virus transmission.

A HealthMap/ProMED-mail interactive map of France showing the location of Corsica can be accessed at <http://healthmap.org/r/07GC>. - Mod.TY]

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[15] France (Alpes-Maritimes)
Date: Wed 22 Sep 2010
Source: Le Generaliste.fr [in French, trans. Corr.SB, edited]
<http://www.legeneraliste.fr
/layout/Rub_ACTU.cfm?espace=3DACTU&id_rubrique=3D101860&id_article=3D27003>

In addition to the 2 confirmed cases of indigenous dengue fever in a residential area west of Nice, there are thought to be more than "6-7 suspects" in the Maritime Alps, according to Xavier Lorre, departmental delegate of the Regional Agency.

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Communicated by:
HealthMap Alerts via ProMED-mail
<promed@promedmail.org>

[See also the end of report [14] above. - Mod.JW]

[The 26 Sep 2010 edition of Le Progres.com
(<http://www.leprogres.fr/fr/france-monde/article/3850667/Provence-apres-la-den
week,

A HealthMap/ProMED-mail interactive map of France showing the location of Nice can be accessed at <http://healthmap.org/r/07GC>. - Mod.TY]

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[16] Puerto Rico
Date: 25 Sep 2010
Source: Google / EPA [in Spanish, trans. & summ. Mod.TY, edited]
<http://www.google.com/hostednews/epa/article/ALeqM5geAK-Sr_eqRLNZ8hjJbMx93rHz6

The death of a 50-year-old man raised to 23 the number of dengue deaths in Puerto Rico so far this year [2010], according to a report issued today [25 Sep 2010] by the Department of Health. This report indicated that according to the Dengue Division of the [USA] CDC, during [epidemiological] week 35, which corresponds to the 27 Aug - 2 Sep [2010] period, 835 new dengue cases have been reported ... and the DHF cases remained at 28.

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[A HealthMap/ProMED-mail interactive map showing the location of Puerto Rico in the Caribbean can be accessed at
<http://healthmap.org/r/018->. - Mod.TY]

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[17] Mexico (Oaxaca)
Date: Sun 26 Sep 2010
Source: ADN Sureste [in Spanish, trans. & summ. Mod.TY, edited]
<http://www.adnsureste.info/index.php/notas-del-dia/18242-30-de-los-casos-de-de

The dengue outbreak remains at 1668 cases, of which 30 percent are hemorrhagic, although for now a reoccurrence of the outbreak cannot be considered to exist, nor have cases doubled, so that one hopes to continue in a more or less stable trend even with the contingency of increased rains.

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[The occurrence of 30 percent of DHF of the total dengue cases is unusually high. Either the classification of DHF cases is in error, or there is serious underreporting of non-DHF dengue cases.

A map showing the location of Oaxaca state in Mexico can be accessed at <http://www.lib.utexas.edu/maps/americas/mexico_pol97.jpg>. A HealthMap/ProMED-mail interactive map of Mexico can be accessed at <http://healthmap.org/r/04jP>. - Mod.TY]

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[18] Honduras
Date: 19 Sep 2010
Source: European Pressphoto Agency [in Spanish, trans. & summ. Mod.TY, edited]
<http://www.google.com/hostednews/epa/article/ALeqM5q_ltcZj5pU3GSlFXcLaVDCT5FZj

The Honduras Ministry of Health has registered at least 68 deaths from DHF this year [2010], said the head of the National Dengue Program, Roxana Araujo. Also, at least 60 258 classical dengue fever cases have been registered and 2276 of DHF, the official added. Araujo reiterated that the trend of disease incidence remains in decline in recent weeks, and the suspension of national emergency to combat [this disease] issued this past June 2010 is under study.

In 2009, DHF caused the deaths of at least 12 people, and in 2010, the greatest incidence of this disease has been registered in the past 15 years in Honduras, according to health authorities.

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Communicated by:
ProMED-ESP
<promed-esp@promedmail.org>

[A HealthMap/ProMED-mail interactive map showing the location of Honduras in Central America can be accessed at
<http://healthmap.org/r/072r>. - Mod.TY]

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[19] Brazil (Roraima)
Date: Fri 24 Sep 2010
Source: BV News [in Portuguese, trans. & summ. Mod.TY, edited]
<http://www.bvnews.com.br/cotidiano7149.html>

The total of confirmed dengue [virus] type 4 cases has increased to 12 in Roraima. The confirmation was sent from the Instituto Evandro Chagas (IEC) of Belem [national reference laboratory] after analysis of 70 samples.

Two technicians from the Ministry of Health are in Boa Vista to accompany actions to combat the vector [mosquito]. According to the chief of the State Nucleus for Dengue and Yellow Fever of the state Secretariat of Health, Joel Lima, despite the number of cases of dengue 4 [virus infection] not increasing, it is of concern, because the disease could spread with time. Due to this problem, health authorities decided to intensify action in Vila Central, with active searching for and elimination of breeding sites of the _Aedes aegypti_ mosquito.

As of now, there are 6383 confirmed cases of classical dengue fever in Roraima (including the 12 of dengue [virus] 4), 181 with complications and 77 with DHF. Last year [2009] from January - September 2009, 2878 cases were confirmed.

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[Dengue virus serotype 4 is now part of the national patrimony of dengue. It came to stay. - Mod.LJS]

[A map showing the states in Brazil can be accessed at
<http://www.lib.utexas.edu/maps/americas/brazil.jpg>. A HealthMap/ProMED-mail interactive map of Brazil can be accessed at
<http://healthmap.org/r/008S>. - Mod.TY]

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[20] Brazil (Sao Paulo)
Date: Wed 22 Sep 2010
Source: O Globo [in Portuguese, trans. & summ. Mod.TY, edited]
<http://oglobo.globo.com/cidades/mat/2010/09/22/dengue-atinge-4-100-pessoas-mat

Confirmed dengue cases reached 4100 people and killed 17 in Sao Vicente in Sao Paulo [state]. Although the worst period has passed, mosquito populations will increase more easily in the [upcoming] hot months, a continuing cause of concern. Last year [2009], for example, the municipal Secretariat of Health registered 44 cases of the disease, with no deaths since 2007.

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[There is a lengthy discussion of concerns and details of mosquito vector control activities at the above URL. - Mod.TY]

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[21] Paraguay
Date: Sat 25 Sep 2010
Source: Paraguay.com [in Spanish, trans. Mod.TY, edited]
<http://www.paraguay.com/nacionales/mas-de-13-mil-paraguayos-afectados-por-el-d

The Ministry of Public Health confirmed 13 678 dengue cases in the entire country this year [2010]. Health authorities issued a report calling for the populace to eliminate possible _Aedes aegypti_ mosquito breeding sites, since the [upcoming] hot season is ideal for the reproduction of this [virus] vector. The Health [Ministry] did not provide details of the origin of the cases but specified that of the 21 443 notifications [of suspected cases], 13 678 were confirmed.

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<promed-port@promedmail.org>

[Additional details are available at the above URL.

A map of Paraguay can be accessed at
A HealthMap/ProMED-mail interactive map of Paraguay can be accessed at
<http://healthmap.org/promed/en?vs=-23.2,-58%4.5>. - Mod.TY]

[see also:
Dengue/DHF update 2010 (49) 20100921.3399
Dengue/DHF update 2010 (48) 20100915.3345
Dengue/DHF update 2010 (47) 20100913.3308
Dengue/DHF update 2010 (46) 20100906.3198
Dengue/DHF update 2010 (45) 20100830.3085
Dengue/DHF update 2010 (44) 20100826.3010
Dengue/DHF update 2010 (43) 20100819.2891
Dengue/DHF update 2010 (42) 20100817.2847
Dengue/DHF update 2010 (41) 20100810.2726
Dengue/DHF update 2010 (40) 20100805.2651
Chikungunya and dengue - France (02): risk 20100731.2564
Dengue/DHF update 2010 (30) 20100627.2152
Chikungunya and dengue - France ex overseas 20100616.2008
Dengue/DHF update 2010 (20) 20100426.1347
Dengue/DHF update 2010 (10) 20100304.0707
Chikungunya & dengue - India: (TN) conf. 20100212.0500
Dengue/DHF update 2010 (01) 20100104.0038]
.....mpp/sb/ty/msp/jw
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Archive Number 20100915.3343
Published Date 15-SEP-2010
Subject PRO/AH/EDR> Salmonellosis, serotype Typhimurium DT8 - Ireland: duck eggs

SALMONELLOSIS, SEROTYPE TYPHIMURIUM DT8 - IRELAND: DUCK EGGS
****

A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Tue 14 Sep 2010
Source: The Clare Herald [edited]
<[illegible]>

The investigation into the current outbreak of salmonellosis linked with the consumption of duck eggs is ongoing, with 5 new cases in August 2010. The latest confirmed cases brings the total number to date to 24 and it is now the largest food poisoning outbreak of salmonellosis recorded in recent years in Ireland.

In light of this, the Food Safety Authority of Ireland (FSAI) today, 14 Sep 2010, reiterated its advice on the safe consumption of duck eggs. The people infected have ranged from 5 months to 80 years of age. The latest cases tend to be linked with the consumption of duck eggs from small backyard flocks or private farms. The confirmed cases are nationwide. Hen eggs are not implicated in this outbreak.

The FSAI has advised to only consume duck eggs that have been thoroughly cooked and to cease using raw duck eggs in any dishes that will not be cooked thoroughly prior to eating. It also cautions on the importance of good hygiene practices being followed, such as washing hands and preparation surfaces after handling or using duck eggs.

Prof Alan Reilly, chief executive, FSAI said: "We are advising caterers, retailers, and consumers to treat duck eggs in the same way as they would raw chicken. We all know that we should never eat raw chicken. This is a risk that is well understood by everyone, both in terms of ensuring it is cooked thoroughly and also by maintaining good hygiene practices, thereby preventing cross contamination between raw food and ready-to-eat food."

He continued: "However, people may have forgotten that duck eggs have been associated with _Salmonella_ in the past and therefore, are not taking the correct precautions today. The fact that the outbreak is ongoing, underlines the huge importance attached to maintaining stringent hygiene practices when handling raw duck eggs. Even when duck eggs look clean, they may still have salmonellae on the outside of the shell and sometimes carry it on the inside of the egg."

"The symptoms of _Salmonella_ [_enterica_ serotype] Typhimurium DT8 infection vary from mild discomfort due to vomiting and diarrhea, to life threatening illness. Infants, pregnant women, the frail elderly, and the sick are most at risk from food poisoning. Anyone who may have these symptoms and suspects it may have been from recently eating duck eggs should contact their doctor for advice," added Prof Reilly.

The FSAI is working in collaboration with the Department of Agriculture, Fisheries and Food on control measures for commercial flocks and also smaller backyard flocks on private farms. A code of practice has been published for commercial flock owners and also guidelines have been published for producers of small quantities of

111

別紙様式第2-1　　　　　　　　　　　　　　　　　　　　No. 17

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. 10. 4	該当なし	
一般的名称	人赤血球濃厚液	研究報告の公表状況	ProMED 20100915.3343,15-SEP-2010. Source: The Clare Herald	公表国	
販売名（企業名）	赤血球濃厚液-LR「日赤」（日本赤十字社） 照射赤血球濃厚液-LR「日赤」（日本赤十字社）			アイルランド	

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
○アヒル卵によるサルモネラ症：血清型ネズミチフス菌DT8－アイルランド アヒル卵を摂取することによるサルモネラ症は計24例が報告され、アイルランドで近年記録された食中毒の中でも最大のものとなっている。感染者の年齢層は生後5ヶ月～80歳にわたり、全国的である。これを考慮してアイルランド当局では、2010年9月14日、アヒル卵の安全な摂取法に関する助言を行った。また、アヒル卵に触った後の手洗い等の衛生管理を継続することの重要性について警告している。 サルモネラネズミチフス菌DT8感染の症状は、嘔吐や下痢による軽度の症状から生命を脅かす疾患に変化してきている。乳児や妊婦、高齢者や病人は最も危険にさらされているため特に注意が必要である。 この食中毒の集団発生に鶏卵は関係していない。	赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
アヒル卵の摂取によるサルモネラ症の症例数が計24例となり、アイルランドで近年に記録された食中毒発生の中で最大の原因となっているとの報告である。	日本赤十字社では、輸血による細菌感染予防対策として問診時に献血者の健康状態を確認し、発熱を伴う食中毒様の激しい下痢症状がある場合は1カ月間献血不適としている。また、全ての輸血用血液製剤について保存前白血球除去及び初流血除去を導入している。さらに、輸血情報リーフレット等により、細菌感染やウイルス感染について医療機関へ情報提供し注意喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

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71

duck eggs from backyard flocks. Work is also underway by Bord Bia [Irish Food Board] to develop a new quality assurance scheme to ensure a safe source of duck eggs in the future.

The FSAI is continuing to work closely with the Health Protection Surveillance Centre, the Department of Agriculture, Fisheries and Food, and various local authorities to control this outbreak and to prevent further cases of illness.

[Byline: Mike Fanning]

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ProMED-mail
<promed@promedmail.org>

[Duck eggs are larger than hen eggs and richer in flavor, lending a creamy depth to baked dishes. The shells of duck eggs are slightly tougher to crack. The tougher shell gives duck eggs a longer shelf life, about 6 weeks in the refrigerator, inside is a yolk that is larger in proportion to the white than that of a chicken egg.

We commonly associate raw or poorly cooked chicken eggs (alone or in recipes) with a risk of salmonellosis acquisition. The risk is also present for the eggs of other fowl such as ducks (as shown in this report), ostriches, and quail.

