2$ subclass deficient is diminished.

Pregnancy is a known risk factor for increased severity of both seasonal and pandemic influenza infections [19–23], which is thought to be attributable to pregnancy-related physiologic and immunologic changes, such as decreased lung capacity and increased cardiovascular demand, as well as a shift away from cell-mediated immunity to humoral immunity [24]. Our finding that a substantial number (10 of 17) of our healthy pregnant cohort had mildly low IgG$_1$ and/or IgG$_2$ levels is consistent with the known decrease in immunoglobulin levels that occurs during normal pregnancy and resolves after delivery [25, 26]. Low IgG$_2$ levels in pregnant women could therefore potentially explain why pregnancy appears to be a risk factor for severe H1N1 infection [2–4]. However, this alone does not appear to explain the significantly lower levels of IgG$_2$ observed among pregnant patients with H1N1 infection, compared with levels among our healthy pregnant control subjects ($P = .001$), nor the fact that IgG$_2$ deficiency persisted postpartum in some women with severe H1N1 infection.

Although IgG$_2$ deficiency appears to be associated with H1N1 infection severity, it remains uncertain whether administration of immunoglobulin to patients who are IgG$_2$ deficient is likely to be therapeutically beneficial. We administered pooled immunoglobulin to some of our patients with severe H1N1 infection who had IgG$_2$ deficiency, but our observations were uncontrolled. Nevertheless, convalescent blood products were administered during the Spanish influenza pandemic with a reduction in mortality [27], and more recently, convalescent-phase plasma samples obtained from a patient who recovered from H5N1 influenza infection was used successfully [28]. Further investigation of the use of convalescent-phase blood products in severe pandemic H1N1 infection is needed.

Our study has a number of important limitations, including being of relatively limited size and lacking suitable specimens to analyze patient cellular immunity or to assess influenza virus neutralization, and we have not compared our findings with those that might be expected among healthy nonpregnant control subjects. Furthermore, with the number of cases of H1N1 infection now decreasing in Australia, our findings need to be confirmed in other geographical locations (although the H1N1 strain circulating in Victoria appears to be the same as that isolated in the Northern Hemisphere) [4].

Nevertheless, we considered our finding of a statistically significant association between IgG$_2$ deficiency and H1N1 infection severity to be sufficiently notable and hypothesis-generating in terms of potential clinical therapeutic importance that prompt notification of these data to clinicians managing cases of H1N1 infection was warranted.

Acknowledgments

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医薬部外品　　研究報告　調査報告書
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一般的名称	乾燥濃縮人アンチトロンビンⅢ		研究報告の公表状況	CDC/Emerging Infections Disease/2010/03/25	公表国 日本	
販売名（企業名）	①ノイアート静注用500単位（ベネシス） ②ノイアート静注用1500単位（ベネシス） ③ノイアート（ベネシス）					

研究報告の概要

世界的に流行している(H1N1)2009 ウイルスの献血血液の輸血を介した伝播の可能性に関心が寄せられている。
日本赤十字血液センターは、献血後に世界的に流行している(H1N1)2009 ウイルス感染の可能性を示す情報のある血液製剤を止め、NAT で血液製剤のウイルス遺伝子を確認することを試みた。
2009 年 6～11 月の間の血液サンプルは献血から製造された血漿そして赤血球製剤から集められた、献血後情報は、献血後まもなく世界的に流行している(H1N1)2009 ウイルス感染との診断を示唆した。
ウイルス RNA は、血漿サンプルそして赤血球画分はそれぞれ QIAamp Virus Biorobot MDx kit(QIAGEN, Valencia, CA, USA)そして High Pure Viral Nucleic Acid Large Volume Kit(Roche Diagnostics, Indianapolis, IN, USA)によって抽出した。
RNA サンプルは、PRISM 7900(Applied Biosystem, Foster City, CA, USA)を用いてインフルエンザ A 型の赤血球凝集素(HA)とマトリックス(M)の遺伝子をリアルタイム逆転写-PCR(RT-PCR)にかけた。
HA の RT-PCR は世界的に流行している(H1N1)2009 ウイルスに特異的であったが、M の PT-PCR は世界的に流行している(H1N1)2009 ウイルスと季節性インフルエンザ A ウイルスの両方検出できるように設計された。
プローブそしてプライマーの配列は日本の国立感染症研究所によって開発されたプロトコルに従って合成された。
献血血液サンプルを用いての試験前に、NAT システムの感度はスパイク実験によって確認した。
NAT は献血後 7 日以内にインフルエンザの症状を示した 96 人のドナーから 96 の血漿と 67 の赤血球サンプルを用いて実施された。
20 人のドナーについては、世界的に流行している(H1N1)2009 は献血後 1 日以内に、そして他の 20 人については献血後 2 日以内に診断された。
世界的に流行している(H1N1)2009 ウイルスはどの試験サンプルからも検出されなかった、しかし外部陽性コントロールでは一貫して検出された。
これらの結果は、世界的に流行している(H1N1)2009 ウイルスによるウイルス血症は、あるとしても非常に低く現行の NAT では見逃されているかもしれないこと、あるいはウイルス血症の期間がウイルス血症を確認するにはあまりにも短いことを示唆している。
輸血による世界的に流行しているインフルエンザの伝播のリスクは低いようであるが、にもかかわらず、さらにこのリスクを解明する調査が必要である。

使用上の注意記載状況・その他参考事項等

代表としてノイアート静注用 500 単位の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT(GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アンチトロンビン III を濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見

日本赤十字血液センターの献血後にインフルエンザの症状を示したドナーにおける、NAT によるパンデミック A(H1N1)2009 インフルエンザウイルスの調査報告である。
インフルエンザA(H1N1)はオルソミクソウイルス科に属し、ビリオンは球形で、直径80～120nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、インフルエンザA(H1N1)が原料血漿に混入したとしてもBVDをモデルウイルスとしたウイルスバリデーション試験成績から、製造工程にて十分に不活化・除去されると考えている。

今後の対応

本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。

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アンチトロンビンⅢ

BENESIS 2010-007

Risk for Transmission of Pandemic (H1N1) 2009 Virus by Blood Transfusion

To the Editor: Influenza A pandemic (H1N1) 2009 virus emerged in early 2009 in Mexico and has since spread worldwide. In Japan, the first outbreak of the novel influenza was reported in May 2009 (*1*) and became pandemic in November. Although no cases of transfusion-transmitted influenza have been published, evidence exists of brief viremia before onset of symptoms (*2,3*). The possibility of transmission of this virus through transfusion of donated blood is of concern. The Japanese Red Cross Blood Centers have intercepted blood products with accompanying postdonation information indicating possible pandemic (H1N1) 2009 infection and attempted to identify the viral genome in those products by using nucleic acid amplification technology (NAT).

During June–November 2009, blood samples were collected from plasma and erythrocyte products that had been processed from donations; postdonation information indicated diagnosis of pandemic (H1N1) 2009 infection soon after donation. Viral RNA was extracted from plasma samples and erythrocyte fractions by using a QIAamp Virus Biorobot MDx kit (QIAGEN, Valencia, CA, USA) and a High Pure Viral Nucleic Acid Large Volume kit (Roche Diagnostics, Indianapolis, IN, USA), respectively. RNA samples were subjected to real-time reverse transcription–PCR (RT-PCR) of hemagglutinin (HA) and matrix (M) genes of influenza A by using PRISM 7900 (Applied Biosystems, Foster City, CA, USA). The RT-PCR of HA was specific for pandemic (H1N1) 2009 virus, whereas the RT-PCR of M was designed to detect both pandemic (H1N1) 2009 and seasonal influenza A viruses. The sequences of probes and primers were synthesized according to the protocols developed by the Japanese National Institute of Infectious Diseases (*4*). Either 200 μL of a plasma sample or 100 μL of packed erythrocytes was used for each test, and the test was performed 2× for each gene in each sample. Before the investigation using donated blood samples, the sensitivity of the NAT system was checked by spiking experiments. Viral particles of pandemic (H1N1) 2009 virus (A/California/04/2009 [H1N1]), donated by the National Institute of Infectious Diseases, were spiked into plasma and erythrocyte samples from healthy volunteers. Viral RNA was detected in the plasma samples spiked with viral particles corresponding to 300 genome equivalents/mL and in the packed erythrocyte samples spiked with viral particles corresponding to 3,000 genome equivalents/mL.

NAT was conducted by using 96 plasma and 67 erythrocyte samples obtained from 96 blood donors who had symptoms of influenza within 7 days postdonation. For 20 donors, pandemic (H1N1) 2009 was diagnosed within 1 day postdonation and, for another 20, within 2 days postdonation (Figure). Pandemic (H1N1) 2009 virus was not found in any of the samples tested, but it was consistently detected in the external positive control. These results suggest that the viremia with pandemic (H1N1) 2009 virus, if any, is very low and can be missed by current NAT or that the viremic period is too brief to identify viremia. Although the risk for transmission of pandemic influenza by transfusion seems to be low, further investigation is needed to elucidate this risk.