Illustrative references include
1. Miller AA: Human _Salmonella typhimurium infection due to duck eggs. Brit Med J, 1952; 2(4776): 125-8; available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2021319/>.
2. Stokes R: Salmonella food poisoning traced to duck eggs. Ir J Med Sci, 1959; 34: 481-501.
3. Baker RC, Qureshi RA, Sandhu TS, Timoney JF: The frequency of salmonellae on duck eggs. Poult Sci: 1985; 64(4): 646-52; abstract available at <http://www.ncbi.nlm.nih.gov/pubmed/4001051>.
4. Erdogrul OF, Ozkan N, Cakiroglu E: _Salmonella_ Enteritidis in quail eggs. Turk J Vet Anim Sci 2002; 26: 321-3; available at <http://journals.tubitak.gov.tr/veterinary/issues/vet-02-26-2/vet-26-2-20-0102-Mod.LL]

[The HealthMap/ProMED-mail interactive map of Ireland is available at <http://healthmap.org/r/00bn>. - Sr.Tech.Ed.MJ]

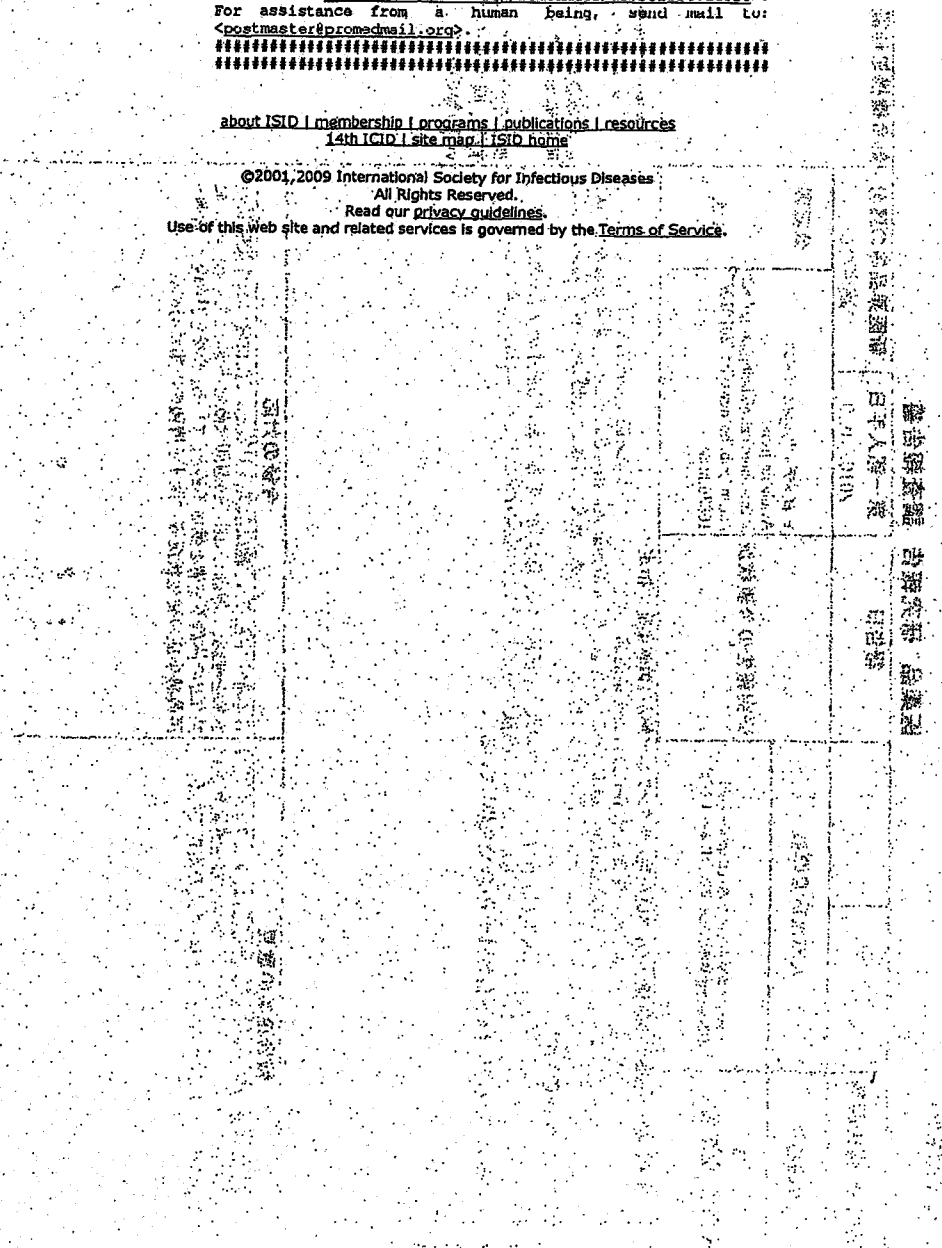
[see also:
Salmonellosis, serotype Enteritidis - USA (07): eggs 20100910.3276
Salmonellosis, serotype Enteritidis - USA: eggs, alert, recall 20100817.2846
Salmonellosis, restaurant - USA: (CO), undercooked eggs 20100805.2653
2008

Salmonellosis, eggs - Australia: (NSW) 20080104.0048
2007

Salmonellosis, eggs - Australia: (NSW) 20071228.4171
Salmonellosis, eggs - Australia (QLD) (03) 20070308.0811
Salmonellosis, eggs - Australia (QLD): recall 20070303.0749
2005

Salmonellosis, raw eggs - Australia (TAS) 20051209.3556
2004

Salmonella, eggs - UK ex Spain 20041019.2835]
.....ll/mj/lm



別紙

一般的名称	①乾燥弱毒生風しんワクチン*、②乾燥弱毒おたふくかぜワクチン
販売名（企業名）	①乾燥弱毒生風しんワクチン*、②乾燥弱毒おたふくかぜワクチン「化血研」
報告企業の意見	コレラはコレラ菌で汚染された水や食物を摂取することで感染する代表的な経口感染症であると共に、重大な感染症である。今回の報告では、患者、死亡者共に短期に著しく増加し、拡大を続けていることが報告されている。当所製剤の安全性に対し、現時点で特段の対応が急がれるものではないが、その動向には注意しておく必要があると考える。 上記製剤の製造には、当所において国内献血血漿から製造した血漿分画製剤である「人血清アルブミン」を使用している。当所の人血清アルブミンの製造工程には、孔径約 0.2μm の無菌ろ過工程が導入されており、その効果はバクテリアチャレンジテストにより確認されている。また、当所の人血清アルブミンの製造工程には細菌より小型であるウイルスの除去を目的としたウイルス除去膜ろ過工程も導入されており、その効果は「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）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。よって、上記製剤に使用している人血清アルブミンはコレラに対する安全性を確保していると考える。更に、これまでに上記製剤によるコレラへの感染報告例は無い。 以上の点から、上記製剤はコレラ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

別紙様式第 2－1

2

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日 2010 年 10 月 29 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり	研究報告の公表状況	MMWR. 2010;Dispatch/59	公表国 ハイチ	
販売名（企業名）	別紙のとおり				

研究報告の概要

問題点：ハイチでコレラのアウトブレークが発生し、2010 年 10 月 27 日時点で 303 名の死亡が報告されている。

ハイチにおいてコレラのアウトブレークが発生しており、2010 年 10 月 21 日にハイチの National Laboratory of Public Health of the Ministry of Public Health and Population によって Viblio cholerae O1-serotype Ogawa-biotype El Tor が同定された。2010 年 10 月 27 日現在で 4,722 人が発症し、この内 303 人の死亡が報告されている。ほとんどの症例が田舎の人口密集地である Artibonite Department で報告されているが、首都のある Ouest Department において可能性例が報告されており、感染の拡大を見せている。これまでハイチではコレラが流行したことはなく、国民はコレラへの感受性が高いと考えられる。

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.13.1

別紙様式第2-1

No. 21

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
			2010. 9. 15	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N. Lancet Infect Dis. 2010 Sep;10(9):597-602. Epub 2010 Aug 10.	インド

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
○インド、パキスタン、英国での新規抗生物質耐性メカニズムの出現:分子学的、生物学的ならびに疫学的研究 背景:New Delhi metallo-β-lactamase 1(NDM-1)によるカルバペネム耐性グラム陰性腸内細菌は、世界的に重大な健康問題となる可能性があるため、インド、パキスタン、英国の多剤耐性腸内細菌におけるNDM-1陽性率を調査した。 方法:インドの2つの中心地 ― チェンナイ(南インド)、ハリヤーナー(北インド)で腸内細菌の分離株が研究され、英国の国立リファレンス研究所に参照された。抗生物質の感受性を評価し、カルバペネム耐性遺伝子 bla_{NDM-1} の存在がPCRによって明らかになった。分離株はパルスフィールドゲル電気泳動法によってタイピングされた。プラスミドは、S1ヌクレアーゼ分解およびPCRタイピングによって分析した。英国患者については、インドまたはパキスタンへの渡航および最近の入院の有無を調査した。 所見:NDM-1産生分離株は、チェンナイ44株、ハリヤーナー26株、英国37株、その他のインド、パキスタン地域で73株が確認された。NDM-1は、ほとんどが *Escherichia coli*(36株)と *Klebsiella pneumoniae*(111株)に見つかり、チゲサイクリンとコリスチンを除く全ての抗生物質に高度耐性があった。ハリヤーナーから分離した *K. pneumoniae* 分離株は遺伝的に均一であったが、英国およびチェンナイの分離株は遺伝的多様性を示した。ほとんどの分離株は、プラスミド上にNDM-1遺伝子を有し、英国およびチェンナイ分離株では受容株に容易にプラスミドが伝達されたが、ハリヤーナーの分離株は伝達性がなかった。英国のNDM-1陽性患者の多くは、前年にインド、パキスタンへの渡航歴があるか、これらの国と関連があった。 結論:NDM-1が世界的な公衆衛生問題となる可能性は大きく、国際的共同調査が必要である。	解凍赤血球濃厚液「日赤」 照射解凍赤血球濃厚液「日赤」 解凍赤血球-LR「日赤」 照射解凍赤血球-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
インド、パキスタン、英国で、多剤耐性腸内細菌におけるNew Delhi metallo-β-lactamase 1(NDM-1)陽性率を調査したところ、NDM-1はほとんどが *Escherichia coli* や *Klebsiella pneumoniae* で見つかり、また英国のNDM-1陽性患者の多くは、インド、パキスタンへの渡航歴やこれらの国と関連があることが分かったとの報告である。	日本赤十字社では輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に、保存前白血球除去及び初流血除去を導入している。さらに、輸血情報リーフレット等により、細菌感染やウイルス感染について医療機関へ情報提供し注意を喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

91

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Cholera Outbreak — Haiti, October 2010

An outbreak of cholera is ongoing in Haiti. On October 21, 2010, toxigenic *Vibrio cholerae* O1, serotype Ogawa, biotype El Tor was identified by the National Laboratory of Public Health of the Ministry of Public Health and Population in Haiti. Identification of the isolate was confirmed by CDC. Antimicrobial susceptibility testing of selected *V. cholerae* O1 isolates conducted at the National Laboratory of Public Health and at CDC demonstrated susceptibility to tetracycline (susceptibility to this drug predicts doxycycline susceptibility), ciprofloxacin, and kanamycin; and resistance to trimethoprim-sulfamethoxazole, furazolidone, nalidixic acid, sulfisoxazole, and streptomycin.

As of October 27, a total of 4,722 cholera cases with onset during October 21–27 and 303 deaths had been reported in Haiti (*1*). Most cases have been reported from Artibonite Department (*1*), a rural but densely settled area with several small urban centers. In addition, probable cases have been identified elsewhere in Haiti, including Ouest Department, where the capital city of Port-au-Prince is located.

Cholera is transmitted through fecal contamination of water or food and causes an acute, severe, watery diarrhea that can result in hypovolemic shock and death if not treated with fluid replacement promptly. Epidemic cholera has not been reported previously from Haiti; the population is immunologically naive and therefore highly susceptible to infection with *V. cholerae* (*2–4*). The outbreak appears to have spread from an initial concentration of cases in Artibonite Department. An international public health response, led by the Ministry of Public Health and Population and including technical support from the Pan American Health Organization, CDC, and other governmental and nongovernmental organizations, is under way. The emphasis of the response is on 1) minimizing morality by using oral rehydration for most cases and intravenous rehydration for severely ill patients and 2) preventing infection by promoting water treatment, adequate sanitation and hygiene, and safe food preparation (*5*).

No cases of cholera in travelers from Haiti to the United States have been reported to CDC. Cholera is notifiable in all U.S. states and territories. Clinicians should promptly report known or suspected cases of cholera to state or local health departments. Health departments that identify suspected or confirmed cases of cholera in travelers who have arrived recently from Haiti should e-mail CDC at eocreport@cdc.gov. The potential for spread in the United States is low because U.S. water, sanitation, and food systems minimize the risk for fecal contamination of food and water.

CDC has provided prevention and treatment guidance for travelers to and from Haiti online (available at http://wwwnc.cdc.gov/travel/default.aspx). Health departments, especially in areas with large Haitian populations that might be more likely to include recent travelers to Haiti, should consider providing cholera information to clinicians. Clinicians serving Haitian populations should be aware of the recommendations for diagnosis and treatment.

More information on cholera, including recommendations for treatment, laboratory testing, and scientific publications, is available at http://www.cdc.gov/cholera. Further information regarding the outbreak in Haiti is available at http://www.cdc.gov/haiticholera.

Reported by
Ministry of Public Health and Population, Haiti. Pan American Health Organization. CDC.

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91

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Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study

Karthikeyan K Kumarasamy[a], Mark A Toleman[b], Timothy R Walsh[b,*], Jay Bagaria[c], Fathana Butt[d], Ravikumar Balakrishnan[e], Uma Chaudhary[e], Michel Doumith[c], Christian G Giske[f], Seema Irfan[g], Padma Krishnan[a], Anil V Kumar[h], Sunil Maharjan[c], Shazad Mushtaq[c], Tabassum Noorie[c], David L Paterson[i], Andrew Pearson[c], Claire Perry[c], Rachel Pike[c], Bhargavi Rao[c], Ujjwayini Ray[j], Jayanta B Sarma[k], Madhu Sharma[e], Elizabeth Sheridan[c], Mandayam A Thirunarayan[l], Jane Turton[c], Supriya Upadhyay[m], Marina Warner[c], William Welfare[c], David M Livermore[c], and Neil Woodford[c]

[a]Department of Microbiology, Dr ALM PG IBMS, University of Madras, Chennai, India

[b]Department of Infection, Immunity and Biochemistry, School of Medicine, Cardiff University, Cardiff, UK

[c]Health Protection Agency Centre for Infections, London, UK

[d]Department of Microbiology, Shaukat Khanum Cancer Hospital, Lahore, Pakistan

[e]Department of Microbiology, Pandit B D Sharma PG Institute of Medical Sciences, Haryana, India

[f]Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden

[g]Department of Pathology and Microbiology, The Aga Khan University, Karachi, Pakistan

[h]Department of Microbiology, Amrita Institute of Medical Sciences, Kerala, India

[i]University of Queensland Centre for Clinical Research, University of Brisbane, Herston, QLD, Australia

[j]Department of Microbiology, Apollo Gleneagles Hospital, Kolkata, India

[k]Department of Medical Microbiology, Northumbria Healthcare NHS Foundation Trust, Tyne and Wear, UK

[l]Department of Microbiology, Apollo Hospitals, Chennai, India

[m]Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Summary

Background—Gram-negative Enterobacteriaceae with resistance to carbapenem conferred by New Delhi metallo-β-lactamase 1 (NDM-1) are potentially a major global health problem. We investigated the prevalence of NDM-1, in multidrug-resistant Enterobacteriaceae in India, Pakistan, and the UK.