Chieko Matsumoto, Rieko Sobata, Shigeharu Uchida, Takao Hidaka, Syunya Momose, Satoru Hino, Masahiro Satake, and Kenji Tadokoro

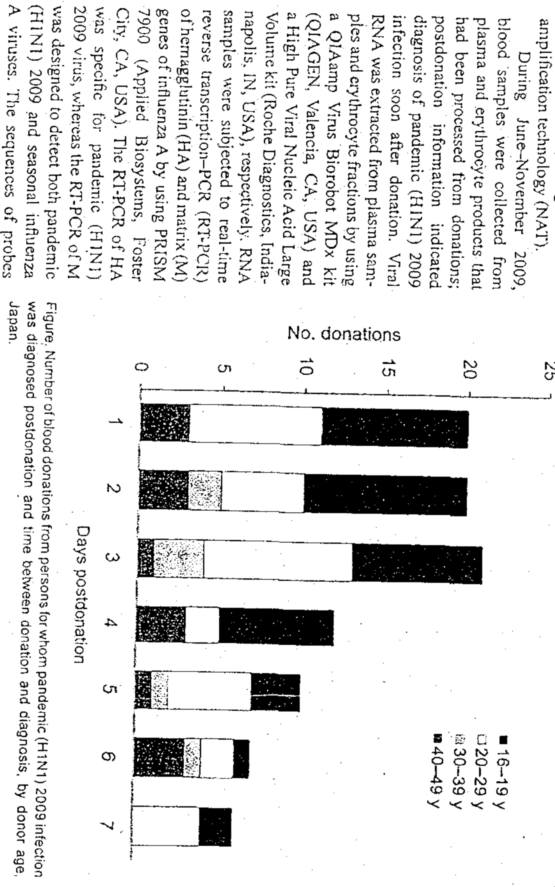

Figure. Number of blood donations from persons for whom pandemic (H1N1) 2009 infection was diagnosed postdonation and time between donation and diagnosis, by donor age, Japan.

Author affiliation: Japanese Red Cross Society Blood Service Headquarters, Tokyo, Japan

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Address for correspondence: Chieko Matsumoto, Infectious Disease Research Department, Japanese Red Cross, Central Blood Institute, JRC 2-1-67 Tatsumi, Koto-ku, Tokyo 135-8521, Japan; email: c-matsumoto@jrc.or.jp

Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Rapid Emergence of Oseltamivir Resistance

To the Editor: The influenza A pandemic (H1N1) 2009 virus has spread globally since it first appeared in Mexico in April 2009. This third influenza pandemic since the Spanish influenza pandemic of 1918 (1) has caused at least 400,000 infections within 6 months; estimated mortality rate is 1.2% (2). Emergence of oseltamivir resistance in the pandemic (H1N1) 2009 virus is a rising challenge to global control of the pandemic. So far, 39 oseltamivir-resistant pandemic (H1N1) 2009 viruses have been reported worldwide (3). Among the 32 resistant strains reported in October 2009, a total of 13 (41%) were associated with postexposure chemoprophylaxis and 16 (50%) were from samples of patients receiving oseltamivir (3). We report rapid emergence of resistance (H275Y mutation) in a patient, 4 days after early treatment with standard doses of oseltamivir for pandemic (H1N1) 2009 pneumonia.

On September 1, 2009, a 20-year-old man with mental retardation consulted the emergency department of Kaohsiung Veterans General Hospital after 1 day of fever, sore throat, and nonproductive cough. A rapid diagnostic antigen test (Quick Vue Influenza test; Quidel, San Diego, CA, USA) showed the man to be positive for influenza A. He was hospitalized for bilateral pneumonitis and treated with oseltamivir (75 mg 2×/day for 5 days), ampicillin/sulbactam, and erythromycin. However, a progressive increase in bilateral perihilar interstitial infiltration developed on the third day, accompanied by increasing dyspnea. Influenza A pandemic (H1N1) 2009 virus was isolated from the patient's nasopharyngeal secretions on days 1 and 4 by using MDCK cells. After DNA sequence analysis of the neuraminidase gene, the mutation of H275Y was not found in the first isolate, but sequence analysis of the second isolate detected mixed populations (C/T) in the 823-nt position of the neuraminidase gene. Only a single pattern (T) was found from the cultured viruses, indicating a mixed quasispecies of oseltamivir-resistant and -susceptible viruses emerging after 4 days of oseltamivir treatment. The oseltamivir-resistant viruses become dominant in the cell culture–propagated viruses. Chan et al. reported a similar case in which the original clinical specimens contained a mixed population of variants, and oseltamivir-resistant viruses become dominant after the passage in MDCK cells (4).

On his 9th day in the hospital, the patient was intubated because of acute respiratory distress syndrome (Figure) and given levofloxacin. Urine samples were negative for *Pneumococcus* and *Legionella* spp. antigens. The patient improved and was extubated on hospital day 16.

Paired serologic test results were negative for *Mycoplasma pneumoniae* and *Legionella* spp. antibody; however, immunoglobulin G for *Chlamydia pneumoniae* increased 4-fold. By 37 days after illness onset, clinical signs and symptoms resolved and bilateral lineoreticular infiltration was reduced.

On August 8, 2009, Taiwan had the most devastating typhoon (Typhoon Morakot) in 50 years. The patient reported here had stayed in a typhoon evacuation camp for 1 week before his influenza signs and symptoms developed. Although 4 sporadic cases of pandemic (H1N1) 2009 infections were reported from the same camp, none of the isolated viruses harbored the H275Y mutation in the neuraminidase gene. No evidence of virus transmission was found among healthcare personnel, family members, and camp members who had been in close contact with the patient.

Oseltamivir has been recommended by the US Centers for Disease Control and Prevention for the treatment of

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番号12

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販売名（企業名）	①ノイアート静注用500単位（ベネシス） ②ノイアート静注用1500単位（ベネシス） ③ノイアート（ベネシス）				

研究報告の概要

最近現れた世界的に流行しているインフルエンザ A(H1N1)2009 ウイルスの感染は散発的に非常に重篤な場合があるが大多数の症例は軽症例である。ウイルス赤血球凝集素(D222G)の特異的変異は、ノルウェーでの致命的及び重篤な症例で相当な頻度で見られたが、臨床的に軽度の症例では実質的に存在しなかった。この違いは統計学的に有意であり、我々のデータは突然変異と臨床転帰の間の因果関係の可能性と整合している。

2009年に世界的に流行しているインフルエンザA(H1N1)は症例の圧倒的多数では軽度そして自己限定的疾患によって特徴づけられた。しかしながら、重篤そして致命的な症例（主にウイルス性肺炎で多い）は、そのような臨床転帰が季節性インフルエンザではあまり見られない年齢層に起こっていた。どんなウイルス及び宿主関連因子がこの二分化を決定するのかをより理解することが重要である。

我々の知る限りでは、これは重篤な臨床転帰と相関する世界的に流行しているウイルスの変化の最初の同定である。しかしながら、我々のデータがD222G突然変異と重症度の間に関係あることを統計学的に有意な支持をする一方、軽度の症例数では、非重篤症例で変異ウイルスの頻度が本当に低いのか大規模な同定が必要である。D222G突然変異ウイルスが広まっていないならば、すなわち、それは伝播性でないため、公衆衛生への影響は限定的である。しかしながら、もし大規模な曝露を通して伝播したならば、普通に伝播している異型より毒性があるかもしれない、ウイルスは重症症例の管理に関連がある可能性がある。

更に、それは現在世界的に流行しているウイルスの一般的に非常に低い毒性は固定された特徴ではない、しかも、それは個人及び集団レベルでの感染を制限するための手段を実行する際の安心感のための理由にはならない。

使用上の注意記載状況・その他参考事項等

代表としてノイアート静注用500単位の記載を示す。

2. 重要な基本的注意

(1)本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人アンチトロンビンIIIを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
パンデミックインフルエンザ A(H1N1)の重症化にウイルス赤血球凝集素(D222G)の突然変異が関係しているとの報告である。 インフルエンザA(H1N1)はオルソミクソウイルス科に属し、ビリオンは球形で、直径80～120nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、インフルエンザA(H1N1)が原料血漿に混入したとしてもBVDをモデルウイルスとしたウイルスバリデーション試験成績から、製造工程にて十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。

Observed association between the HA1 mutation D222G in the 2009 pandemic influenza A(H1N1) virus and severe clinical outcome, Norway 2009-2010

A Kilander[1], R Rykkvin[1], S G Dudman[1], O Hungnes (olav.hungnes@fhi.no)[1]
1. Department of Virology, Norwegian Institute of Public Health, Oslo, Norway

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Infection with the recently emerged pandemic influenza A(H1N1) virus causes mild disease in the vast majority of cases, but sporadically also very severe disease. A specific mutation in the viral haemagglutinin (D222G) was found with considerable frequency in fatal and severe cases in Norway, but was virtually absent among clinically mild cases. This difference was statistically significant and our data are consistent with a possible causal relationship between this mutation and the clinical outcome.

The 2009 influenza A(H1N1) pandemic has been characterised by mild and self-limiting disease in the overwhelming majority of cases. However, severe and fatal cases, many of them with primary viral pneumonia, have been occurring in age groups where such clinical outcomes are very rarely seen in seasonal influenza [1,2]. It is important to better understand what viral and host-related factors determine this dichotomy.

Genetic characterisation of clinical specimens

As part of the intensified surveillance carried out during the current influenza pandemic, the national reference laboratory for human influenza at the Norwegian Institute of Public Health collected a large number of respiratory specimens from verified and possible cases of pandemic influenza. In the present study we analysed 61 respiratory specimens from severe and fatal cases that occurred between July and December 2009, as well as from 205 cases with mild clinical outcomes collected between May 2009 and January 2010. Genetic characterisation was performed using conventional sequencing, or with a pyrosequencing assay subsequently developed to detect the particular mutations described below and which facilitated investigation of a large number of specimens.