Methods—Enterobacteriaceae isolates were studied from two major centres in India—Chennai (south India), Haryana (north India)—and those referred to the UK's national reference laboratory. Antibiotic susceptibilities were assessed, and the presence of the carbapenem resistance gene bla_{NDM-1} was established by PCR. Isolates were typed by pulsed-field gel electrophoresis of XbaI-restricted genomic DNA. Plasmids were analysed by S1 nuclease digestion and PCR typing. Case data for UK patients were reviewed for evidence of travel and recent admission to hospitals in India or Pakistan.

Findings—We identified 44 isolates with NDM-1 in Chennai, 26 in Haryana, 37 in the UK, and 73 in other sites in India and Pakistan. NDM-1 was mostly found among *Escherichia coli* (36) and *Klebsiella pneumoniae* (111), which were highly resistant to all antibiotics except to tigecycline and colistin. *K pneumoniae* isolates from Haryana were clonal but NDM-1 producers from the UK and Chennai were clonally diverse. Most isolates carried the NDM-1 gene on plasmids: those from UK and Chennai were readily transferable whereas those from Haryana were not conjugative. Many of the UK NDM-1 positive patients had travelled to India or Pakistan within the past year, or had links with these countries.

Interpretation—The potential of NDM-1 to be a worldwide public health problem is great, and co-ordinated international surveillance is needed.

Funding—European Union, Wellcome Trust, and Wyeth.

*Correspondence to: Prof Timothy R Walsh, Professor of Medical Microbiology and Antimicrobial Resistance, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK walshtr@cardiff.ac.uk.

Introduction

Bacteria from clinical and non-clinical settings are becoming increasingly resistant to conventional antibiotics. 10 years ago, concern centred on Gram-positive bacteria, particularly meticillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* spp. Now, however, clinical microbiologists increasingly agree that multidrug-resistant Gram-negative bacteria pose the greatest risk to public health. Not only is the increase in resistance of Gram-negative bacteria faster than in Gram-positive bacteria, but also there are fewer new and developmental antibiotics active against Gram-negative bacteria and drug development programmes seem insufficient to provide therapeutic cover in 10–20 years.

The increase in resistance of Gram-negative bacteria is mainly due to mobile genes on plasmids that can readily spread through bacterial populations. Standardised plasmid typing methods are enhancing our understanding of the host ranges of these elements and their worldwide distribution. Moreover, unprecedented human air travel and migration allow bacterial plasmids and clones to be transported rapidly between countries and continents. Much of this dissemination is undetected, with resistant clones carried in the normal human flora and only becoming evident when they are the source of endogenous infections. The CTX-M-15 extended-spectrum β-lactamase (ESBL) encoded by $bla_{CTX-M-15}$ was first reported in India in the mid-1990s. The gene jumped from the chromosome of its natural hosts, *Kluyvera* spp, to plasmids that have subsequently spread widely, establishing CTX-M-15 as the globally-dominant ESBL and the primary cause of acquired resistance to third-generation cephalosporins in Enterobacteriaceae.

Recent surveys have identified ESBLs in 70–90% of Enterobacteriaceae in India and, although these collections might be a biased sample, they do suggest a serious problem, making the widespread use of reserved antibiotics such as carbapenems necessary. Rates of cephalosporin resistance are lower in other countries but the growing prevalence of ESBL producers is sufficient to drive a greater reliance on carbapenems. Consequently, there is selection pressure for carbapenem resistance in Enterobacteriaceae, and its emergence is a worldwide public

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health concern since there are few antibiotics in reserve beyond carbapenems. Already *Klebsiella pneumoniae* clones with KPC carbapenemase are a major problem in the USA, Greece, and Israel, and plasmids encoding the VIM metallo-carbapenemase have disseminated among *K pneumoniae* in Greece.

We recently reported a new type of carbapenem resistance gene, designated bla_{NDM-1}. A patient, repatriated to Sweden after admission to hospital in New Delhi, India, was colonised by *K pneumoniae* and *Escherichia coli* with bla_{NDM-1} on plasmids of varying size, which readily transferred between bacterial strains in vitro. We sought molecular, biological, and epidemiological data on New Delhi metallo-β-lactamase 1 (NDM-1) positive Enterobacteriaceae in India and Pakistan and investigated importation of the resistance gene into the UK by patients returning from the Indian subcontinent.

Methods

Bacterial isolates

Isolates of bacteria were identified from Chennai and Haryana in India. UK isolates were identified from referrals to the Antibiotic Resistance Monitoring and Reference Laboratory by UK microbiology laboratories between 2003 and 2009. We also identified isolates from other sites around Bangladesh, India, and Pakistan.

Procedures

Bacteria were identified via the Phoenix automated phenotypic identification criteria (Becton Dickinson, Oxford, UK) or with API 20E strips (bioMerieux, Basingstoke, UK). Minimum inhibitory concentrations (MICs) and carbapenem resistance were established by microbroth dilution (Phoenix), British Society for Antimicrobial Chemotherapy (BSAC) agar dilution, or disc diffusion.

Modified Hodge (cloverleaf) test involving distorted carbapenem inhibition zones and imipenem-EDTA synergy tests by disc, or the MBL Etest (AB bioMerieux, Solna, Sweden) were used to screen for metallo-β-lactamase production. The presence of bla_{NDM-1} was established by PCR with specific primers targeting the gene. PCR and sequencing were used to identify other resistant genes (bla_{CMY-4} and $bla_{CTX-M-15}$) carried by the bacterial isolates.

Conjugational transfer of antibiotic resistance to the laboratory strain *E coli* J53 was done on blood agar without selection. After 18 h, the mixed cultures were washed from the plates, suspended in saline, and plated onto MacConkey agar containing sodium azide (100 mg/L) and meropenem (2 mg/L). Transconjugants were confirmed to have bla_{NDM-1} by PCR analysis. Plasmids were subsequently isolated and typed on the basis of their origins of replication, as described by Carattoli and colleagues.

Genomic DNA was prepared in agarose blocks and digested with the restriction enzyme XbaI (Roche Diagnostics, Mannheim, Germany). DNA fragments were separated by pulsed-field gel electrophoresis (PFGE) on a CHEF-DR III apparatus (Bio-Rad, Hercules, CA, USA) for 20 h at 6 V/cm at 14°C with an initial pulse time of 0·5 s and a final pulse time of 30 s. Dendrograms of strain relatedness were created with BioNumerics software.

Genomic DNA in agarose blocks was digested with the restriction enzyme S1 (Invitrogen, Abingdon, UK). DNA fragments were separated by PFGE as above. In-gel hybridisation was done with a bla_{NDM-1} probe labelled with ^{32}P (Stratgene, Amsterdam, Netherlands) with a random-primer method. Plasmid DNA bands that hybridised with bla_{NDM-1} were cut from the gel, purified, and typed as described by Carattoli and colleagues.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From Chennai, 75 *E coli*, 60 *Klebsiella* spp, and six other Enterobacteriaceae resistant to carbapenems were isolated from 3521 (4%) Enterobacteriaceae analysed throughout 2009. Of these 141 carbapenem-resistant Enterobacteriaceae, 44 (19 *E coli*, 14 *K pneumoniae*, seven *Enterobacter cloacae*, two *Proteus* spp, one *Citrobacter freundii*, and one *Klebsiella oxytoca*) were NDM-1-positive (about 1% of all resistant isolates). During that same period, 47 carbapenem-resistant isolates (24%) of 198 from Haryana were identified; from these, 26 (13%) were positive for NDM-1, and all were *K pneumoniae*. The Indian isolates from Chennai and Haryana were primarily from community acquired urinary tract infections, pneumonia, and blood-stream infections. The age range was 4–66 years with a mean of 36 years (SD 20) and a female to male ratio of about two to one.

In the UK resistant isolates increased in both 2008 and 2009 (figure 1). Isolates with the NDM-1 enzyme, which was first detected in the UK in 2008, became the predominant carbapenemase-producing Enterobacteriaceae in 2009, accounting for 32 (44%) of 73 carbapenemase producers. During 2008–09, 37 Enterobacteriaceae isolates with the NDM-1 enzyme were referred from 25 laboratories across England with single representatives also from Scotland and Northern Ireland. These were identified as *K pneumoniae* (21 isolates), *E coli* (seven), *Enterobacter* spp (five), *Citrobacter freundii* (two), *Morganella morganii* (one), and *Providencia* spp (one). They were from 29 patients and had been isolated from urine (15 patients), blood (three), burn or wound swab (four), sputum (two), central line tip (one), throat swab (one), or unknown specimens (three). The mean age of the patients was 60 years (SD 24; range 1–87), with 17 male patients and 12 female patients. At least 17 patients had a history of travelling to India or Pakistan within 1 year, and 14 of them had been admitted to a hospital in these countries. Reasons for these admissions included renal or bone marrow transplantation, dialysis, cerebral infarction, chronic obstructive pulmonary disease, pregnancy, burns, road traffic accidents, and cosmetic surgery.

Isolates, NDM-1-positive bacteria from Mumbai (32 isolates), Varanasi (13), and Guwahati (three) in India, and 25 isolates from eight cities in Pakistan (Charsadda, Faisalabad, Gujrat, Hafizabad, Karachi, Lahore, Rahim Yar Khan, and Sheikhupura) were also analysed in exactly the same manner but in laboratories in India and Pakistan. These isolates were from a range of infections including bacteraemia, ventilator-associated pneumonia, and community-acquired urinary tract infections.

All the isolates producing the NDM-1 enzyme were resistant to several antibiotic classes (table). The 37 UK isolates were all resistant to imipenem and ertapenem, although a single *M morganii* isolate remained susceptible, at least in vitro, to meropenem (MIC 2 mg/L). Only four UK isolates remained susceptible to the monobactam aztreonam (MICs ≤1 mg/L), which is unaffected by metallo-carbapenemases including NDM-1; the other UK isolates were all resistant to all β-lactams, including aztreonam, suggesting the concurrent presence of additional β-lactamases including ESBLs and AmpC enzymes—identified by sequencing as mainly $bla_{CTX-M-15}$ and bla_{CMY-4}. All 37 isolates were resistant to amikacin and tobramycin, although one isolate was susceptible to gentamicin and three to ciprofloxacin. MICs of minocycline were consistently 2 mg/L or greater, interpreted as non-susceptible with the BSAC and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for

doxycycline, but most (33 of 37) were susceptible to colistin (MICs ≤4 mg/L) and 26 were suseptible to tigecycline (MICs ≤1 mg/L; figure 2).

The 44 isolates from Chennai were similarly resistant to all β-lactam antibiotics, fluoroquinolones, and aminoglycosides, apart from two that were sensitive to gentamicin. 39 were resistant to minocycline with MICs >2 mg/L, 19 to tigecycline, and three to colistin (table and figure 2). Two of the three isolates resistant to colistin were *Proteus* spp, which are intrinsically resistant, and the third was a *K pneumoniae* strain (colistin MIC >32 mg/L, tigecycline MIC 8 mg/L). Although several reports from Greece have noted *K pneumoniae* isolates as colistin resistant, we believe our isolate is truly pan-resistant. Most of the 26 Haryana isolates were resistant to all β-lactam and non-β-lactam antibiotics, although three were susceptible to aztreonam and one to ciprofloxacin and amikacin. Minocycline MICs for the Haryana isolates were 8–16 mg/L and ten isolates had intermediate resistance (2 mg/L) to tigecycline by EUCAST criteria. None were resistant to colistin (table and figure 2).

The 21 *Klebsiella* isolates from the UK had different PFGE profiles and were typed to 19 distinct groups with only two related pairs, both of which included isolates from epidemiologically linked patients, probably representing cases of cross-infection. All the UK *E coli* isolates were different. The Chennai isolates were also very different, with none similar to each other. By contrast, the 26 NDM-positive *K pneumoniae* isolates from Haryana belonged to a single PFGE profile suggesting clonal spread. We could not prove statistically significant strain relatedness between the Indian and UK isolates.

Isolates from Chennai, Haryana, and the UK's Antibiotic Resistance Monitoring and Reference Laboratory were analysed for the location of the bla_{NDM-1} gene by S1 digestion of DNA, and then PFGE and direct probing of the gels with a radiolabelled bla_{NDM-1} gene. Each of the three groups of isolates typically carried several plasmids, with some isolates having up to eight plasmids (figure 3).

Indian isolates had bla_{NDM-1} exclusively on plasmids. Plasmids from the non-clonal Chennai isolates ranged from 50 kb to 350 kb, whereas those from the clonal *K pneumoniae* from Haryana were predominately either 118 kb (54%) or 50 kb (36%). The UK isolates had a more diverse range of plasmid sizes, 80 kb to greater than 500 kb. Three UK isolates also carried bla_{NDM-1} on their chromosome, suggesting in-situ movement of bla_{NDM-1}. There were many plasmids of identical size in isolates collected from India and the UK (data not shown), suggesting plasmid movement between bacterial isolates. In some isolates, bla_{NDM-1} was carried on more than one plasmid (figure 4).

47 isolates from Chennai (33) and Haryana (14) were randomly chosen for further investigation with PCR and DNA probing to verify the origin of replication (incompatibility type) for plasmids carrying bla_{NDM-1}. Plasmids carrying bla_{NDM-1} from the 14 isolates from Haryana could not be typed. 13 of the 33 isolates from Chennai carried bla_{NDM-1} on A/C-type plasmids and one bla_{NDM-1} positive plasmid was incompatibility type FI/FII. Similarly, when the 32 randomly selected UK isolates were assessed with the same methods, 22 carried A/C type plasmids. The other bla_{NDM-1} positive plasmids from India and the UK that were A/C and FI/FII negative could not be typed.

Transconjugants were created in *E coli* J53 from the 33 Chennai and 32 UK isolates; however, the isolates from Haryana did not produce transconjugates. All transconjugants were shown by PCR to contain bla_{NDM-1}. We compared the sizes of the plasmids in the clinical strains with those of the transconjugants and, in about 10% of cases, the plasmid had altered in size during transfer. In most cases the plasmid had lost DNA but two of 102 had gained DNA during transfer.

In addition to the collections of isolates from Chennai and Haryana detailed above, we have confirmed by PCR alone the presence of genes encoding NDM-1 in carbapenem-resistant Enterobacteriaceae isolated from Guwahati, Mumbai, Varanasi, Bangalore, Pune, Kolkata, Hyderabad, Port Blair, and Delhi in India, eight cities (Charsadda, Faisalabad, Gujrat, Hafizabad, Karachi, Lahore, Rahim Yar Khan, and Sheikhupura) in Pakistan, and Dhaka in Bangladesh (figure 5) suggesting widespread dissemination.