Here we report the occurrence of an amino acid substitution, aspartic acid to glycine in position 222 (D222G) in the HA1 subunit of the viral haemagglutinin, in clinical specimens from 11 out of 61 cases analysed in Norway with severe outcome. Such mutants were not observed in any of 205 mild cases investigated (Table), thus the frequency of this mutation was significantly higher in severe (including fatal) cases ($p<0.001$, Fisher's exact test, two-sided) than in mild cases. D222G mutants were detected throughout the sampling period, from the first recorded severe cases in July until early December. The frequency of another substitution in the same position, D222E, did not differ significantly between mild and severe cases ($p=0.772$). Yet another substitution, D222N, was observed in a very few cases (n=4), and at a higher rate than expected among severe cases (three of four cases, $p=0.039$). The wild type 222D was, not surprisingly, significantly less frequent in severe than in mild cases ($p<0.001$).

In several of the patients where D222G mutant viruses were found, they coexisted with wildtype 222D viruses. Further analysis of this phenomenon is ongoing.

The cases infected with the D222G-mutated virus were not epidemiologically related to each other, and the mutated viruses do not cluster together in phylogenetic analysis (data not shown).

Validity and limitations of the analysis

Cases with severe clinical outcomes were much more likely to be included in our study for several reasons: they are more likely to seek healthcare, they are more likely to be prioritised for virological testing, and their specimens are more likely to be forwarded to the national reference laboratory where they have a higher chance of being selected for detailed analysis than viruses from mild cases. Because of this, we chose to record the frequency of a given genotype in each severity group and compare it with the corresponding frequency in other severity groups. This approach is not expected to have a selection bias.

Cases were classified as mild, severe non-fatal and fatal based on the patient information that was available to us. Some seemingly mild cases may later have exacerbated to severe outcomes without our knowledge, or the presented patient information may have been incomplete, but we think these cases must be few. On the other hand, all severe and fatal cases were confirmed as non-mild. Thus, the fact remains that only cases confirmed as severe outcomes exhibited the D222G mutation in our investigation.

The sampling period for the cases analysed spans from the initial detections of the pandemic H1N1 virus in early May 2009 until early January 2010. The first severe and fatal cases occurred in July. By the end of December, the epidemic in Norway had largely passed, and a large proportion of cases in our data set is from the peak period in October and November. At all times an effort was made to include a reasonable number of non-severe cases in our analyses, and such cases were well represented throughout the pandemic. The fractions of severe/fatal cases among all analysed cases during the two-month periods July/August (n=21), September/October (n=84), and November/December (n=149), were within the range of 23% to 26%. Severe outcomes were not recorded among the few cases in May and June (n=11) and in January (n=1). We thus do not see a trend over time in the composition of severe versus mild cases in our dataset that could lead to an artificial difference in the frequency of the D222G substitution. Furthermore, the D222G substitution was represented also among the earliest fatal and severe cases in July and August.

Specimens from both the lower and upper respiratory tract were analysed. Lower respiratory tract specimens were available from severe/fatal cases only, and in some cases they were the only materials available. However, in all cases where we had paired upper and lower airway specimens (five cases with 222D and four cases with 222G), the wildtype-versus-D222G pattern was matching between the locations. We have therefore no reason to believe that this difference in proportion of lower airway specimens distorted the analysis.

Discussion

Amino acid position 222 resides in the receptor binding site of the HA protein and may possibly influence the binding specificity and thus the cellular tropism of the virus. The corresponding difference between two viruses from the 1918 Spanish influenza pandemic correlates to a shift in receptor preference [3], which conceivably could make the virus prone to infect a wider range of cells in the lower respiratory tract [4,5]. However, the effect of a mutation depends on the molecular context and it is unclear whether the binding properties are affected likewise in the present pandemic virus as they were in the 1918 influenza virus.

Our observations are consistent with an epidemiological pattern where the D222G substitution is absent or infrequent in circulating viruses, with the mutation arising sporadically in single cases where it may have contributed to severity of infection. This may aid in filling some knowledge gaps identified in a recent preliminary review of this and other mutations in the pandemic virus [6]. The correlation between presence of the D222G substitution and a severe clinical outcome may reflect an increase in pathogenicity caused by the mutation, possibly related to a change in cellular tropism rendering the virus more pneumotropic. Conversely, it is possible that the likelihood of such mutations arising is higher in patients who fail to fight off the virus rapidly and have virus already colonising the lower respiratory tract. These two possibilities are not mutually exclusive. A large proportion of the fatal and severe cases had underlying risk conditions. However, some of the D222G cases manifested themselves as a rapid unexpected deterioration after a period of mild symptoms in previously healthy subjects, and we consider it likely that there is a causal relationship between the occurrence of the D222G mutation in this virus and severe disease.

It should be borne in mind, however, that the majority of severe and fatal cases investigated did not carry the D222G substitution and, clearly, this mutation is not required for a severe outcome.

Conclusions

To our knowledge, this is the first identification of a change in the pandemic virus that correlates with a severe clinical outcome. However, whereas our data lend statistically significant support to an association between the D222G mutation and severity, the number

TABLE

Pandemic influenza A(H1N1) viruses characterised for amino acid position 222 of the haemagglutinin HA1 domain, by clinical outcome, Norway, May 2009–January 2010 (n=266)

HA1 position 222 genotype[b]	Clinical outcome[a]				
	Mild (n=205)	Severe (n=34)	Fatal (n=27)	Severe plus fatal (n=61)	All cases (n=266)
222D (wt)	92% (189)	82% (28)	59% (16)	72% (44)	88% (233)
222G	0% (0)	8.8% (3)	30% (8)	18% (11)	4.1% (11)
222E	7.3% (15)	2.9% (1)	7.4% (2)	4.9% (3)	6.8% (18)
222N	0.5% (1)	5.9% (2)	3.7% (1)	4.9% (3)	1.5% (4)
Total	100%	100%	100 %	100 %	100 %

[a] Clinical outcome based on patient information, assigned into categories by a medical specialist according to WHO guidance criteria [1].
[b] Percentage of genotype within each clinical category is given, with number of cases per category in parentheses.

of mild cases would need to be larger to determine whether mutant viruses are indeed circulating at a very low frequency also in non-severe cases. Provided that D222G mutant viruses are not circulating, i.e. that they are less transmissible, the immediate public health impact of this finding is limited. However, it may have implications for the management of severe cases where the virus, if transmitted through massive exposure, may be more virulent than the commonly circulating variant. Furthermore, it may serve as a reminder that the generally very low virulence of the current pandemic virus is not a fixed characteristic, and that there is no reason for complacency in carrying out measures that limit infection with this virus at individual and population level.

Further virological, clinical and epidemiological investigations are needed to ascertain the role of this and other mutations that may alter the virulence and transmissibility of the pandemic influenza A(H1N1) virus.

Acknowledgements

We gratefully acknowledge the essential contributions of primary diagnostic laboratories, clinicians and pathologists in making virus-containing materials and the relevant patient information available to us. We also acknowledge the Department for Infectious Disease Epidemiology for invaluable help in supplying the clinical data on many of the fatal and intensive care cases. We would like to thank Jan Oksnes, Department of Bacteriology and Immunology, as well as Torstein Aune, Hilde Elshaug, Valentina Johansen, Anne Marie Lund, Grethe Hermansen Krogh, Marianne Morken and Remilyn Ramos-Ocao, Department of Virology, for excellent technical assistance.

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別紙様式第2-1

No. 26

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
				2009. 11. 30	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Zhou JJ, Fang DY, Fu J, Tian J, Zhou JM, Yan HJ, Liang Y, Jiang LF. Virus Genes. 2009 Aug;39(1):76-80.	公表国 中国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○感染者におけるトリインフルエンザH5N1ウイルスの感染および複製
本試験では、感染者の組織中のH5N1型ウイルスの分布を理解し、H5N1型ウイルスが肺以外の組織で複製できるかどうかを調べるため、剖検を実施した。
肺のウイルス量が脾臓より多く、心臓、肝臓、腎臓、大腸、小腸または脳ではウイルスが検出されないことを認めた。具体的には、左肺(7.1 log 10 copies/mL)の方が右肺(5.7log 10 copies/mL)よりウイルス量が多かったため、左肺病変の方が病理学的損傷がひどく、肺組織中はプラス鎖・マイナス鎖ウイルスRNAの双方が存在した。
しかし、脾臓にはH5N1型ウイルス量は少なく(3.8 log 10 copies/mL)、プラス鎖RNAは存在しなかった。この結果は、H5N1型ウイルス複製が主に肺で起こり、肺の損傷程度は肺中ウイルス量と相関が高いことを示している。脾臓中の低いウイルス量は、循環血液、その他の状況によって起こったことが考えられる。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
H5N1型鳥インフルエンザウイルスの複製が主に肺で起こり、脾臓中の低いウイルス量は、循環血液、その他の状況によって起こったことが考えられるとの報告である。 インフルエンザは毎年流行をみる最もポピュラーな疾患であるが、本剤によるいかなるインフルエンザウイルス感染の報告はない。本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれているため、本剤の安全性は確保されていると考える。	日本赤十字社では家禽に高病原性トリインフルエンザの流行が認められた場合、当該飼養農場の関係者や防疫作業従事者の献血制限を行っている。新型インフルエンザが流行した場合、献血者減少につながることも予想される。今後も引き続き情報の収集に努める。

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Infection and replication of avian influenza H5N1 virus in an infected human

Jing-Jiao Zhou · Dan-Yun Fang · Jie Fu · Jiang Tian · Jun-Mei Zhou · Hui-Jun Yan · Yu Liang · Li-Fang Jiang

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Abstract The highly pathogenic avian influenza H5N1 viruses usually cause severe diseases and high mortality in infected humans. However, the tissue tropism and underlying pathogenesis of H5N1 virus infection in humans have not been clearly elucidated yet. In this study, an autopsy was conducted to better understand H5N1 virus distributions in tissues of infected humans, and whether H5N1 virus can replicate in extrapulmonary tissues. We found that the lungs had the higher viral load than the spleen, whereas no detectable viruses in tissues of heart, liver, kidney, large intestine, small intestine, or brain. Specifically, the viral load was higher in the left lung (7.1 log10 copies per ml) in relation to the right lung (5.7 log10 copies per ml), resulting in more severe pathological damage in the left lung, and lung tissues contained both positive- and negative-stranded viral RNA. However, there existed a low level of H5N1 viruses in the spleen (3.8 log10 copies per ml), with the absence of positive-stranded viral RNA. Our results indicate that replication of H5N1 viruses mainly occurs in the lungs, and the degree of lung damage is highly correlated with the viral load in the lungs. The low-load viruses in the spleen might be introduced through blood circulation or other ways.