Discussion

Enterobacteriaceae with NDM-1 carbapenemases are highly resistant to many antibiotic classes and potentially herald the end of treatment with β-lactams, fluoroquinolones, and aminoglycosides—the main antibiotic classes for the treatment of Gram-negative infections. Only a few isolates remained sensitive to individual aminoglycosides and aztreonam, perhaps owing to the loss of resistance genes (eg, those encoding aminoglycoside modifying enzymes, 16S rRNA methylases, or bla_{CMY-4}). Most isolates remained susceptible to colistin and tigecycline.

Typing did not identify common strain types of *E coli* or *K pneumoniae* between the Indian subcontinent and the UK or between north and south India. Nevertheless, the NDM-1-positive *K pneumoniae* isolates from Haryana were clonal, suggesting that some strains could potentially cause outbreaks. Most bla_{NDM-1} positive plasmids were readily transferable and prone to rearrangement, losing or (more rarely) gaining DNA on transfer. This transmissibility and plasticity implies an alarming potential to spread and diversify among bacterial populations. Curiously, many of the plasmids were incompatibility A/C types—a group not commonly associated with multidrug-resistant phenotypes.

Although antibiotic resistance in China has been highlighted as a concern, the rapid emergence of bla_{NDM-1} deserves equal attention. A recent editorial by Abdul Ghafur highlights the widespread non-prescription use of antibiotics in India, leading to huge selection pressure, and predicts that the NDM-1 problem is likely to get substantially worse in the foreseeable future. This scenario is of great concern because there are few new anti-Gram-negative antibiotics in the pharmaceutical pipeline and none that are active against NDM-1 producers. Even more disturbing is that most of the Indian isolates from Chennai and Haryana were from community-acquired infections, suggesting that bla_{NDM-1} is widespread in the environment.

The introduction of NDM-1 into the UK is also very worrying and has prompted the release of a National Resistance Alert 3 notice by the Department of Health on the advice of the Health Protection Agency. Given the historical links between India and the UK, that the UK is the first western country to register the widespread presence of NDM-1-positive bacteria is unsurprising. However, it is not the only country affected. In addition to the first isolate from Sweden, a NDM-1-positive *K pneumoniae* isolate was recovered from a patient who was an Australian resident of Indian origin and had visited Punjab in late 2009. The isolate was highly resistant and carried bla_{NDM-1} on an incompatibility A/C type plasmid similar to those in India and the UK.

Several of the UK source patients had undergone elective, including cosmetic, surgery while visiting India or Pakistan. India also provides cosmetic surgery for other Europeans and Americans, and bla_{NDM-1} will likely spread worldwide. It is disturbing, in context, to read calls in the popular press for UK patients to opt for corrective surgery in India with the aim of saving the NHS money. As our data show, such a proposal might ultimately cost the NHS substantially more than the short-term saving and we would strongly advise against such proposals. The potential for wider international spread of producers and for NDM-1-encoding plasmids to become endemic worldwide, are clear and frightening.

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124

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Acknowledgments

TW, MT, and KK did MIC determinations, all genetic analysis, and plasmid profiling. Authors from HPA Centre for Infections undertook strain typing, MIC determinations, and follow-up for UK cases. DP proofread the manuscript and provided data from Australia. PK and MT (Chennai) and MS and UC (Haryana) characterised strains and provided clinical details. UR and AK provided prevalence data and demographics. All authors were involved in the compiling the report and approved the final version.

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125

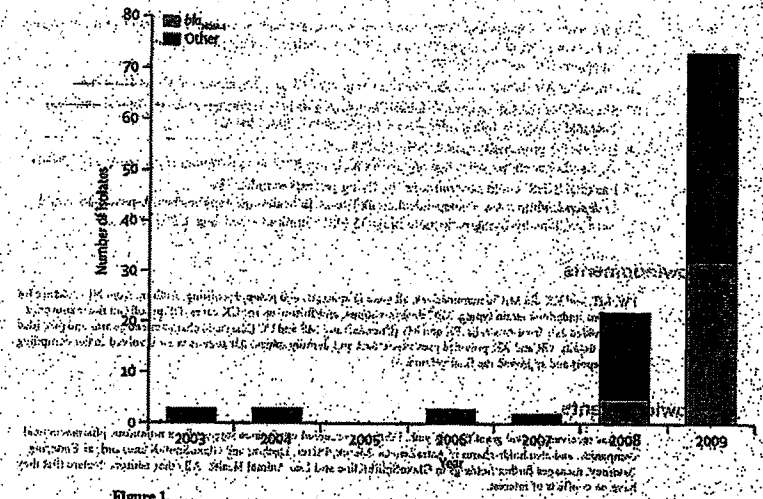

Figure 1.
Numbers of carbapenemase-producing Enterobacteriaceae referred from UK laboratories to the UK Health Protection Agency's national reference laboratory from 2003 to 2009

The predominant gene is *bla*$_{NDM-1}$, which was first identified in 2008. The other group includes diverse producers of KPC, OXA-48, IMP, and VIM enzymes.

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126

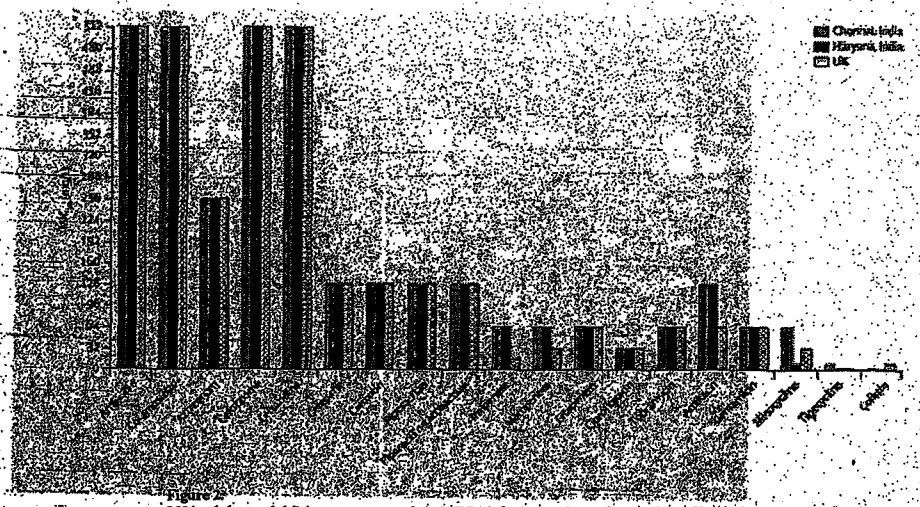

Figure 2
90% minimum inhibitory concentration (MIC_{90}) for Enterobacteriaceae from Chennai and Haryana, India, and the UK

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127

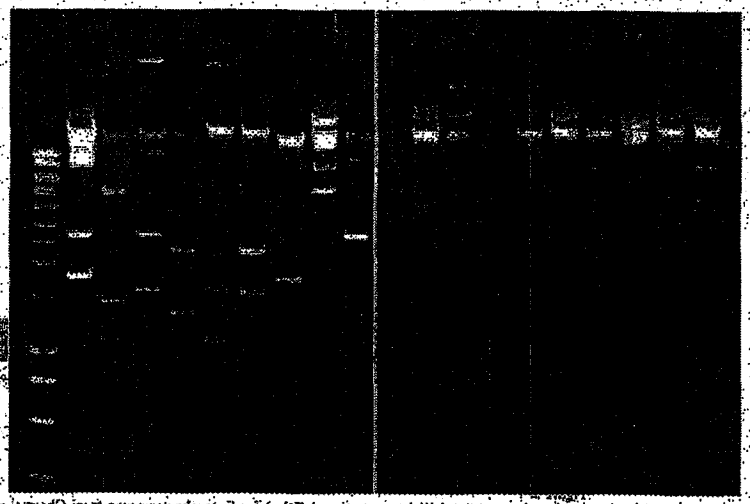

Figure 3.
The difference in plasmid numbers from a selection of Indian isolates
Tracks 1–10 show the number of plasmids in isolates from Chennai (south India) and tracks 11–18 show the number of plasmids in isolates from Haryana (north India). Most isolates contained up to seven plasmids, and in Chennai there was greater variation than in isolates from Haryana showing the bacterial clonality of NDM-1 carriage in Haryana.

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Figure 4.
Hybridisation results of UK isolates with bla_{NDM-1}
Pulsed-field gel of S1-treated plasmid DNA of UK isolates M15–M27 stained with ethidium bromide (A). Molecular weight marker is Lambda concatamer 50–1000 kb. The chromosome of each isolate is the bright band at the top of each lane and bright bands below are plasmids of various sizes. Autoradiogram of gel A probed with a bla_{NDM-1} showing individual or multiple plasmids in each strain carrying bla_{NDM-1} (B).

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Figure 5
Distribution of NDM-1-producing Enterobacteriaceae strains in Bangladesh, Indian, Pakistan, and the UK

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Table
Antibiotic susceptibilities for NDM-1-positive Enterobacteriaceae isolated in the UK and north (Chennai) and south India (Haryana)

	UK (n=65)			Chennai (n=45)			Haryana (n=36)		
	MIC$_{50}$	MIC$_{90}$ (mg/L)	Proportion susceptible[a]	MIC$_{50}$	MIC$_{90}$ (mg/L)	Proportion susceptible	MIC$_{50}$	MIC$_{90}$ (mg/L)	Proportion susceptible
Imipenem	32; 128	96	96	64; 128	96	96	32; 128	96	97
Meropenem	32; 32	31%	31%	32; 32	96%	96%	>32; >32	0%	0%
Piperacillin-tazobactam	>64; >64	0%	0%	>64; >64	0%	0%	>64; >64	0%	0%
Cefuroxime	>256; >256	0%	0%	>256; >256	0%	0%	>256; >256	0%	0%
Ceftriaxone	256; 256	0%	0%	>256; >256	0%	0%	>256; >256	0%	0%
Ceftazidime	>64; >64	0%	0%	>64; >64	0%	0%	>64; >64	0%	0%
Cefepime	>64; >64	11%	0%	>64; >64	0%	0%	>64; >64	0%	0%
Aztreonam	>64; >64	8%	8%	>64; >64	0%	0%	>64; >64	0%	0%
Ciprofloxacin	>8; >8	3%	3%	>8; >8	0%	0%	>8; >8	0%	0%
Gentamicin	>32; >32	3%	3%	>32; >32	3%	3%	>32; >32	3%	3%
Tobramycin	>32; >32	0%	0%	>32; >32	0%	0%	>32; >32	0%	0%
Amikacin	>64; >64	0%	0%	>64; >64	0%	0%	>64; >64	0%	0%
Minocycline	16; >32	0%	0%	32; >32	0%	0%	8; 16	0%	0%
Tigecycline	1; 4	0%	0%	4; 8	96%	96%	1; 2	97%	97%
Colistin	0.5; 8	89%[b]		1; 32	89%[b]		1; 2	100%[b]	

MIC=minimum inhibitory concentration.

[a] Susceptibility defined by British Society for Antimicrobial Chemotherapy and European Committee on Antimicrobial Susceptibility Testing breakpoints; piperacillin-tazobactam breakpoints were used for enterobacteria.

[b] Colistin-resistant UK isolates were one isolate of *Morganella morganii* and one *Providencia* sp. (both intrinsically resistant species), one each *Escherichia coli*, *Klebsiella pneumoniae* and one *Enterobacter* sp.

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The Washington Times

Japan confirms its first case of new superbug gene

By SHINO YUASA

Associated Press

3:01 a.m., Tuesday, September 7, 2010

TOKYO (AP) - Japan has confirmed the nation's first case of a new gene in bacteria that allows the microorganisms to become drug-resistant superbugs, detected in a man who had medical treatment in India, a Health Ministry offici said Tuesday.

The gene, known as NDM-1, was found in a Japanese man in his 50s, Kensuke Nakajima said.

Researchers say the gene _ which appears to be circulating widely in India _ alters bacteria, making them resistant t nearly all known antibiotics.

Drug-resistant bacteria are not new. Many bacteria are resistant to the world's first antibiotic, penicillin, as well as successive generations of drugs. Excessive use and improper use of antibiotics have exacerbated the problem and le to the emergence of superbugs.

"The potential of NDM-1 to be a worldwide public health problem is great, and coordinated international surveillan is needed," according to a widely publicized report in the British medical journal Lancet in August.

The gene has been seen largely in the deadly E. coli bacteria and on DNA structures that can be easily copied and passed onto other types of bacteria.

The man was hospitalized in April 2009 after returning from India where he had medical treatment. Nakajima declined to say what kind of treatment the man had received in India, citing the man's privacy.

The man had a high fever while staying at a hospital in Tochigi, north of Tokyo. He was discharged in October last year.

The hospital _ Dokkyo Medical University Hospital _ kept a preserved sample of the suspected superbug from the man. The hospital examined the sample after the Lancet report.

識別番号・報告回数			報告日	第一報入手日 2010. 9. 15	新医薬品等の区分 該当なし
一般的名称	解凍人赤血球濃厚液				公表国 日本
販売名（企業名）	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		研究報告の公表状況	The Washington Times. Available from: http://www.washingtontimes.com/news/2010/sep/7/japan-confirms-its-first-case-of-new-superbug-gene/	

研究報告の概要	○日本初の超強力薬剤耐性菌症例 細菌を薬剤耐性菌に変化させる新たなNew Delhi metallo-β-lactamase 1（NDM-1）遺伝子が日本で初めて、インドで治療を受けた50歳代日本人男性に確認された。この遺伝子はほとんどすべての流行病原に耐性となるよう細菌を変化させる。この遺伝子は主に病原性大腸菌で見られ、他のタイプの細菌に容易に複写され移入することが出来るDNA構造を有している。当該男性はインドで内科治療を受け、帰国後の2009年4月に入院した。男性がインドでどのような治療を受けたかについては、プライバシー保護のため公表されなかった。男性は入院中に高熱を出したが、2009年10月に退院した。病院は超強力薬剤耐性菌を疑い検体を保管、検査し、NDM-1遺伝子の検出について、厚生労働省に届け出た。院内感染は認められていない。日本初のNDM-1症例確認後、厚生労働省は全国調査を開始した。
報告企業の意見	
今後の対応	日本赤十字社では輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に、保存前白血球除去及び初流血除去を導入しているほか、輸血情報リーフレット等により、細菌感染やウイルス感染について医療機関に情報提供し注意を喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

総合機構処理欄	
使用上の注意記載状況・その他参考事項等	解凍赤血球濃厚液「日赤」 照射解凍赤血球濃厚液「日赤」 解凍赤血球-LR「日赤」 照射解凍赤血球-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

別紙様式第2-1

No. 30

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. 10. 4	該当なし	
一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		USA TODAY. Available from: http://www.usatoday.com/yourlife/health/medical/2010-09-17-1Asuperbug17_ST_N.htm	米国	

研究報告の概要

○強力薬剤耐性菌が米国の35州を襲い、世界へ拡大

抗生物質の機能を一時的に阻害する酵素*Klebsiella pneumoniae* carbapenamase(KPC)を産生する遺伝子を備えた強力な薬剤耐性菌が米国の35州以上の病院で報告されている。この細菌は重篤な疾患患者を襲い、死亡率は全症例の30～60%に及ぶ。米国疾病管理予防センター(CDC)によると、主にインドで治療を受けた患者において認められたNew Delhi metallo-β-lactamase 1(NDM-1)は米国では稀であり、KPCの方が全国的にはるかに一般的で、現在では米国の半分以上の州で報告されているという。

この細菌に対する唯一の薬にポリミキシンがあるが、腎臓に有毒であるため数年前からほとんど使用されていない。従って予防は極めて重要である。2009年3月にCDCは予防に関する新しいガイドラインを示し、特にカルバペネム耐性菌感染症と診断される患者を治療する医師は、ガウンと手袋を着用して自身を守るとともに、他の患者への感染を防がなくてはならないとしている。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

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
抗生物質の機能を一時的に阻害する酵素*Klebsiella pneumoniae*. carbapenamase(KPC)を産生する遺伝子を備えた強力な薬剤耐性菌が米国の35以上の州で報告されているとのことである。	日本赤十字社では輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に、保存前白血球除去及び初流血除去を導入している。さらに、輸血情報リーフレット等により、細菌感染やウイルス感染について医療機関へ情報提供し注意喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

MedDRA/J Ver.13.1J

Japan confirms its first case of new superbug gene - Washington Times 2/2 ページ

The Tochigi hospital notified the Health Ministry about the detection of the NDM-1 gene. It told the ministry that n in-hospital infections were found. Following the confirmation of the discovery _ Japan's first NDM-1 case _ the Health Ministry launched a nationwide survey, asking local health authorities to check on hospitals for evidence of more infections.

Along with India, the new superbug gene has been detected in small numbers in Australia, Canada, the United State the Netherlands, Sweden and the U.K. Researchers say since many Americans and Europeans travel to India and Pakistan for elective procedures like cosmetic surgery, it was likely the superbug gene would spread worldwide.

Antimicrobial resistance _ the ability of microorganisms to escape drugs' efficacy _ is an increasing global health problem that could affect control of diseases such as respiratory infections and dysentery, according to the World Health Organization.

The WHO says NDM-1 requires monitoring and further study. With effective measures, countries have successfully battled multi-drug resistant microorganisms in the past.

It recommends that governments focus their efforts in four areas: surveillance, rational antibiotic use, legislation to stop sales of antibiotics without prescription, and rigorous infection prevention measures such as hand-washing in hospitals.

Japan Newspaper Washington Times MRSA Antibiotic MRSA Treatment Japan Ladies

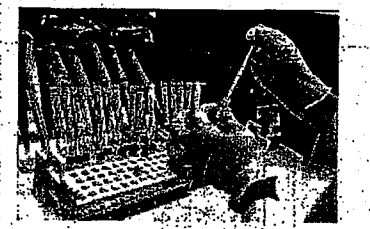
http://www.washingtontimes.com/news/2010/sep/7/japan-confirms-its-first-case-of-new-supe... 2010/10/05

USA TODAY

Superbug spreading

Updated 9/17/2010 5:05 PM

By Steve Sternberg, USA TODAY



By Paul S. Howell for USA TODAY

Medical technician Carla Woodmansee tests nose swabs of new patients for MRSA at UTMB, the University of Texas Medical Center in Nov. 2007. Bacteria that are able to survive every modern antibiotic are cropping up in many U.S. hospitals and are spreading outside the USA, public health officials say.

Bacteria that are able to survive every modern antibiotic are cropping up in many U.S. hospitals and are spreading outside the USA, public health officials say.

The bugs, reported by hospitals in more than 35 states, typically strike the critically ill and are fatal in 30% to 60% of cases. Israeli doctors are battling an outbreak in Tel Aviv that has been traced to a patient from northern New Jersey, says Neil Fishman, director of infection control and epidemiology at the University of Pennsylvania and president of the Society of Healthcare Epidemiologists.

The bacteria are equipped with a gene that enables them to produce an enzyme that disables antibiotics. The enzyme is called Klebsiella pneumoniae carbapenamase, or KPC. It disables carbapenam antibiotics, last-ditch treatments for infections that don't respond to other drugs.

"We've lost our drug of last resort," Fishman says.

Carbapenam-resistant germs are diagnosed mostly in hospital patients and are not spreading in the community. They're far more common nationwide than bacteria carrying a gene called NDM-1 that made headlines this week, Fishman says.

Those NDM-1 bacteria, named for the city of New Delhi, are rare in the USA and have been found mainly in people who obtain medical treatment in India, Arjun Srinivasan of the U.S. Centers for Disease Control and Prevention (CDC) said Thursday.

Although KPCs are most common in New York and New Jersey, Srinivasan says, "they've now been reported in more than half of the states." A decade ago, only 1% of Klebsiella pneumoniae bacteria reported to CDC by hospitals were carbapenam-resistant. Today, resistance has spread to more than 8% of these bacteria. No one knows precisely how many people have KPC infections because cases aren't routinely reported to the CDC.

"We see a ton of the KPC organisms," says Yoko Furuya, medical director of infection control in New York Presbyterian Hospital. "It started in 2002-2003. They just somehow established themselves in nursing homes and hospitals. We always have some patients, five to 10 at a time, in the hospital with this problem."

Doctors say the bacteria are more worrisome than another well-known superbug, methicillin-resistant Staphylococcus aureus (MRSA), because more drugs are available to treat MRSA, Fishman says. "When MRSA started to develop 15 years ago, the industry started producing antibiotics now coming onto the

USA TODAY

market," he says. "We're in the same position with KPCs as we were with staph aureus 15 years ago, except that the pharmaceutical industry isn't rushing to produce new drugs."

One of the only drugs that combats these bugs is polymbin, which was all but abandoned years ago because it is so toxic to the kidneys, Fishman says. As a result, he says, prevention is crucial.

In March 2009, the CDC gave hospitals new guidelines for prevention. Among other things, doctors treating any patient diagnosed with carbapenam-resistant infections are advised to wear gowns and gloves to protect themselves and make sure they don't infect other patients.

Centers for Disease Control and Prevention
MMWR Morbidity and Mortality Weekly Report
Weekly / Vol. 59 / No. 36 September 17, 2010

Balamuthia mandrillaris Transmitted Through Organ Transplantation — Mississippi, 2009

On December 14, 2009, a physician in Mississippi contacted CDC to report possible transplant-transmitted encephalitis in two kidney transplant recipients who shared the same organ donor. Histopathologic testing of donor autopsy brain tissue at CDC showed amebae, and subsequent testing of specimens from the donor and the two kidney recipients confirmed transmission by transplantation of *Balamuthia* granulomatous amebic encephalitis (GAE), a rare disease caused by *Balamuthia mandrillaris*, a free-living ameba found in soil (*1*). One kidney recipient, a woman aged 31 years, died; the other recipient, a man aged 27 years, survived with neurologic sequelae. Recipients of the heart and liver from the same donor received preemptive therapy and have shown no signs of infection. The donor, a previously healthy boy aged 4 years, was presumed to have died from acute disseminated encephalomyelitis (ADEM), an autoimmune neurologic disease, after infection with influenza A. An investigation was conducted by the state health departments in Mississippi, Kentucky, Florida, and Alabama and CDC to characterize the cases, elucidate possible exposures in the donor, and develop recommendations for early detection and prevention. This is the first reported transmission of *Balamuthia* by organ transplantation. Clinicians should be aware of *Balamuthia* infection as a potentially fatal cause of encephalitis. Organ procurement organizations (OPOs) and transplant centers should be aware of the potential for *Balamuthia* infection in donors with encephalitis of uncertain etiology, and OPOs should communicate this elevated risk for infection to transplant centers so they can make an informed risk assessment in the decision to accept an organ.

Organ Donor

The organ donor, a boy aged 4 years from Kentucky, was living with relatives in Mississippi in October 2009, when he developed a transient febrile illness. He was diagnosed with influenza A infection by rapid influenza test on October 25 and prescribed antivirals; his symptoms resolved without hospitalization. On November 3, the boy had sudden onset of headache and seizures and was hospitalized (Table 1). Cerebrospinal fluid (CSF) demonstrated lymphocytic pleocytosis (170 white blood cells/mm^3) and normal protein (29 mg/dL); magnetic resonance imaging (MRI) of the brain showed numerous small enhancing lesions and edema (Table 2). An extensive search for viral, bacterial, and fungal etiologies of encephalitis was unrevealing. His clinical presentation, CSF findings, and MRI were thought to be most consistent with a diagnosis of ADEM, an immune-mediated encephalitis that can follow influenza or other infections. He was treated symptomatically and discharged on November 6.

The boy was readmitted on November 10 with recurrent seizures. MRI of the brain demonstrated progression of several of the enhancing lesions; CSF again demonstrated lymphocytic pleocytosis (150 cells/mm^3) and normal protein (44 mg/dL) (Table 2). He was treated for presumed worsening ADEM with intravenous corticosteroids and immunoglobulin. He developed subarachnoid hemorrhage and brain stem herniation on November 18 and was pronounced brain dead the next day. His heart, liver, and kidneys were transplanted into four recipients at three different transplant centers on November 20. On December 16, histopathologic examination of the donor's

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
www.cdc.gov/mmwr

医薬品 研究報告 調査報告書 No. 21

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一般的名称	人赤血球濃厚液
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研究報告の概要

○臓器移植による*Balamuthia mandrillaris*の伝播―ミシシッピー州、2009年

2009年12月14日、ミシシッピー州の医師により、同じドナーからの腎臓移植レシピエント2名が移植により脳炎を発症した可能性があると米国疾病管理予防センター（CDC）に報告された。CDCはドナーの剖検脳組織からアメーバを発見し、その後、ドナーと2名のレシピエントからの検体で実施した検査により、バラムチア・アメーバ性肉芽腫性脳炎（GAE）の伝播を確認した。これは*Balamuthia mandrillaris*（土壌中に生息する自由生活アメーバ）に起因するまれな疾患である。

腎臓移植レシピエント2名のうち1名（31歳女性）は死亡し、もう1名（27歳男性）は右肺、両脚、視力に後遺症があるが生存している。同じドナーから心臓移植と肝臓移植を受けたレシピエントにはそれぞれ感染の徴候は見られていない。ドナー（4歳の健常男児）はインフルエンザA感染症を発症後、急性散在性脳脊髄炎で死亡したと推定される。これは臓器移植によるバラムチア感染症の初めての報告である。

研究報告の概要

報告企業の意見	今後の対応
臓器移植による初のバラムチア感染症によるアメーバ性肉芽腫脳炎が米国疾病管理予防センターに報告されたとのことである。	今後も引き続き、新規・再興感染症の発生状況等に関する情報の収集に努める。

brain tissue at CDC revealed the presence of abundant amebae morphologically suggestive of *Balamuthia* (Figure); empiric treatment for both kidney recipients was initiated later that day, in consultation with CDC. On December 17, immunohistochemical and indirect immunofluorescent stains (Figure) revealed antigens of free-living amebae in the donor's brain tissue; polymerase chain reaction (PCR) results confirmed *Balamuthia* infection.

Kidney Recipient A

Kidney recipient A, a woman aged 31 years, underwent transplantation for end-stage renal disease resulting from hypertension and diabetes. On December 10, post-transplant day (PTD) 20, she reported onset of right leg twitching and neck spasms, numbness, headache, nausea, and seeing flashing lights (Table 1). She was evaluated in an emergency department, where she was treated with benzodiazepines and discharged with muscle relaxants; no neuroimaging or lumbar puncture was performed. On December 12, she was found unresponsive at home and taken back to the emergency department, where she had a generalized seizure and was admitted; the next day, she was transferred to the intensive-care unit. MRI of the brain demonstrated numerous ring-enhancing lesions. CSF initially showed a normal white blood cell count (3 cells/mm^3) and elevated protein (75 mg/dL); however, another specimen collected on December 15 revealed a neutrophilic pleocytosis (507 cells/mm^3) and increased protein (142 mg/dL) (Table 2). On December 16, she underwent brain biopsy. On December 18, histopathologic examination of the brain tissue at CDC revealed amebae; immunohistochemical stains detected antigens of free-living amebae, and PCR confirmed *Balamuthia* infection. She was treated with pentamidine, sulfadiazine, flucytosine, fluconazole, and azithromycin. Miltefosine, an antileishmanial and antineoplastic agent, was added on December 25 under an emergency investigational new drug (IND) protocol. Despite several weeks of intensive care, she deteriorated neurologically and died on February 3 (PTD 75).

Kidney Recipient B

Kidney recipient B, a man aged 27 years, underwent transplantation for end-stage renal disease resulting from focal segmental glomerulosclerosis. On December 10 (PTD 20), he had sudden onset

TABLE 1. Timeline of events involving transmission of *Balamuthia* infection from an organ donor to two kidney recipients — Mississippi, 2009–2010

Date	Donor	Kidney recipient A	Kidney recipient B
2009			
November 3	Hospitalized with headaches and seizures.		
November 5	Initial brain MRI performed.		
November 6	Discharged from hospital.		
November 10	Hospitalized after recurrence of seizures.		
November 18	Developed subarachnoid hemorrhage and brain stem herniation.		
November 19	Pronounced brain dead.		
November 20	Heart, liver, and kidneys transplanted into four recipients.	Received kidney from donor.	Received kidney from donor.
December 10		Onset of right leg twitching and neck spasms, numbness, headache, nausea, and seeing flashing lights.	Onset of severe headache and vomiting.
December 11			Onset of altered mental status and seizures. Hospitalized.
December 12		Found unresponsive at home. Hospitalized.	
December 13		Admitted to intensive-care unit.	Admitted to intensive-care unit. Initial brain MRI performed.
December 15		Initial brain MRI performed.	
December 16	Histopathologic examination of brain tissue at CDC revealed amebae suggestive of *Balamuthia*.	Underwent brain biopsy. Started on multiple drug regimen.	Started on multiple drug regimen.
December 17	*Balamuthia* infection confirmed by PCR.	Amebae seen on brain histopathology at CDC.	
December 18		*Balamuthia* infection confirmed by PCR of brain tissue at CDC.	
2010			
January 5			*Balamuthia* infection confirmed by PCR on CSF specimen drawn December 29.
February 2			*Balamuthia* infection cultured from CSF specimen drawn December 29.
February 3		Died after 7 weeks of intensive care.	
April 28			Discharged to a rehabilitation facility.
June 11			Discharged home with neurologic sequelae.