Keywords Influenza virus · H5N1 · Replication · Viral load · Tissue distribution

Jing-Jiao Zhou and Dan-Yun Fang equally contributed to this work.

J.-J. Zhou · D.-Y. Fang · J. Fu · J. Tian · J.-M. Zhou · H.-J. Yan · Y. Liang · L.-F. Jiang (✉)
Department of Microbiology, Zhongshan School of Medicine, Sun Yat-sen University, 74 Zhongshan Road II, Guangzhou 510080, People's Republic of China
e-mail: Jianglf@mail.sysu.edu.cn

Introduction

The highly pathogenic avian influenza H5N1 viruses could replicate systemically in a variety of organs in poultry, such as respiratory tract, intestine, and spleen, affecting these organs, even the central nervous system [1–4]. H5N1 viruses could also cause disseminated infections in either naturally or experimentally infected mammalian species, i.e., tiger, leopard, and ferret [5–10]. However, evidence for extrapulmonary replication of H5N1 viruses in humans, in general, has been lacking at present. Some studies had found H5N1 viruses exclusively in respiratory tract (mainly in lung) [11, 12]. Other studies had found the presence of H5N1 viruses in many extrapulmonary organs, such as intestine, liver, and brain, which indicated that virus dissemination seems to occur in some humans through blood circulation or other ways [13–17]. In March 2006, the first case of avian influenza H5N1 virus infection was identified in Guangdong province of China. An autopsy was then conducted to detect the virus distribution and load, which, we hoped, would provide some insights into H5N1 infection and replication in both pulmonary and extrapulmonary organs.

Materials and methods

Patient and virologic diagnosis

A thirty-six-year-old male patient, who had a 4-day history of discomfort of fever, throat pain, and dry cough, was admitted to hospital on February 26, 2006. A chest radiograph obtained on admission showed evidence of left lower pneumonia. His condition was rapidly deteriorated, featured by consecutive high body temperature and

dysfunctions of multiple systems, including respiratory system, circulatory system, central nervous system, liver, kidney, and gastrointestinal system. Chest radiograph revealed a massive consolidation shadow in both lungs on February 28, which, however, was much more severe in the left lung. Despite active treatment, the patient finally died from adult respiratory distress syndrome (ARDS) complicated with multiple organ failure (MOF) on March 2. None of the anti-influenza drugs, such as oseltamivir or amantadine, were used in the treatment.

This patient had been to the market where live chickens were slaughtered for sale 1 week prior to onset of symptoms, so the patient's tracheal aspirates were detected for H5N1 viral RNA using H5N1 real-time RT-PCR Kit (PG Biotech, China) on March 1. The full-length gene segments of hemagglutinin (HA) and neuraminidase (NA) were amplified by using one-step RT-PCR Kit (Qiagen, Germany) with the specific primer pairs (HA-F 5′-AGCAAAAGCAGGGGTTCAAT-3′, HA-R 5′-AGTAGAAACAAGGGTGTTTT-3′; NA-F 5′-AGCAAAAGCAGGAGTTCAAA-3′, NA-R 5′-AGTAGAAACAAGGAGTTTTTTT-3′), the reaction was subjected to a pre-cycle condition consisting of 30 min at 50°C(for reverse transcription), 15 min at 95°C followed by 25 circles of amplification. Each cycles consisted of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min 45 s. The amplification ended with a final extension at 72°C for 10 min. The PCR products were purified and cloned into the pGEM-T vector (Promega, USA). The positive clones were sequenced with T7 and SP6 primers by a 3730 automated DNA sequencer (ABI, USA).

Analysis of viral load and replication in autopsy specimens

On March 4, 2006, an autopsy was carried out in Zhongshan School of Medicine, Sun Yat-sen University. Tissues of the left lung, right lung, brain, heart, spleen, liver, kidney, large intestine, and small intestine were obtained, respectively. Some specimens were used for pathological analysis, and the remaining was stored at −80°C in small pieces for future study.

The obtained tissues were minced on ice with presence of culture medium, which were then centrifuged at the speed of 1,500 rpm for 15 min at 4°C. Supernatant was collected and added into lysis buffer of QIAamp Viral RNA Kit (Qiagen, Germany). RNA was then extracted according to the manufacturer protocol. Viral RNA was detected using H5N1 real-time RT-PCR Kit (PG Biotech, China) on ABI 7000 Real-Time PCR System (ABI, USA). Standard curve was used in the quantitative analysis of H5N1 RNA isolated from the autopsy tissues. In our study, the preparation of reagents, nucleic acids extraction, and nucleic acid amplification were performed in three physically separated laboratories.

To analyse viral replication in autopsy tissues, strand-specific RT-PCR was performed with H5 specific primer pairs H5F (5′-GCCATTCCACAACATACACCC-3′, 943–963) and H5R (5′-CTCCCCTGCTCATTGCTATG-3′, 1158–1139). Briefly, two-step reactions were used. First, RT reaction was done in the presence of the primer H5F or H5R. cDNA products then underwent PCR with H5F and H5R. The amplified fragment was about 216 bp and detected by agarose gel electrophoresis.

Results

Real-time RT-PCR had revealed H5N1 viral RNA in the patient's tracheal aspirates. HA and NA gene sequences amplified were the most related to those of avian influenza H5N1 viruses, Duck/Guangxi/5165/05 and Duck/Hunan/1265/05 (99.5 and 99.1% homologous, respectively). Therefore the patient was identified as avian influenza H5N1 virus infected.

Real-time RT-PCR had detected H5N1 viral RNA in the lungs and spleen, whereas there was no detectable viral RNA in tissues of heart, liver, kidney, large intestine, small intestine, or brain. Specifically, the viral load was higher in the left lung (7.1 log10 copies per ml) in relation to the right lung (5.7 log10 copies per ml); and there existed a lower level of H5N1 viruses in the spleen (3.8 log10 copies per ml) (Fig. 1). To confirm a successful H5N1 viral RNA isolation from the autopsy tissues, GAPDH mRNA amplified using RT-PCR served as the internal reference in our study (data not shown). At the same time negative controls did not produce H5 genes, which suggests there is no cross contamination in RT-PCR amplification.

To further elucidate whether H5N1 viral RNA in the lungs and spleen was H5N1 genome RNA, or alternatively, was replicated by H5N1 viruses, we performed strand-specific RT-PCR amplification. Our results indicated that negative- and positive-stranded RNA were detectable in both the left and the right lung, but there was only negative-stranded RNA in the spleen (Fig. 2). An independent duplication RT-PCR was performed under the same condition to confirm the result.

Discussion

In our study, a high viral load was detected only in the lungs in which both positive- and negative-stranded RNA coexisted, which was consistent with previous findings that replication of H5N1 viruses mainly occurs in the lungs of humans and mammals [18–20]. In line with the finding that

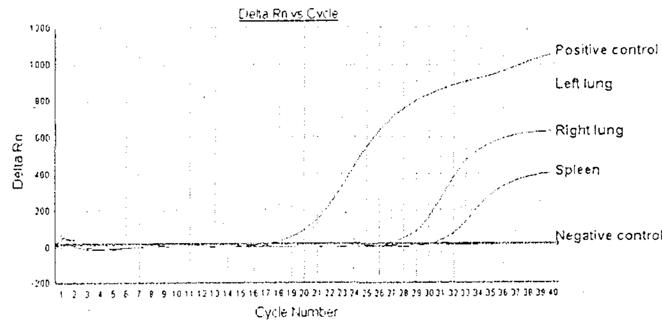

Fig. 1 Interpretation of H5N1 influenza viral RNA in autopsy tissues by single real-time RT-PCR. Different load of H5N1 influenza viral RNA existed in the left lung, right lung, and spleen

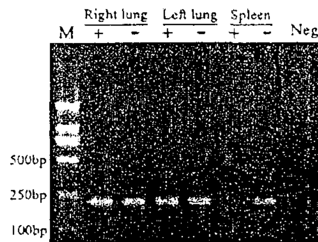

Fig. 2 Detection of positive- and negative-stranded viral RNA in the lungs and spleen by strand-specific RT-PCR. *M* DNA mark, − Negative-stranded RNA, + Positive-stranded RNA, *Neg* Negative control

the viral load was higher in the left lung in relation to the right lung, a chest radiograph obtained on admission showed evidence of left lower pneumonia, and histopathological analysis of the autopsy specimens had also suggested more severe pathological damage of the left lung, featured by more severe alveolar damage and diffuse exudation [21]. These results had demonstrated that the viral load in the lungs was related to the degree of lung damage. However, whether the observed damage was a direct result of the viral replication or a consequence of the dysfunction of cytokines and chemokines induced by these high-load viruses were still unclear.

Previous studies had shown that avian influenza H5N1 viruses could also target immune organs, in addition to the lung [22, 23]. In our study, a low viral load was detected in the spleen, but with absence of positive-stranded H5N1 viral RNA. Influenza viruses contain negative-stranded RNA, and they first replicate positive-strand RNA, which served as mRNA and the template for genome replication of progeny virus. In our study no positive-stranded viral RNA was detected, which suggested that the H5N1 virus did not replicate in the spleen, or that only little replication occurred [24]. The H5N1 viruses of low load in the spleen might be introduced through blood circulation or other ways.

When compared with that of the human- or swine-derived influenza viruses, NA activity of the avian influenza viruses is more resistant to the low pH environment in the upper digestive tract [25, 26]. Accordingly, the highly pathogenic avian influenza H5N1 viruses can replicate in human intestine, resulting in gastrointestinal symptoms, so that H5N1 viruses were detected in the intestine of infected humans [24, 27, 28]. Clinical data had suggested that the patient presented gastrointestinal symptoms in early stages of disease progression, which finally developed into gastrointestinal dysfunctions. But viral RNA was detected neither in large intestine nor small intestine in our study. Some literature suggested that antiviral drugs can lower the level of viral replication and interfere with the detection of viruses in the examined tissues [24, 29]. However, none of the anti-influenza drugs, such as oseltamivir or amantadine, were used in the treatment.

The HA cleavage site of highly pathogenic H5N1 viruses contains multiple basic amino acids, which could be hydrolyzed by a broader range of cellular proteases, so that the tissue tropism for H5N1 viruses is not restricted to the lungs, but extends to other organs, including the brain [30, 31]. A boy confirmed as H5N1 infected presented with severe diarrhea and acute encephalitis symptoms, and H5N1 virus was isolated from patient's throat, serum, feces, and the cerebrospinal fluid [32]. In addition to lung tissues, some studies had detected both positive- and negative-stranded RNA in large intestine, small intestine, and liver, suggesting the possibility of viral replication in the intestines and liver [15, 24]. Furthermore, viral gene sequences and antigen were detectable in neurons of the brain, T cells of the lymph node, and Hofbauer cells of the placenta, which was indicative of viral replication in extrapulmonary tissues [29]. The H5N1 virus obtained from the patient has multiple basic amino acids at the HA

cleavage site, which has molecular characteristics of the highly pathogenic avian influenza viruses [33]. The viral RNA was detectable in the patient's lung and spleen in our study. These findings suggested that H5N1 viruses might be transmitted to extrapulmonary tissues, causing disseminate infection. However, viral distribution and replication vary to a certain extent from individual to individual, which might be explained by tissue tropism differences of viral strains, or that viral distribution might differ in different stages of disease progression, or that different individuals reacted differently to H5N1 viruses.

The autopsy tissues of H5N1 infected cases can often not be obtained due to various reasons (e.g. religion), so reports concerning the tissue tropism and distribution of H5N1 viruses are lacking. We studied H5N1 viral load and replication in autopsy tissues, and the relationship between the viral load and tissue damage, which had significant implication for the further investigation of the tissue tropism and pathogenesis of H5N1 viruses.

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別紙様式第２−１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2009年11月4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	Schweiz Arch Tierheilkd 151:433-436		公表国 スイス	
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：新規なプロテアーゼ抵抗性プリオン蛋白質検出系による検査の結果、BSE罹患牛から生まれた仔牛のBSE罹患率は、非BSE罹患牛から生まれた仔牛のBSE罹患率より高率であった。 出産後にBSEを発症した母ウシから生まれ、過去の組織検査及び免疫組織検査ではBSE陰性とされた仔牛の血液検体を現在バリデーション中である新規なプロテアーゼ抵抗性蛋白質（PrP^{res}）検出系で検査したところ、BSEに罹患した母ウシから生まれた仔牛のBSE罹患率が16.1%であったのに対し、BSEに罹患していない母ウシから生まれた仔牛のBSE罹患率は4.2%であり、BSE罹患牛を母ウシに持つ仔牛の方がBSE罹患率が高率であった。また、出産後1年以内にBSEと診断された母ウシから生まれた仔牛のBSE罹患率は、出産後1年以上経過してからBSEと診断された母ウシから生まれた仔牛のBSE罹患率よりも有意に高率であった。但し、検出されたPrP^{res}が感染性のプリオン蛋白質であるかは動物実験による検証が必要である。これらの結果は、神経組織学的異常が認められる前に、血液検体による感染確認の可能性を示唆すると共に、ウシにおけるBSE垂直感染の可能性が未だ払拭できないことを示している。	別紙のとおり

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.12.1

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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人免役グロブリン、⑥人免役グロブリン、⑦乾燥ペプシン処理人免疫グロブリン、⑧乾燥ペプシン処理人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン、⑫乾燥スルホ化人免疫グロブリン、⑬乾燥スルホ化人免疫グロブリン、⑭乾燥スルホ化人免疫グロブリン*、⑮乾燥濃縮人活性化プロテインC、⑯乾燥濃縮人血液凝固第Ⅷ因子、⑰乾燥濃縮人血液凝固第Ⅷ因子、⑱乾燥濃縮人血液凝固第Ⅷ因子、⑲乾燥濃縮人血液凝固第Ⅷ因子、⑳乾燥濃縮人血液凝固第Ⅸ因子、㉑乾燥濃縮人血液凝固第Ⅸ因子、㉒乾燥濃縮人血液凝固第Ⅸ因子、㉓乾燥濃縮人血液凝固第Ⅸ因子、㉔乾燥抗破傷風人免疫グロブリン、㉕乾燥抗破傷風人免疫グロブリン、㉖抗 HBs 人免疫グロブリン、㉗抗 HBs 人免疫グロブリン、㉘トロンビン、㉙フィブリノゲン加第ＸⅢ因子、㉚フィブリノゲン加第ＸⅢ因子、㉛乾燥濃縮人アンチトロンビンⅢ、㉜乾燥濃縮人アンチトロンビンⅢ、㉝ヒスタミン加人免疫グロブリン製剤、㉞ヒスタミン加人免疫グロブリン製剤、㉟人血清アルブミン*、㊱人血清アルブミン*、㊲乾燥ペプシン処理人免役グロブリン*、㊳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ガンマーグロブリン筋注 450mg/3mL「化血研」、⑥ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑦献血静注グロブリン "化血研"、⑧献血グロブリン注射用 2500mg「化血研」、⑨献血ベニロンーⅠ、⑩献血ベニロンーⅠ静注用 500mg、⑪献血ベニロンーⅠ静注用 1000mg、⑫献血ベニロンーⅠ静注用 2500mg、⑬献血ベニロンーⅠ静注用 5000mg、⑭ベニロン*、⑮注射用アナクトC2,500 単位、⑯コンファクトF、⑰コンファクトF注射用 250、⑱コンファクトF注射用 500、⑲コンファクトF注射用 1000、⑳ノバクトM、㉑ノバクトM注射用 250、㉒ノバクトM注射用 500、㉓ノバクトM注射用 1000、㉔テタノセーラ、㉕テタノセーラ筋注用 250 単位、㉖ヘパトセーラ、㉗ヘパトセーラ筋注 200 単位/mL、㉘トロンビン "化血研"、㉙ボルヒール、㉚ボルヒール組織接着用、㉛アンスロビンＰ、㉜アンスロビンＰ500 注射用、㉝ヒスタグロビン、㉞ヒスタグロビン皮下注用、㉟アルブミン 20%化血研*、㊱アルブミン 5%化血研*、㊲静注グロブリン*、㊳アンスロビンＰ1500 注射用
使用上の注意記載状況・その他参考事項等	製剤①②④⑤⑥⑦⑧⑨⑩⑪⑫⑬⑮⑯⑰⑱⑲⑳㉑㉒㉓㉔㉕㉖㉗㉘㉙㉚㉛㉜㉝㉞㊳ ・重要な基本的注意 「現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分に行い、治療上の必要性を十分検討の上投与すること。」
報告企業の意見	異常プリオンについては、血中における存在様態等未だ不明な点が多い。今回の報告は血中の異常プリオン検出及び BSE の垂直感染の可能性を示唆するものである。 上記製剤の製造にはウシの肺臓に由来するアプロチニンを使用しているが、当所では医薬発第 1226 号（平成 12 年 12 月 12 日）等の通知に基づいて牛由来原材料に係る原材料の原産国、使用部位等の調査、確認を行い、同通知等で BSE 発生リスクが低いとされる国をウシの肺臓の原産国としている。また、「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）」を参考に実施したクリアランス試験により、異常プリオンのクリアランス効果を有することを確認したウイルス除去膜ろ過工程を含む工程により製造を行い、安全性確保に努めてきている。更に、これまでに上記製剤による異常プリオン感染の報告例は無い。 以上の点から、上記製剤は BSE 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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Untersuchung von BSE-Nachkommen auf Protease-resistentes Prion Protein (PrP[res]) im Blut

U. Braun[1], A. Tschuor[1], M. Hässig[1], S. Franitza[1], E. Berli[1], A. El Gedaily[2], N. Franscini[2], U. Matthey[1], R. Zahn[2]