Abbreviations: MRI = magnetic resonance imaging; PCR = polymerase chain reaction; CSF = cerebrospinal fluid.

of severe headache and vomiting and was examined at a local emergency department early the next morning, where he was diagnosed with sinusitis and discharged on amoxicillin-clavulanic acid (Table 1). Later that day, he developed altered mental status and seizures and was admitted to a regional hospital. A lumbar puncture was performed; CSF demonstrated 1 white blood cell/mm^3 and slightly elevated protein (69 mg/dL) (Table 2). On December 13, he was transferred to the intensive-care unit at the same hospital as kidney recipient A. CSF that day revealed mild pleocytosis (19 cells/mm^3) and slightly increased protein (74 mg/dL). MRI of the brain showed numerous ring-enhancing lesions. The man was treated with the same combination of drugs as kidney recipient A, including miltefosine obtained under IND. *Balamuthia* infection was confirmed by PCR and culture on a CSF specimen drawn December 29. After 2 months in a coma, the man had a slow but significant recovery of cognitive and motor function and was discharged to a rehabilitation facility on April 28 (PTD 159). He was discharged home June 11. His neurologic sequelae included residual right arm paralysis, bilateral leg weakness, and intermittent vision loss; however, he performed most activities of daily living independently.

Heart Recipient

The heart recipient, a boy aged 2 years, underwent transplantation for restrictive cardiomyopathy. When the

140

141

TABLE 2. Demographic, clinical, and laboratory features of cases involving transmission of *Balamuthia* infection from an organ donor to two kidney recipients — Mississippi, 2009–2010

Patient	Age/Sex	Race/ Ethnicity	Time from transplant to symptom onset	Initial clinical symptoms	Initial lumbar puncture (LP) results (2nd LP results) WBC*	Protein†	Glucose§	Neuroimaging results	Mode of initial *Balamuthia* GAE diagnosis	Preliminary diagnosis	Outcome
Donor	4 yrs/male	White, non-Hispanic	N/A	Personality changes, loss of appetite, muscle twitching, headache, seizure	170 (150)	29 (44)	49 (46)	Multiple focal enhancing lesions	Autopsy	ADEM	Death
Kidney recipient A	31 yrs/female	Black, non-Hispanic	20 days	Paresthesias, muscle spasms, headache, nausea, altered mental status, seizure	3 (507)	75 (142)	114 (67)	Multiple large ring-enhancing lesions	PCR of brain biopsy	Muscle spasms	Death
Kidney recipient B	27 yrs/male	Black, non-Hispanic	20 days	Headache, nausea, altered mental status, seizure	1 (19)	69 (74)	77 (62)	Ring-enhancing lesions	PCR and culture of CSF	Sinusitis	Survived, but with neurologic sequelae¶

Abbreviations: GAE = granulomatous amebic encephalitis; ADEM = acute disseminated encephalomyelitis; PCR = polymerase chain reaction; CSF = cerebrospinal fluid.
* White blood cells per mm³; normal range: 0–5 (aged >12 yrs), 0–20 (aged 1–4 yrs).
† mg/dL; normal range: 12–60.
§ mg/dL; normal range: 40–70.
¶ Including intermittent hemianopsia, bilateral leg weakness, and right arm paralysis.

kidney recipients were diagnosed with *Balamuthia* GAE, the boy was asymptomatic. On December 17 (PTD 27), he was hospitalized for evaluation. MRI of the brain was normal, and testing of CSF, serum, and endomyocardial tissue at CDC showed no evidence of *Balamuthia* infection. The boy was treated for presumed *Balamuthia* exposure with a 6-week course of intravenous pentamidine, azithromycin, and fluconazole, followed by 5 weeks of oral azithromycin. He remains well.

Liver Recipient

The liver recipient, a boy aged 7 years, underwent transplantation for end-stage liver disease resulting from alpha-1-antitrypsin deficiency. The boy was asymptomatic when the kidney recipients were diagnosed with *Balamuthia* GAE, and he was hospitalized for evaluation on December 17. MRI of the boy's brain was normal, and testing of CSF, serum, and liver tissue at CDC showed no evidence of *Balamuthia* infection. He was treated for presumed *Balamuthia* exposure with a 1-month course of intravenous pentamidine, fluconazole, azithromycin, and sulfadiazine. He remains well.

Public Health Investigation

Interviews with the donor's family revealed that he had lived in Kentucky, Florida, and Mississippi during the 2 years before his death. He frequently played outdoors and had soil exposure in all of these locations. He occasionally played in a wading pool; the water supply for drinking and recreation in Florida was untreated well water. No environmental sampling was performed because *Balamuthia* is thought to be ubiquitous in the environment.

Approximately 4 months before his first seizure, the boy had become more irritable and emotionally labile. His family also noted regression of toilet training and an infrequent, sporadic tremor of the right hand that began at about the same time. He had no history of immunocompromising conditions. No medical evaluation of family members was conducted.

Reported by

S Schlesinger, MD, Mississippi Organ Recovery Agency, Flowood; K Kokko, MD, J Fratkin, MD, F Butt, MD, A Hawxby, MD, M Todaro, PharmD, H Henderson, MD, A Seawright, DNP, C Parker, MD, Univ of Mississippi Medical Center; P Byers, MD, Mississippi Dept. of Health. R Gonzalez-Peralta, MD, L Kayler, MD, L Fauerbach, R Lawrence, MD, A Haafiz, MD, Shands Hospital, Univ. of Florida; D Stanek, DVM, K Hammond, Florida Dept of Health. D Thoroughman, PhD, Kentucky Dept for Public Health. T Pippen, S Johnson, Alabama Dept of Public Health. W Mable, MD, G Lyon III, MD, K Laporte, K Kanter, MD, Children's Healthcare of Atlanta, Emory Univ, Atlanta; M Ivey, MPH, K Arnold, MD, S Lance, DVM, Georgia Div of Public Health. E Navarro-Almario, Food and Drug Admin. E Farnon, MD, M Kuehnert, MD, Div of Healthcare Quality Promotion; W Shieh, MD, C Paddock, MD, S Zaki, MD, C Drew, DVM, A Schmitz, DVM, J Sejvar, MD, Div of High-Consequence Pathogens and Pathology; R Sriram, PhD, G Visvesvara, PhD, M Beach, PhD, J Yoder, MPH, S Roy, MD, Div of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases; Y Quarnstrom, PhD, R Bandea, PhD, A daSilva, PhD, E Bosserman, MPH, Div of Parasitic Diseases and Malaria, Center for Global Health; P Budge, MD, E Lutterloh, MD, EIS officers, CDC.

Editorial Note

This report is the first to describe transmission of *Balamuthia* through organ transplantation. However, a second cluster of patients with transplant-transmitted *Balamuthia* was confirmed at CDC on August 27, 2010 (*2*). *Balamuthia* infection is extremely rare, with fewer than 200 human cases recognized worldwide since *Balamuthia* was found to be a human pathogen in 1990 (*3,4*). The true magnitude of disease caused by *Balamuthia* is unknown because *Balamuthia* GAE often is misdiagnosed as other neurologic diseases (*1,3*). Once infection progresses to encephalitis, it is almost always fatal. Infection occurs in both immunocompromised and otherwise healthy persons, and often in children, although cases have occurred in patients across the age spectrum (*5*). Because of the rarity of *Balamuthia* GAE, risk factors are poorly defined, but might include exposure to soil or stagnant water, young age, and Hispanic ethnicity (*3*).

Balamuthia has been isolated from soil and dust and is thought to be present worldwide (*6*). Routes of infection might include exposure of mucous membranes or nonintact skin to cysts or trophozoites in soil. *Balamuthia* has not been isolated from water, but water also might serve as a vehicle for infection (*1*). Cutaneous lesions have preceded *Balamuthia* GAE in some cases, primarily those reported in South America (*7*). These lesions often are on the central face, suggesting nasal exposure; but they also have been reported on the extremities. Extension to the brain might occur through hematogenous spread or by direct extension through the nasal cavity or sinuses (*1*). Why some patients develop cutaneous lesions before onset of neurologic disease and others do not is unknown. In a series of 10 *Balamuthia* cases in California, common signs and symptoms of *Balamuthia* GAE were headache, altered mental status, and cranial nerve abnormalities (*3*). Although the incubation period for *Balamuthia* GAE has been postulated as ranging from weeks to 2 years, the two kidney recipients in this report had onset of symptoms only 20 days after transplantation.

Successful treatment of *Balamuthia* GAE has been reported in some, but not all, patients administered a combination of flucytosine, pentamidine, sulfadiazine, fluconazole or amphotericin B, azithromycin or clarithromycin, and miltefosine (*3,8*). However, optimal therapy has not been determined. Optimal preemptive therapy for asymptomatic recipients after transplant of an infected organ also is unknown. Miltefosine is active against *Balamuthia* in vitro and was recently used with success in combination therapy for *Balamuthia* GAE in Peru (*9*). Miltefosine is not marketed in the United States but can be available through single patient IND.*

Balamuthia is one of several agents of severe or fatal encephalitis (e.g., West Nile virus, lymphocytic choriomeningitis virus, and rabies virus) that have been transmitted through organ transplantation in recent years (*10*). Organ donors are screened to identify infectious risks in accordance with policies set by the Organ Procurement and Transplantation Network,† which is overseen by the United Network for Organ Sharing through a contract with the Health Resources and Services Administration. However, the number of pathogens screened is limited and creating standards that eliminate all risk for infectious disease transmission is not feasible. Therefore, physicians and organ procurement organizations should be aware of the possibility of transmitting *Balamuthia* and other potentially fatal infections from donors with encephalitis of uncertain etiology, even after testing for usual agents of encephalitis has shown negative results (*10*). *Balamuthia* infection should be considered in patients who might have an infectious encephalitis,

FIGURE. Organ donor brain tissue revealing amebae suggestive of *Balamuthia* (indicated by arrows) (A), and immunohistochemical staining showing antigens (red) of free-living amebae (B)*

Photomicrographs/CDC
* Original magnifications: 158x (A), 100x (B).

* For information regarding a single patient IND for miltefosine, contact the Food and Drug Administration's Division of Special Pathogen and Transplant Products at 301-796-1600 (1-888-INFO-FDA after hours).
† Additional information available at http://optn.transplant.hrsa.gov/policiesandbylaws/policies.asp.

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医薬部外品　研究報告　調査報告書
化粧品

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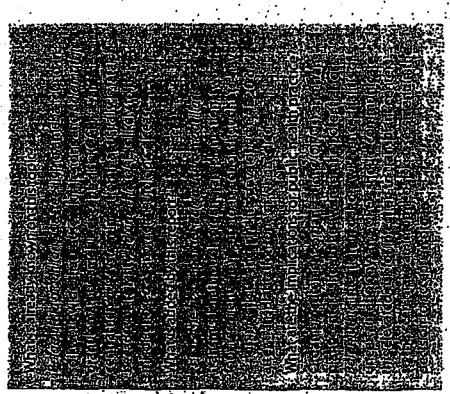
販売名 (企業名)	①テタノブリン IH 静注 250 単位　(ベネシス) ②テタノブリン IH 静注 1500 単位　(ベネシス) ③テタノブリン-IH　(ベネシス) ④テタノブリン筋注用 250 単位　(ベネシス) ⑤テタノブリン　(ベネシス)					

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
英国で集められた扁桃腺標本のリンパ網内プリオン蛋白の大規模な免疫組織化学試験についての報告である。 BSE の結果として、2010/7/5 現在までに英国では 173 の vCJD 症例がある。無症候性感染の数および最終的な患者数は不確かである。この問題を解決する計画で、63,007 の扁桃腺組織標本で過去に PrPres 関連疾患の存在について EIA 法により調査され、陰性であった。この結果の信頼性を確認するために、最もリスクのあるコホート（1961-1985 年生まれ）について免疫組織化学的に試験された。 扁桃腺 9160 検体の中の 1 検体が陽性であり、英国人口 100 万人あたり 109 人のプリオン蛋白関連疾患の保有率であった。	代表としてテタノブリン IH 静注 250 単位の記載を示す。 2. 重要な基本的注意 (1)略 1)略 2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

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

20

抗破傷風人免疫グロブリン



with encephalitis of uncertain etiology and OPOs should communicate this elevated risk for infection to transplant centers so they can make an informed risk assessment in the decision to accept an organ.§

§Additional information available at http://www.cdc.gov/balamuthia.

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particularly those with elevated CSF protein, CSF pleocytosis (white blood cells ≥5/mm³), and enhancing lesions on MRI (3).

Clinicians should be aware of *Balamuthia* as a cause of skin lesions and encephalitis and should report all suspected cases of transplant-transmitted infection to public health departments and organ procurement organizations to enable prompt evaluation and treatment of all recipients from an infected donor. OPOs and transplant centers should be aware of the potential for *Balamuthia* infection in donors

1/41

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Large-scale immunohistochemical examination for lymphoreticular prion protein in tonsil specimens collected in Britain

Mar Fernandez de Marco,[1] Jacqueline Linehan,[2] O Noel Gill,[3] Jonathan P Clewley[3]* and Sebastian Brandner[1]*

[1] Division of Neuropathology and Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK
[2] MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK
[3] Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ, UK

*Correspondence to: Jonathan P Clewley, (Regarding the tonsil archive) Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ, UK e-mail: jonathan.clewley@hpa.org.uk
*Correspondence to: Sebastian Brandner, (Regarding the IHC) Division of Neuropathology and Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK e-mail: sebastian.brandner@prion.ucl.ac.uk

Abstract

There have been 173 cases of variant Creutzfeldt–Jakob disease (vCJD) in the UK, as of 5 July 2010, as a result of the bovine spongiform encephalopathy epidemic. The number of individuals subclinically infected with vCJD, and thus the eventual number of cases, remains, however, uncertain. In an attempt to address this problem, 63 007 tonsil tissue specimens were previously tested by enzyme immunoassay (EIA) for the presence of disease-related prion protein (PrP^{res}) and found to be negative. To confirm the reliability of this result, all those in the birth cohort most at risk (1961–1985) and a few others, including controls, have now been tested by immunohistochemistry (IHC). Histological slides were prepared from 10 075 anonymized formalin-fixed, paraffin-embedded tissues and examined for PrP^{res} with two anti-prion protein antibodies, ICMS35 and KG9. One specimen showed a single strongly positive follicle with both antibodies, on two slides from adjacent sections. As this specimen was negative when it was further investigated by EIA, IHC, and immunoblotting, it is unclear whether the patient from whom the tonsil came will go on to develop vCJD. If, however, this is the case, then a finding of 1 out of 9160 gives a prevalence of disease-related prion protein in the British population of 109 per million, with a 95% confidence interval (CI) of 3–608 per million, which is not statistically different (exact $p = 0.63$) from population prevalence estimates based on finding three positives out of 10 278 in a previous IHC study of appendix tissue. If this is not the case, a finding of 0 out of 9160 gives a prevalence of 0–403 per million (95% CI) for the 1961–1985 cohort, which is also not different (exact $p = 0.25$) from previous population prevalence estimates. Therefore, the results of this work could be summarized as finding, by IHC, no or one vCJD-positive individual.