[1]Departement für Nutztiere der Universität Zürich, [2]Alicon AG, Schlieren

Zusammenfassung

Das Ziel der vorliegenden Arbeit war, zu untersuchen, ob im Blut von schweizerischen BSE-Nachkommen (Gruppe A) Protease-resistentes Prion Protein (PrP[res]) vorkommt und ob sich die Häufigkeit des Vorkommens von derjenigen einer gesunden Kontrollpopulation aus dem Jahr 2006 (Gruppe B) unterscheidet. Die Gruppe A bestand aus 181 Nachkommen von an BSE erkrankten Kühen, die Gruppe B aus 240 gesunden Rindern aus einem Gebiet, in welchem in den Jahren 2001 bis 2006 keine BSE diagnostiziert worden war. Die Blutproben wurden mit einem BSE-Lebendtest (Alicon PrioTrap®) zum Nachweis von Protease-resistentem Prion Protein untersucht. Um abzuklären, ob zwischen der Zeitdifferenz von der Geburt des Nachkommens bis zur Erkrankung der Mutter an BSE eine Beziehung in Bezug auf den Nachweis von PrP[res] beim Nachkommen bestand, wurde diese Zeitdauer bei jedem Nachkommen errechnet. Bei 29 (16.1%) von 181 untersuchten BSE-Nachkommen wurde im Blutplasma PrP[res] nachgewiesen, 152 Tiere waren negativ. Nachkommen, die innerhalb eines Jahres vor dem Auftreten von klinischen Symptomen des Muttertieres geboren worden waren, wiesen im Blut signifikant häufiger PrP[res] auf als Tiere, bei denen der zeitliche Abstand von der Geburt bis zur Erkrankung mehr als ein Jahr betragen hatte ($P < 0.05$). In der Kontrollgruppe wurden 10 von 240 Tieren (4.2%) positiv auf PrP[res] getestet. Die Untersuchungen haben gezeigt, dass beim Rind im Blut Protease-resistentes Prion Protein nachgewiesen werden kann und dass dieses bei Nachkommen von BSE-Kühen häufiger vorkommt als bei Tieren aus einer gesunden Kontrollpopulation.

Schlüsselwörter: Rind, BSE-Nachkommen, Protease-resistentes Prion Protein

[1]*Die vorliegende Publikation beruht auf den Ergebnissen der Dissertation von Dr. Andreas Tschuor*

Protease-resistant prion protein (PrP[res]) in the blood of offspring of cows that developed BSE

The goal of the present study was to investigate whether protease-resistant prion protein (PrP[res]) occurs in plasma samples of offspring of cows that developed bovine spongiform encephalopathy (BSE; group A) and to compare the prevalence with that of a healthy control group in 2006 (Group B). Group A consisted of 181 offspring of cows that developed BSE and group B consisted of 240 healthy animals from a region in Switzerland where no cases of BSE occurred from 2001 to the end of 2006. All plasma samples were evaluated using Alicon PrioTrap®, an antemortem test for PrP[res]. The time between birth of the offspring and onset of BSE in the dam was calculated to determine its relationship with the presence of PrP[res] in the plasma of the offspring. From 181 offspring, 29 (16.1%) had PrP[res]-positive plasma samples. Offspring that were born within one year of the onset of BSE in the dam had a significantly higher prevalence of PrPres-positive plasma samples than those born more than one year before the onset of BSE in the dam. Ten (4.2%) of 240 control cattle had PrP[res]-positive plasma samples. Thus, PrP[res] can be detected in bovine blood and occurs more frequently in the offspring of cows that develop BSE than in cattle of a healthy control population.

Keywords: cattle, offsprings of BSE cows, PrP[res]

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Einleitung

Bei Nachkommen von britischen BSE-Kühen wurde neurohistologisch signifikant häufiger BSE diagnostiziert als bei Nachkommen von Kühen, die nicht an BSE erkrankt waren (SEAC, 1996). Das Risiko eines BSE-Nachkommens, selbst an BSE zu erkranken, wurde vom britischen Ministry of Agriculture, Fisheries and Food (MAFF) auf ca. 10% geschätzt, und es wurde proklamiert, dass eine Übertragung von BSE von der erkrankten Mutter auf das Kalb nicht ausgeschlossen werden kann (Masood, 1996). Diese Untersuchungen veranlassten die schweizerischen Behörden im Jahr 1996, alle Nachkommen von BSE-Kühen klinisch untersuchen, euthanasieren und danach auf BSE abklären zu lassen. Bei keinem Tier wurden damals Hinweise für eine BSE-Infektion gefunden (Braun et al., 1998; Fatzer et al., 1998). In der seither vergangenen Zeit haben sich viele Forschergruppen mit der Entwicklung eines Bluttests zum Nachweis von BSE beschäftigt. Einer dieser Gruppen ist es gelungen, einen Test zum Nachweis von Protease resistentem Prion Protein (PrPres) in Körperflüssigkeiten zu entwickeln (Firma Allcon, unveröffentlicht). In der vorliegenden Arbeit wurde ein in der Validierung befindlicher Prototyp des ante mortem Testverfahrens zum Nachweis von BSE eingesetzt. Das Ziel der vorliegenden Arbeit war es, mit Hilfe des neuen Testverfahrens zu untersuchen, ob im Blut von schweizerischen BSE-Nachkommen Protease-resistentes Prion Protein (PrPres) vorkommt und ob sich die Häufigkeit des Vorkommens von derjenigen einer gesunden Kontrollpopulation aus dem Jahr 2006 unterscheidet.

Tiere, Material und Methoden

Tiere

Blutproben von 2 Tiergruppen (A und B) wurden auf das Vorhandensein von Protease resistentem Prion Protein (PrPres) im Blut untersucht. Die Gruppe A bestand aus 181 Nachkommen von an BSE erkrankten Kühen. Die Nachkommen dieser Kühe waren im Winter 1996/97 auf Anordnung des schweizerischen Bundesrats an der Klinik für Wiederkäuer untersucht und danach euthanasiert worden (Braun et al., 1998). Bei den Müttern dieser Tiere konnte BSE in allen Fällen histologisch und immunhistochemisch nachgewiesen werden. Bei den 181 Nachkommen dieser Tiere war BSE postmortal weder bei der neurohistologischen noch bei der immunhistochemischen Untersuchung gefunden worden (Fatzer et al., 1998). Für den Nachweis von Protease-resistentem Prion Protein stand von jedem Nachkommen eine Blutplasmaprobe zur Verfügung, die seit 1996/97 bei -80 °C gelagert worden war. Die Gruppe B bestand aus 240 gesunden Rindern der Schweizer Braunviehrasse im Alter von 1 bis 9 Jahren aus dem Vorderrheintal des Kantons Graubünden. Die Blutplasmaproben von diesen Tieren waren im Jahr 2006 speziell für diese Untersuchung gewonnen worden. BSE war in diesem Gebiet in den Jahren 2001 bis 2006 bei keinem einzigen Tier nachgewiesen worden.

Untersuchung der Blutproben auf PrPres

Die Blutproben wurden mit dem Ante Mortem BSE-Test untersucht. Das Testprinzip beruht darauf, dass in einem ersten Schritt die Prion Proteine PrPC und PrPres an ein Liganden-gekoppeltes Harz (Franscini et al., 2006) gebunden werden, welches die Prion Proteine mit hoher Affinität und Spezifität bindet. In einem zweiten Schritt wird PrPres nach Behandlung der Probe mit Proteinase K wie beschrieben (McKinley 1983) im Westernblot nachgewiesen. Das normale Prion Protein, PrPC, ist Protease-sensitiv und wird daher im Test nicht nachgewiesen. Ob es sich bei dem nachgewiesenen Protein tatsächlich um infektiöses Prion Protein (PrPSc) handelt, muss im Tierexperiment noch bestätigt werden.

Zeitdifferenz von der Geburt der BSE-Nachkommen zur BSE-Erkrankung der Mutter

Um abzuklären, ob zwischen der Zeitdifferenz von der Geburt des Nachkommens bis zur Erkrankung der Mutter an BSE eine Beziehung in Bezug auf den Nachweis von PrPres beim Nachkommen besteht, wurde diese Zeitdauer für jeden Nachkommen errechnet.

Statistik

Die statistische Auswertung der Häufigkeiten erfolgte mit dem Programm StatView 5.0 (SAS Institut, 8602 Wangen, Schweiz).

Ergebnisse

Nachweis von PrPres

Bei 29 (16.1%) der 181 untersuchten BSE-Nachkommen wurde im Blutplasma PrPres nachgewiesen, 152 Tiere waren im Test negativ. In der Kontrollgruppe wurden 10 von 240 Tieren (4.2%) positiv auf PrPres getestet (Differenz $P < 0.05$).

Zeitdifferenz von der Geburt der BSE-Nachkommen zum Diagnosedatum BSE beim Muttertier

41 Nachkommen waren ein Jahr, 79 Nachkommen zwei Jahre, 41 Nachkommen drei Jahre und 20 Nachkommen 4 oder mehr Jahre vor der BSE-Diagnose beim Muttertier geboren worden. Nachkommen, die innerhalb eines Jahres vor der Erkrankung des Muttertieres geboren worden waren, wiesen im Blut signifikant häufiger PrPres

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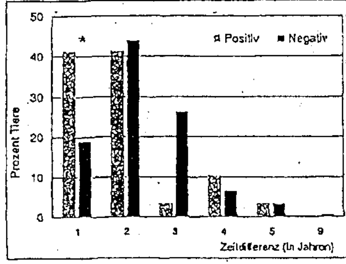

Abbildung 1: Häufigkeitsverteilung der zeitlichen Abstände von der Geburt der BSE-Nachkommen bis zur Erkrankung der Muttertiere an BSE bei Rindern mit positivem und negativem Nachweis von PrPres im Blut (*Differenz zwischen den 2 Gruppen zum Zeitpunkt 1 $P < 0.05$).

auf als Tiere, bei denen der zeitliche Abstand von der Geburt bis zur Erkrankung mehr als ein Jahr betragen hatte ($P < 0.05$, Mann-Whitney-U-Test, Abb. 1).