Keywords: variant Creutzfeldt–Jakob disease; bovine spongiform encephalopathy; vCJD prevalence; PrP

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No conflicts of interest were declared.

Introduction

Variant Creutzfeldt–Jakob disease (vCJD) is understood to have arisen from bovine spongiform encephalopathy (BSE) [1–3]. There was widespread population exposure in the UK and some other countries to BSE and as of 5 July 2010, at least 220 people have developed clinical vCJD worldwide (173 in the UK) [4]. The number of currently subclinically infected individuals, and thus the eventual number of cases, remains uncertain [4–6]. This represents an ongoing public health concern with the risk of iatrogenic transmission through blood and surgical instruments [7,8], since prions resist most conventional decontamination procedures [9]. Four instances of vCJD infection resulting from blood transfusion have been reported, establishing the existence of an infective asymptomatic stage [10–14]. There has been a report of autopsy finding of abnormal prion protein (PrP^{res}) in the spleen of a person with haemophilia [15]. Iatrogenic transmission of sporadic CJD has also been reported to occur through neurosurgical instruments, and experimental studies have shown stainless steel-bound prions to transmit disease with remarkable efficiency when implanted into mice [16–18]. These factors, together with the unknown maximum length of the asymptomatic incubation period and the influence of the host's genotype [19–21], all contribute to the uncertainty about the underlying prevalence of vCJD.

Preclinical colonization of the lymphoreticular system in vCJD is lent support by the detection of PrP^{res} in an appendix removed 8 months before onset of overt neurological symptoms in a patient whose diagnosis was confirmed at autopsy [22,23]. The finding of PrP^{res} in the spleen removed at autopsy from a person with haemophilia is consistent with lymphatic spread of disease [15], as is the report of PrP^{res}, but not disease, in spleen and lymph tissue at post-mortem from a recipient of red blood cells donated by a vCJD case approximately 18 months before onset of clinical symptoms [12]. Moreover, tonsil biopsies are successfully used for the diagnosis of vCJD, showing 100% sensitivity and specificity [24,25]. These collective data indicate that large-scale screening of surgical tonsillectomy tissues for PrP^{CJD} could provide early warning of a high level of subclinical prevalence of vCJD prion in the general population [6,22,24,26,27].

Three previous studies analysed appendix and tonsil specimens for the presence of PrP^{res} [6,26,27]. In the first study, 11 247 archived fixed appendix specimens, and 1427 tonsil specimens, were screened by immunohistochemistry (IHC), revealing PrP^{res} deposition in three appendix specimens, all from the 1961–1985 birth cohort [26]. The prevalence of detectable PrP^{res} in Britain was therefore calculated to be 292 (95% confidence interval 60–853) per million [26]. In a second study, 2000 tonsils were screened by both immunoblotting (IB) and IHC, showing no positive cases [27]. A third study examined 63 007 tonsil specimens from a national anonymous tissue archive and screened for the presence of the PrP^{res} by the use of two enzyme immunoassays (EIAs) based on different analytical principles [6]. No samples contained detectable levels, allowing a prevalence estimate of 0 per million (upper 95% confidence limit of 113 per million). These results suggest that the prevalence of subclinical vCJD infection in Britain may be lower than, but still consistent with, that given by the survey of appendix tissue [26], with an upper limit in tonsil tissue of 289 per million in the 1961–1985 birth cohort [6]. These two surveys may not, however, be directly comparable, particularly because the study of Hilton *et al* [26] screened all of the samples by IHC, whereas the study of Clewley *et al* [6] used EIA as the screening method and only employed IHC as a confirmatory method on a limited subset of the 63 007 tonsils.

The aim of the present study was to investigate further, by IHC, the prevalence of subclinical vCJD in Britain in anonymized samples derived from patients in the 1961–1995 birth cohort of the 63 007 tonsils collected by Clewley *et al* [6].

Materials and methods

Tonsil archive

The tonsils used in this study came from an opportunistic sample of 63 007 tonsils removed for clinical reasons at 131 hospitals across England and Scotland representing 11 Strategic Health Authorities (South West, South Central, South East Coast, East of England, London, East Midlands, West Midlands, Yorkshire and the Humber, North East, North West, and Scotland) [6]. All available tonsils from patients in the 1961–1985 birth cohort were selected for testing by IHC, as well as others chosen for control and technical reasons. The study received ethical approval from the Trent Multi-Centre Research Ethics Committee (MREC/03/4/073) [6].

Sectioning and conventional staining

Sections were prepared from tissue blocks at a nominal thickness of 5 µm at two levels using a standard rotary microtome (LEICA RM 2135, LEICA, Milton Keynes, UK). One section per block was stained with H&E for morphological assessment using standard staining procedures, on a LEICA Autostainer ST5020.

Immunohistochemical staining

For immunohistochemical analysis, sections of formalin-fixed tonsils were dewaxed and rehydrated, immersed into 98% formic acid for 5 min, and then washed in PBS. Thereafter, the slides were loaded onto a BondMax automated immunostaining instrument (LEICA). All antigen retrieval, staining, washing, and haematoxylin counterstaining steps were carried out on this instrument. The antigen retrieval was performed using Bond™ Epitope Retrieval solution 1 and Bond™ Enzyme (LEICA Microsystems, Milton Keynes, UK). Endogenous peroxidase was neutralized and the sections were incubated with either anti-PrP monoclonal antibody ICSM35 (D-Gen, London, UK; dilution 1:1500) or anti-PrP monoclonal antibody KG9 (Institute for Animal Health, TSE Resource Centre, UK; dilution 1:3000). ICSM35 recognizes the region encompassing residues 93 and 102 of human PrP, and KG9 recognizes residues 140–180 of human PrP. The sections were then incubated with the secondary antibody for signal amplification and detection (Vision Biosystems Bond Polymer Detection System, visualized with diaminobenzidine and Bond DAB Enhancer). After counterstaining with haematoxylin, the sections were dehydrated in ascending concentrations of alcohols and xylene and coverslipped with a LEICA ST5020 automated coverslipper.

Autopsy brain tissues from confirmed cases of CJD were used as a positive control for each machine cycle, while omission of the primary antibody on a CJD brain section served as a negative control. Blinded positive control sheep scrapie tonsil tissue cases were randomly included among the human tonsil tissue blocks as an internal quality control, to test the overall sensitivity of the IHC screening.

Each section was labelled with the unique identifier number, the date of the run, and a unique identifier that can be linked to all data related to the machine cycle, reagent and batch number, incubation times, and other parameters.

Microscopic examination

A first quality control was performed by evaluating the quality of the staining in the controls and in the tonsil specimens. We examined and scored entire sections of every anonymous tonsil specimen, at one or more levels, after inspection of a minimum of 15 lymphoid follicles or a minimum of 20 mm^2 tonsil area.

Additional testing

EIA screening, immunoblotting (IB), and codon 129 genotyping were carried out as previously described [6]. For further investigatory IHC, the existing and new wax blocks were sent to two independent laboratories (CJD Surveillance Unit, Edinburgh and Derriford Hospital, Plymouth, UK), where they were tested as previously described [6,26]. Confirmatory enhanced chemiluminescent IB was carried out by the MRC Prion Unit [25,27] and the National CJD Surveillance Unit [11,28].

Statistical methods

95% confidence intervals for prevalence estimates were calculated using the exact binomial method, and comparisons of prevalence between surveys were made using Fisher's exact test.

Results

We examined a total of 24 360 slides by IHC, of which 17% were repeated because of failure of IHC or insufficient control staining (11%), enzymic overdigestion, irregular distribution of DAB, poor sectioning, etc (5%). One per cent of the slides were repeated for further investigation, due to the presence of suspicious staining. From 10 075 tonsil specimens, 4% were rejected due to the absence or too small an amount of lymphoid tissue, or because of poor tissue quality. Of the 9675 tonsil specimens accepted for the study, 94% had more than 30 lymphatic follicles, 5.5% contained 15–30 follicles, and only 0.5% had a minimum diagnosable area. We screened about 20% of anonymous tonsil specimens more than once. We found three samples with positive labelling requiring further examination to confirm or exclude specific labelling for PrP^{res}.

The first specimen, 38 660 (Figures 1A–1D, and Table 1), when stained with ICSM35 (Figure 1A) and KG9 (Figure 1B), showed intense but diffuse staining in an identical follicle. However, while immunoreactivity was not seen elsewhere in the section stained with KG9, we could detect non-specific staining when the specimen was stained with ICSM35. Therefore, new sections were stained with ICSM35 or KG9 (Figures 1C and 1D), showing no positive staining in the follicle that was positive before. These slides were independently examined, with the conclusion that although the pattern of follicular dendritic cell (FDC) staining in this case does not have the coarse granularity seen in positive tonsils from symptomatic cases, it should be classed as positive on the basis of the one strongly positive follicle with both KG9 and ICSM35 antibodies (J Ironside and D Hilton, personal communication).

A second specimen, 18 824 (Figures 1E–1H, and Table 1), showed strong but diffuse, rather than specific FDC staining. This tonsil showed three adjacent follicles positive in one margin of the specimen when stained with ICSM35 (Figure 1E), together with non-specific staining elsewhere in the tonsil. The same follicles were positive in the KG9 staining in an adjacent section (Figure 1F). Because the staining was suspicious but not typical, the staining was repeated on an adjacent section on a different instrument using antibody ICSM35 and established protocols (dilution 1:3000). Still there was speckled positive labelling of unclear significance in the three follicles that were positive before and no staining elsewhere (Figure 1G). On the basis of this result, new sections were prepared and stained, showing perinuclear positivity in the same (immediately adjacent) follicles (Figure 1H). These slides were independently examined, with the conclusion that the staining seen was 'background' (J Ironside and D Hilton, personal communication).

A third specimen, 40 751 (Figures 1I–1N, and Table 1), was stained with ICSM35 (Figure 1I) and KG9 (Figure 1L). Although no immunoreactivity was found in both slides, ICSM35 staining was repeated because of poor staining quality. Therefore, a new section was stained using ICSM35. Immunoreactivity was then detected in one lymphoid follicle, showing a fine granular pattern suggesting FDC positivity (Figures 1J and 1M). We therefore repeated the staining again on new sections, again with ICSM35 (Figure 1K) and KG9 (Figure 1N), respectively. This (second) repeat showed no immunoreactivity. These slides were independently examined, with the conclusion that the staining seen was 'probable background' (J Ironside and D Hilton, personal communication).

These three samples were further investigated by EIA, IB, IHC, and codon 129 genotyping (Table 2). They had all given negative results in the initial EIA screening, and this was confirmed on repeat testing after the IHC findings were reported. IB by both the Prionics (G Mallinson, personal communication) and the Bio-Rad methods was negative for all three samples. In addition, multiple tissue homogenates were referred as blinded samples for enhanced chemiluminescent IB [11,25,27,28] and were reported as negative (J Wadsworth and M Head, personal communication). Additional tissue blocks in wax were made from each of these three samples and they were independently examined and reported as negative (J Ironside and D Hilton, personal communication).

Tonsils 38 660 and 40 751 were both MV heterozygotes, whereas tonsil 18 824 originated from a patient homozygous for valine at codon 129 of the *PRNP* gene.

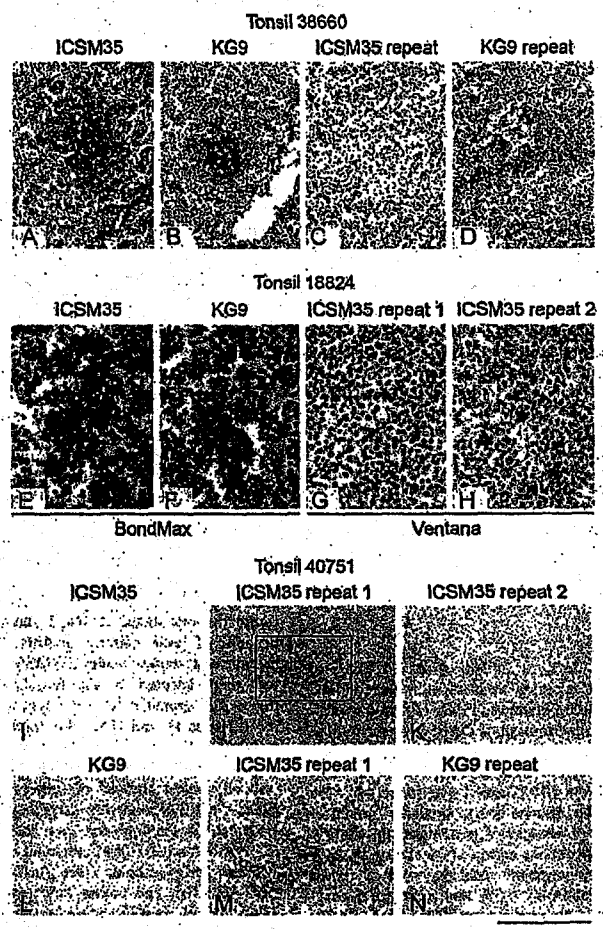

Figure 1. Tonsil specimens with positive immunolabelling. (A–D) Immunoreactivity in tonsil specimen 38660 stained with ICSM35 (A), KG9 (B), ICSM35—new sections (C), and KG9—new sections (D). (E–H) Non-specific immunoreactivity in tonsil specimen 18824 stained with anti-PrP antibody ICSM35 (E), anti-PrP antibody KG9 (F), ICSM35—first repeat (G), and ICSM35—second repeat (H). (I–N) Immunoreactivity in tonsil specimen 40751 stained with ICSM35 (I), KG9 (L), ICSM35—first repeat (J, M), ICSM35—second repeat (K), and KG9—repeat (N). Scale bar: 320 µm (all images).

All three samples were from patients in the 1981–1985 birth cohort.