Diskussion

Bei 16.1% der schweizerischen BSE-Nachkommen konnte im Blut PrPres nachgewiesen werden, obschon diese Tiere neurohistologisch und immunhistochemisch BSE-negativ waren. Im Vergleich dazu wurde PrPres nur bei 4.2% der gesunden Kontrollpopulation aus dem Jahr 2006 gefunden. Unsere Befunde sind ähnlich wie diejenigen der britischen Kohortenstudie. Bei dieser Studie zeigten 14% von 301 BSE-Nachkommen die für BSE charakteristischen neurohistologischen Befunde (SEAC, 1997). Die Ergebnisse können verschieden interpretiert werden. Einerseits ist es möglich, dass bei den BSE-Nachkommen deshalb mehr positive Fälle entdeckt wurden als bei den Kontrolltieren, weil die Nachkommen von ihren an BSE erkrankten Müttern infiziert wurden. Andererseits kann die niedrigere Häufigkeit bei den Kontrolltieren mit dem starken Absinken der BSE-Häufigkeit in der Schweiz erklärt werden. Die Tatsache, dass nur 16.1% der BSE-Nachkommen PrPres positiv reagiert haben, zeigt, dass der maternalen Übertragung von BSE nur eine Nebenrolle bei der Verbreitung der BSE zukommt. Für die Bekämpfung der BSE ist es aber wichtig, dass auch dieser Infektionsweg durch die Keulung der Nachkommen von BSE-Kühen unterbrochen wird. Eine weitere Frage stellt sich, weshalb in der britischen Studie 14% der Nachkommen neurohistologische Symptome zeigten und in unserer Studie kein einziges Tier. Dieser Unterschied ist damit zu erklären, dass die von uns untersuchten Nachkommen im Alter von 3.1 ± 0.8 Jahren getötet und untersucht wurden, das heisst zu einem Zeitpunkt, wo es nur in seltensten Fällen zur BSE-Erkrankung gekommen ist. Im Gegensatz dazu wurden die britischen Tiere erst im Alter von 7 Jahren neurohistologisch untersucht. Die im Blut positive Reaktion bei gleichzeitig negativem neurohistologischem Befund weist darauf hin, dass die Infektion im Blut nachweisbar ist, bevor es zur Ausbildung neurohistologischer Veränderungen kommt.

Bei den BSE-Nachkommen mit einem einjährigen Zeitabstand zwischen ihrer Geburt und der BSE-Erkrankung der Mutter wurde signifikant häufiger PrPres detektiert als bei den Tieren mit längerem Zeitabstand. Die Befunde decken sich mit den Befunden der MAFF-Studie, in welcher für den gleichen Zeitabstand ebenfalls eine signifikante Häufung der neurohistologisch positiven Ergebnisse festgestellt wurde (Donnelly et al., 1997). Eine mögliche Erklärung für die Befunde liegt darin, dass PrPSc gegen Ende der Inkubationszeit ausser im Nervengewebe auch vermehrt im Blut vorkommt und über den Blutweg den Fetus transplazentar infiziert (Aguzzi, 2006). Die maternale Transmission einer TSE konnte allerdings bisher erst bei Scrapie nachgewiesen werden, als in den plazentären Kotyledonen von präklinisch und klinisch kranken Scrapie-Schafen PrPSc gefunden wurde (Andreoletti et al., 2002). Die maternale Übertragung von BSE bei einer Antilope (Aldhous, 1990) und die BSE-Erkrankung einer Kuh, bei deren Mutter ebenfalls BSE diagnostiziert worden war, liessen allerdings den Verdacht aufkommen, dass eine maternale Transmission auch bei mit BSE infizierten Rindern vorkommen könnte (Aldhous, 1991).

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Korrespondenz

Ueli Braun
Departement für Nutztiere
Winterthurerstrasse 260
CH-8057 Zürich
E-Mail: ubraun@vetclinics.uzh.ch
Fax: + 41 44 63 58 904

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Examens de descendants d'animaux BSE quant à la présence de protéines prioniques protéases résistantes (PrP^{res}) dans le sang

Le but du présent travail était d'étudier si les protéines prioniques protease résistantes (PrP^{res}) étaient présentes dans le sang de descendants d'animaux BSE (groupe A) et de voir si la fréquence de cette présence était différente de celle constatée dans une population de contrôle en 2006 (groupe B). Le groupe A se composait de 181 descendants de vaches atteintes de BSE, le groupe B de 240 bovins d'une région dans laquelle de 2001 à 2006 aucun cas de BSE n'avait été diagnostiqué. Les échantillons ont été testés avec Alicon Prio Trap ® pour mettre en évidence la protéine prionique protéase résistante (PrP^{res}). Afin de savoir s'il y avait une relation entre l'intervalle de temps séparant la naissance du veau de la maladie de la mère par rapport à la mise en évidence de PrP^{res} chez le veau, cette durée a été calculée pour chaque animal. Chez 29 (16.1 %) des 181 descendants BSE, la PrP^{res} a été trouvée dans le plasma, 152 animaux étaient négatifs. Les animaux qui étaient nés dans l'année précédant l'apparition des symptômes cliniques chez leur mère avaient de façon significative plus souvent la PrP^{res} dans le sang que les animaux chez lesquels l'intervalle entre la naissance et la maladie dépassait une année ($P < 0.05$). Dans le groupe de contrôle, 10 des 240 animaux (4.2 %) ont été positifs au PrP^{res}. Ces examens montrent que la protéine prionique protéase résistante peut être mise en évidence chez les bovins dans le sang et qu'elle est plus souvent présente chez les descendants d'animaux BSE que dans une population de contrôle saine.

Esame sanguigno della proteina prionica resistente alle proteasi (PrP^{res}) nella discendenza da mucche affette da BSE

Scopo del seguente studio è di esaminare se, nel sangue della discendenza da BSE svizzera (gruppo A), era presenta la proteina prionica resistente alle proteasi (PrP^{res}) e se si distingueva, nella sua frequenza, dalla popolazione di controllo sana del 2006. Il gruppo A era composto da 181 discendenti di mucche malate di BSE, il gruppo B era formato da 240 bovini sani provenienti da una regione nella quale dal 2001 al 2006 non sono stati diagnosticati casi di BSE. Le prove di sangue sono state analizzate con un test BSE (Alicon PrioTrap®) per la ricerca della proteina prionica resistente alle proteasi. Per chiarire se nel lasso di tempo tra la nascita della discendenza alla malattia (BSE) della madre ci sia un rapporto in relazione alla presenza di PrP^{res} nella discendenza, questa durata temporale è stata calcolata per ogni discendente. In 29 (16.1 %) dei 181 discendenti da BSE è stato rilevato nel plasma sanguigno la presenza di PrP^{res}, mentre 152 animali sono risultati negativi. I discendenti nati nell'arco di un anno dall'apparizione dei sintomi clinici della madre mostravano nel sangue una frequenza più significativa di PrP^{res} che gli animali nei quali il lasso di tempo dalla nascita fino alla malattia della madre era maggiore di un anno ($P < 0.05$). Nel gruppo di controllo 10 dei 240 animali (4.2 %) sono risultati positivi al test. Gli esami hanno rilevato che, si può ritrovare nei bovini, la proteina prionica resistente alle proteasi (PrP^{res}) e che la discendenza da mucche con BSE appare più di frequente che negli animali provenienti da una popolazione di controllo sana.

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Protease-resistant prion protein (PrPres) in the blood of offspring of cows that developed BSE

はじめに

英国においては、BSE に罹患した母ウシから生まれた仔ウシは、BSE に罹患していない母ウシから生まれた仔ウシと比較して、神経組織学的に BSE と診断される率が有意に高いとするデータが得られている（英国海綿状脳症諮問委員会 SEAC、1996）。英国農漁業食糧省（MAFF）は、BSE の母ウシから生まれた仔ウシが BSE に罹患するリスクを約 10%と見積もっており、BSE に罹患した母ウシから仔ウシへの BSE 感染は排除できないとの見解をとっている（Masood, 1996）。こうしたデータを踏まえて、1996 年にスイス当局は、BSE の母ウシから生まれたすべての仔ウシに対して臨床所見検査を実施し、安楽死させ、その後に BSE の有無について検査を行った。その結果、BSE 感染の徴候を示す仔ウシは 1 頭も発見することができなかった（Braun et al., 1998; Fatzer et al., 1998）。その後は今日に至るまで、数多くの研究グループが BSE 検出用の血液検査の開発に取り組んできた。そのうちのある研究グループは、プロテアーゼ抵抗性プリオン蛋白（PrPres）を体液中から検出する方法の開発に成功した（Alicon 社、未公開）。本研究の実施に際しては、この BSE 死亡前検査法の、現時点ではバリデーションの段階にあるプロトタイプを利用した。本研究は、スイスの BSE に罹患した母ウシから生まれた仔ウシの血液中からプロテアーゼ抵抗性プリオン蛋白（PrP^{res}）が検出されるかどうか、また、対照群としての 2006 年時点における正常ウシと比較して、その検出率に差が示されるかどうかについて、この新規検査法を利用して検討することを目的として実施した。

動物、材料、方法

動物

2 群（A 群と B 群）の動物から採取した血液検体を対象に、血液中からプロテアーゼ抵抗性プリオン蛋白（PrP^{res}）が検出されるか否かについて検査を実施した。BSE に罹患した母ウシから生まれた仔ウシ 181 頭を A 群とした。これらの仔ウシは 1996 年から 1997 年にかけての冬季間に、スイス連邦評議会の命令を受けて、反芻動物病院にて検査を実施した後、安楽死させた（Braun et al., 1998）。これらの仔ウシの母ウシは、組織検査の際にも免疫組織化学検査の際にもすべてが BSE 陽性であった。これに対して、181 頭の仔ウシに対する死後検査では、組織検査の際にも免疫組織化学検査の際にもすべてが BSE 陰性であった（Fatzer et al., 1998）。プロテアーゼ抵抗性プリオン蛋白（PrP^{res}）の検査用として、すべての仔ウシから血液検体を採取し、1996 年から 1997 年にかけての冬季間以降は−80℃の温度で保存しておいた。グラウビュンデン州のフォルデルラインタールで飼育された、年齢 1～9 歳の健康なスイス褐色牛 240 頭を B 群とした。B 群のウシからは、

2006 年に本研究のために特別に血液検体を採取した。2001 年から 2006 年の期間中に、この地域に飼育されていたウシで BSF に罹患したケースは皆無であった。

血液検体に対する PrPres の検査

血液検体に対してこの新規の BSE 死亡前検査を実施した。この検査法の原理について順を追って説明すると、第一段階としてはプリオン蛋白 PrP^C と PrP^{res} をリガンド固定化樹脂（Franscini et al., 2006）に結合させる。このリガンド固定化樹脂は、高い親和性および特異性のもとにプリオン蛋白と結合する。第二段階としては、文献に記述の方法（McKinley 1983）に準拠して、検体に対してプロテイナーゼ K 処理を行った後に、ウェスタンブロット法によって PrP^{res} を検出する。正常プリオン蛋白 PrP^C はプロテアーゼに感受性を示すので、検出されることはない。検出された蛋白が実際に感染性のプリオン蛋白（PrP^{Sc}）であるかどうかについては、動物実験を実施して検証する必要がある。

母ウシの BSE 発症と BES 仔ウシ誕生との時間的な差

仔ウシが生まれてから母ウシが BSE を発症するまでの時間的長さと、仔ウシにおいて PrP^{res} が検出される率との関係を探るために、すべての仔ウシについて、上記の時間的長さの調査記録を行った。

統計処理

度数の統計評価は、Programm StatView 5.0（SAS Institut、8602 Wangen、スイス）を利用して実施した。

結果

PrPres の検出

BSE の母ウシから生まれた仔ウシ 181 頭中 29 頭（16.1%）の血液検体から PrP^{res} が検出され、残りの 152 頭は陰性であった。対照群で PrP^{res} 陽性の結果を示したのは、240 頭中 10 頭（4.2%）であった（危険率 $p>0.05$）。

仔ウシ誕生から母ウシが BSE と診断されるまでの時間的長さ

母ウシが BSE と診断された時点を基準とした場合、仔ウシ全体(181 頭)のうちの 41 頭が 1 年前、79 頭が 2 年前、41 頭が 3 年前、残りの 20 頭が 4 年またはそれ以前に生まれていた。母ウシが BSE と診断される 1 年前以内に生まれていた仔ウシでは、それ以外の時期に生まれた仔ウシと比較して、血液中から PrP^{res} が検出される率が有意に高かった（$p<0.05$、Mann-Whitney U 検定、図 1）。

考察

スイスの BSE に罹患した母ウシから生まれた仔ウシの血液検体から 16.1%の割合で PrP^{res} が検出された。ただし、これらの仔ウシに対する神経組織検査結果や免疫組織化学検査結果はいずれも BSE 陰性であった。これに対して、対照群としての 2006 年時点における正常ウシの PrP^{res} の検出率は 4.2%程度にすぎなかった。著者のこの所見は英国で実施されたコホート研究の結果と類似していた。英国のコホート研究の際には、BSE に罹患した母ウシから生まれた仔ウシ 301 頭のうちの 14%が、BSE に特徴的な神経組織学的所見を示した（英国海綿状脳症諮問委員会 SEAC、1997）。これらの研究結果については、様々な解釈が可能であろう。そのひとつは、BSE に罹患した母ウシから生まれた仔ウシでは、BSE の母ウシから BSE に感染してしまったために、対照群と比較して BSE の陽性率が高くなったのではないかとする考え方である。他方では、対照群において BSE 陽性率がかなり低かったのは、スイスにおける極度に低い BSE 発症率から説明することができるだろう。また、BSE の母ウシから生まれた仔ウシにおいて PrP^{res} 陽性率が 16.1%程度にすぎなかった事実からは、BSE の伝播拡散にとって、BSE の垂直感染は副次的な意味しか持たないと考えることができるだろう。しかし、BSE との戦いにおいては、BSE に罹患した母ウシから生まれた仔ウシを殺処分することによって、こうした感染経路を絶つのも大切なことであろう。ここで疑問となるのは、英国で実施された研究の際には 14%の仔ウシにおいて神経組織検査結果が陽性であったが、本研究の際には神経組織検査結果が陽性の仔ウシが何故皆無であったかという点である。こうした相違は次のように説明できるだろう。著者の研究の際、殺処分ならびに検査の実施時の仔ウシの年齢は 3.1±0.8 歳であり、これは BSE がほとんど発症することのない年齢である。これに対して、英国で実施された研究の際は 7 歳のウシに対して神経組織検査が実施された。また神経組織検査結果が陰性であったにもかかわらず、血液中の PrP^{res} が陽性という結果を示したことは、神経組織学的異常が開始される前に、血液検体を用いて感染の有無を確認することが可能なことを示している。

母ウシが BSE と診断される 1 年前以内に生まれていた仔ウシでは、それ以外の時期に生まれた仔ウシと比較して、血液中から PrP^{res} が検出される率が有意に高かったが、この所見は MAFF（英国農漁業食糧省)研究の所見と一致している。ただし、MAFF 研究の際にも、同じ 1 年前以内に生まれていた仔ウシでみた場合、神経組織検査結果の陽性率が有意に高くなった（Donnelly et al., 1997）。こうした所見に対しては、PrP^{res} は潜伏期の終わりごろになると、神経組織以外に血液中にも多く存在するようになり、血行路から胎盤を経て胎仔に移行するためとする解釈が可能かもしれない（Aguzzi, 2006）。しかし、伝達性海綿状脳症（TSE）の垂直感染が確認されているのは、現時点ではスクレイピーに限られている。つまり、無症候性または症候性スクレイピーヒツジの胎盤葉からは PrP^{Sc} が検出されている（Andreoletti et al., 2002）。しかし、母動物が BSE と診断されている場合、

アンテロープにおいてはBSEの垂直感染（Aldhous, 1990）が認められ、ウシにおいてはBSEの発症が認められることから、ウシがBSEに感染する際にも垂直感染による可能性も存在するという疑いを払拭することができない（Aldhous, 1991）。

図1：仔ウシが生まれてから母ウシがBSEを発症するまでの時間的長さと、仔ウシの血液検体におけるPrP^{res}の陽性率と陰性率との関係の度数分布（* 母ウシの発症までの時間的長さが1年の際の差、$p<0.05$）

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	Neuropathology. 2009 Oct;29(5):625-31.	公表国 オーストラリア	
販売名（企業名）	－					

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
脳神経外科用器具、脳波計（EEG）用脳内電極、ヒト下垂体ホルモン、硬膜移植片、角膜移植、輸血を介してクロイツフェルト・ヤコブ病（CJD）に罹患した患者は400名を超えている。 医原性CJD患者の新規の罹患数は減少しているが、輸血を介して伝播された多様なCJD症例が2004年以降報告されている。 CJDの医原性感染は、依然として明らかに深刻な問題である。近年、我々はこの9年間に日本CJDサーベイランス委員会（CJD Surveillance Committee）の登録患者に実施された医療（全ての外科処置、脳神経外科処置、眼科手術、および輸血）を調査した。 孤発性CJD（sCJD）患者753名と対照被験者210名で構成した症例対照試験で、プリオン病がsCJD発症以前に調査対照の医療を介して伝播したことを示すエビデンスを見出せなかった。 これまでに報告された症例対照試験のレビューでは、輸血がCJDの有意なリスク因子であることは一度も明らかにされておらず、我々の研究でも同じ結果が得られている。 手術がsCJDの有意なリスク因子であることを報告している症例対照試験もいくつかあるが、外科処置を手術のタイプ別に分類すると、その結果は相互に相容れないものがあり、これは外科処置を介してのプリオン伝播の可能性がほとんどないことを示唆している。我々の試験では、sCJD患者の4.5%がsCJD発症後に手術を受けており、これには脳神経外科処置0.8%および眼科手術1.9%が含まれる。sCJD発症後ですら、脳神経外科処置を含めて、手術を受けた患者がいるという事実は、医療処置を介したプリオン伝播の可能性を除外できないことを示唆している。 医原病リスクを低減するためには、我々はプリオン病に対して警戒を続けなければならない。	重要な基本的注意 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
輸血がCJDの有意なリスク因子であることは明らかにされていないが、警戒は続ける必要があるとの報告である。 現時点まで血友病以外で血漿分画製剤からvCJD伝播が疑われた報告はなく、血漿分画製剤の製造工程でプリオンが除去できるとの情報もある。 なお、当社血漿分画製剤の原料血漿は現在まで英国の血漿を使用していない。	今後ともvCJDに関する安全性情報等に留意していく。

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