Three internal quality control sheep scrapie tonsil tissues were successfully detected, although only when stained with anti-PrP monoclonal antibody ICSM35. None of these tonsils showed immunoreactivity when stained with anti-PrP antibody KG9 (Figures 2G–2I and Table 1). The sheep tonsils showed a different morphology to that of human tonsils. The first positive control sample showed immunoreactivity with ICSM35 in most of the follicles, with a fine granular pattern in a distribution compatible with FDCs, including also some coarse granular aggregates (Figure 2A). The presence of granular staining outside the follicles was also detected. The second positive control sample showed a mixture of fine granular staining in cells with the morphology of FDCs, including coarse granular aggregates, and accumulation within the cytoplasm

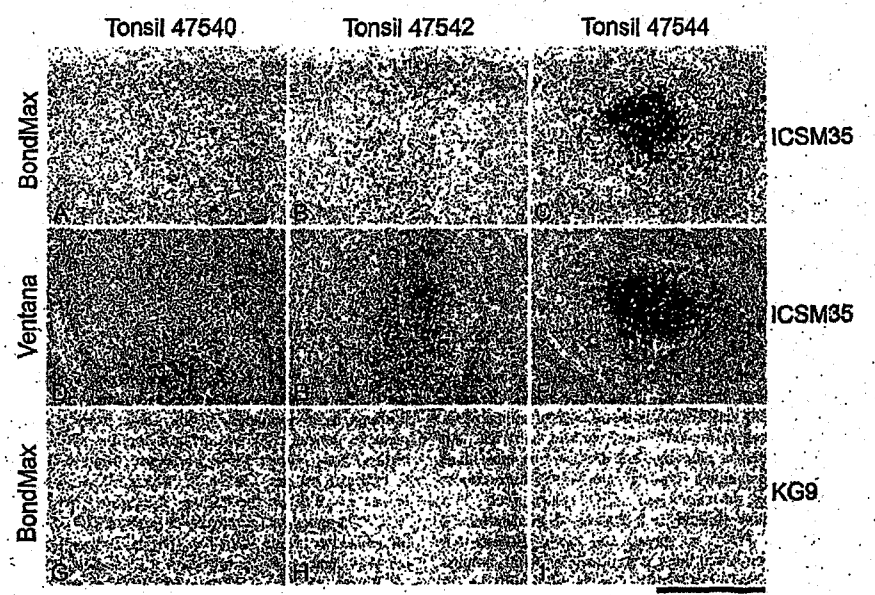

Figure 2. Immunoreactivity in the three blinded positive control sheep scrapie tonsil tissue specimens. Specimen 47540 stained with ICSM35 (A, D) or KG9 (G). Specimen 47542 stained with ICSM35 (B, E) or KG9 (H). Specimen 47544 stained with ICSM35 (C, F) or KG9 (I). Scale bar: 180 μm.

Table 1. Human tonsil tissues reactive by IHC in the 1981–1985 birth cohort

ID no.	Path.	ICSM35	KG9	Description of IHC	Neg*	Neg†	vCJD
38660	1A–1D	Initial reactivity	Initial reactivity	One strongly positive follicle	Neg*	Neg†	MV
40751	1E–1H	Initial reactivity	Initial reactivity	Probable background staining	Neg*	Neg†	MV
18824	1I–1N	Initial reactivity	Initial reactivity	Background staining	Neg*	Neg†	W

*By four independent methods: Bio-Rad, Prionics, and two 'in-house' methods. †By two independent methods: Bio-Rad and Microsens.

of macrophages in most of the lymphoid follicles when stained for ICSM35 (Figure 2B). The third positive control sample showed strong staining in a large area within most of the lymphoid follicles when stained with ICSM35, with a distribution suggesting that it was within FDCs (Figure 2C). These three control specimens were then additionally stained on a different instrument (Ventana Medical Systems) using ICSM35 and established protocols (primary antibody dilution 1 : 3000), confirming the positive result described previously (Figures 2D, 2E, and 2F, respectively).

Discussion

Of the 9675 samples for which an IHC result was obtained, 9160 were in the 1961–1985 birth cohort. The remainder of the samples were selected for IHC because they showed some reactivity in the original serological screening of the 63 007 tonsils by EIA with Bio-Rad and Microsens kits [6]. In addition, there were three positive controls (sheep scrapie) among the 9675 samples submitted for IHC. Three samples (18 864, 38 660, and 40 751) gave IHC results that needed to be investigated more fully. Two of these IHC results were concluded to be background staining by three experts, while for the third it was concluded that there was one strongly positive follicle with both KG9 and ICSM35 antibodies. This could not be confirmed by analysis of slides made from further tissue samples embedded in wax, neither could it be confirmed by IB. This result raises the question of the significance and interpretation of a single positive follicle among the thousands from several sections that were examined, particularly in the light of the failure of IB to confirm the presence of PrP^{CJD} in the tissue. Further investigation of tissue from this specimen by bioassay or protein misfolding cyclic amplification (PMCA) was considered not to be

Table 2. Prevalence of disease-associated prion protein (PrP^{CJD}) in Britain: positive/total and rate per million with 95% confidence intervals

Survey	Tissue	1961–1985	All ages
2004–September 2008 national tonsil survey by IHC Interpretation: one positive patient		1/9160* 109 (3–608)†	1/9672 103 (3–576)
2004–September 2008 national tonsil survey by IHC Interpretation: zero positive patients		0/9160 0 (0–403)	0/9672 0 (0–378)
2004–September 2008 national tonsil survey by EIA		0/12 753 0 (0–289)	0/63 007 0 (0–59)
1995–1999 national tissue survey by IHC	Appendices	1/10 278 292 (60–853)	3/11 247 267 (55–779)
	Tonsils	0/694 NA	0/1427 0 (0–2582)

*Positive/total. †Rate per million with 95% CIs. NA = not applicable, as the 95% CI is calculated only when the denominator exceeds 1000.

worthwhile because bioassay is unlikely to be more sensitive than enhanced chemiluminescent IB tests [11,25,27,28] and PMCA is insufficiently robust [29].

Our finding of one PrP^{res}-positive follicle by IHC can be interpreted as showing that there is one individual in the 9160 samples from the 1961–1985 birth cohort who will go on to develop vCJD. Alternatively, if a single positive follicle is indicative of an insufficient amount of PrP^{res} to spread and cause disease, the interpretation is that there is no one in the 9160 samples from the 1961–1985 birth cohort who will go on to develop vCJD. The decision between these two interpretations needs to be considered in the context of the relative sensitivities of the different tests that were used, and also in the context of the pathological significance of a small quantity of PrP^{res} in a tonsil. Although all three methods (EIA, IB, and IHC) are based on the recognition of PrP^{res} by specific anti-PrP antibodies, they are qualitatively and quantitatively different. As just a few stained cells can be seen by IHC, it could be argued that it is the more sensitive technique. Conversely, however, as a greater volume of tissue and therefore a larger number of cells can be tested by EIA and IB, it can be argued that they are the more sensitive methods [15]. However, the distribution of PrP^{res} in the tissue is likely to be an important factor in assessing the comparative sensitivities of different tests: when there is a very focal deposition of PrP^{res}, IHC may be assumed to have the advantage.

Therefore, while we cannot say whether the patient from whom this tissue came will go on to develop vCJD, we can be reasonably certain, however, that the patient has not yet developed disease as the codon 129 *PRNP* genotype is MV, and all probable and definite vCJD cases to date have been MM at this loci. There have been four 'possible' cases of clinical vCJD, one of which was MV, but this was not biochemically confirmed and it was in a different birth cohort from the person from whom the tonsil in our study came [30]. Also, the two IHC positives (out of three) from the previous study [26] for which a codon 129 genotype could be determined were *PRNP* codon 129VV [31] and no vCJD cases of this genotype have been reported.

The prevalence in the British population of underlying disease-related prion protein calculated from these findings is, if specimen 38 660 came from a vCJD-positive person, 109 per million for the 1961–1985 birth cohort, with a 95% confidence interval (CI) of 3–608 per million (Table 2), which is not different (exact $p = 0.63$) to the finding of three positives from 10 278 samples for the appendix survey [26]. If tonsil 38 660 did not come from a vCJD-positive person, then the prevalence is 0 per million with an upper 95% CI of 403 for the 1961–1985 cohort and 0 per million for the 1961–1995 cohort with an upper 95% CI of 394 (Table 2), which is not different (exact $p = 0.25$) from the previous study.

It is possible that infection arising from exposure to BSE could cause more than one type of prion disease [32–34]. Strains other than that resulting in vCJD, if they exist, may have markedly different pathogenesis, tissue distributions, and structural forms of PrP^{res}. In addition, it is possible that genetic variability in the population may alter the pathogenesis of vCJD, in that the timing and rate of PrP^{res} in appendix and tonsil tissues may differ between individuals. Indeed, genetic differences may even determine the extent of lymphoreticular pathogenesis [31].

Given that the collection of tonsils in our study has occurred later than the collection of appendix samples in the earlier appendix survey, it is conceivable that tonsils have been collected from infected individuals further into the incubation period than is the case for those individuals whose appendices were tested in the earlier survey [26]. Moreover, should the incubation period for prion disease be considerably longer in people with different genotypes, uncertainty about the timing of the appearance of detectable PrP^{res} in these will increase, with concomitant implications for the interpretation of results of PrP^{res} prevalence surveys [6].

Animal experiments have shown that high infectivity, and even disease, can be present in the absence of detectable PrP^{res} [35]. However, this observation cannot be generalized, as PrP^{res} has always been detectable in the lymphoid tissues that have been tested from

vCJD patients [6,25,28]. Data from animal experiments also show 'clearance' of PrP^{res} after inoculation [35,36]. Therefore, the PrP^{res} found in the earlier survey of appendix tissue [26] may conceivably have been transient and eventually cleared without resulting in clinical disease, and therefore the result of the appendix survey result may not be replicable by the current tonsil survey [6].

Although, statistically, the vCJD prevalence estimates in this work do not differ significantly from those obtained by calculating from the previous Hilton study [26], qualitatively they suggest that prevalence estimates may be cautiously lowered. However, in an attempt to provide statistically significant evidence to demonstrate this, a large-scale IHC survey of recently collected appendix tissue specimens for the presence of PrP^{res} is underway.

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Abbreviations

BSE	bovine spongiform encephalopathy
CI	statistical confidence interval
DAB	diaminobenzidine
EIA	enzyme immunoassay
FDC	follicular dendritic cell
H&E	haematoxylin and eosin
HPA	Health Protection Agency
IB	immunoblotting (western blotting)
IHC	immunohistochemistry
PBS	phosphate buffered saline
PMCA	protein misfolding cyclic amplification
PRNP	gene encoding the prion protein
PrP^{res}	disease-related prion protein, specifically the proteinase-K resistant core (it is also referred to in the literature as PrP^{Sc} and PrP^{CJD})
vCJD	variant Creutzfeldt–Jakob disease

Author contribution statement

MM, JL, and SB performed the IHC. JPC was responsible for the tonsil archive. ONG originally initiated the project. MM, SB, and JPC wrote the manuscript.

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の感染リスク、vCJD の発生率としてより低い推定値を用い、製剤の使用量が少ない場合には 9,400,000 人年に 1 回のリスクと予測される。現在までに行われたクリアランス研究に用いられた方法、それらの結果自体、および情報のギャップは様々であったので、現時点である特定の製品が他の製品よりもより安全もしくは安全でないと断言することは不可能である。
本評価モデルでの結果は vCJD 原因物質への暴露の可能性があること、また非常に低いながら潜在的感染のリスクがあることを示唆してはいるものの、本評価モデルでは一般性のある vCJD リスクの正確な推定、または個々の患者への実際上のリスクを正確に提示することは不可能である。実際上のリスクは非常に不確実ではあるが、本リスク評価モデルは、感染リスクに影響を及ぼす最も重要な因子が、製造ステップでの vCJD 原因物質のクリアランス、個々の患者がどの程度の量の製剤を用いるか、および英国のドナー集団における vCJD の発生率であることを示している。
我々の評価モデルでの結果は、pdFVIII 製剤による vCJD 感染の実際上のリスクが非常に小さいことを示唆している。米国で vCJD 症例は出ていないが、そのことが将来の何らかの時点で、いくらかのレシピエントで vCJD への暴露が起こり vCJD がもたらされる可能性を排除するものではない。

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

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医薬品
医薬部外品　研究報告　調査報告書
化粧品

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研究報告の概要

英国の HPA (Health Protection Agency : 健康保護局) が 2009 年の 2 月に公表した報告では、70 歳超の男性血友病患者で臨床症状の出現前の変異型クロイツフェルト-ヤコブ病(vCJD)がほぼ確実な症例について報告されており、また遺伝的にみて vCJD に感染しやすいと考えられるヒトの集団がこれまでに考えられていた範囲よりも広いとの情報から、米国食品医薬品庁(FDA)は米国血漿由来第 VIII 因子(pdFVIII)のレシピエントに vCJD 罹患の危険性があるか再検討することとした。上述の男性血友病患者は死亡時に 70 歳代で、11 年前に英国血漿由来第 VIII 因子製剤での治療を受けており、その製剤は「vCJD に関連している」とされたロットのもの、すなわちドネーション後に確実またはほぼ確実とされた vCJD によって死亡したドナーからの少なくとも 1 回のドネーションを含んだプール血漿から製造された pdFVIII の 1 ロットであった。
FDA は TSEAC の 2009 年 6 月 12 日の会合で、FDA が行った 2006 年 10 月 15 日付のリスク評価「米国内採取血漿から米国承認のもとに製造されたヒト血漿由来第 VIII 因子製剤の使用に伴う vCJD 罹患のリスクの定量的評価　ドラフト」の最新化を提示した。FDA は新たに集積された科学的情報に基づいてこのリスク評価の最新化作業を 2009 年に開始した。この文書は、潜在的 vCJD 罹患リスクの FDA リスク評価文書および評価モデル、ならびに米国内採取血漿から米国承認のもとで製造された血漿由来第 VIII 因子製剤の使用についての 2010 年最新化文書の完全版である。
この文書「米国内採取血漿から米国承認のもとに製造されたヒト血漿由来第 VIII 因子製剤の使用に伴う vCJD 罹患リスクの定量的評価　ドラフト　2010 年最新化版」は、米国血漿から製造されたヒト血漿由来第 VIII 因子(pdFVIII)製剤が用いられた重症の血友病 A(HA)患者および重症のフォン・ヴィレブランド病(vWD)患者における vCJD 原因物質に対する暴露の可能性(確率)とそのレベル、ならびに vCJD に感染するリスクを定量的に推定するものである。
FDA の pdFVIII リスク評価モデルで得られた結果は、米国で製造された pdFVIII からの vCJD 感染のリスクは非常に低いと考えられるが、ゼロではない可能性を示唆している。米国の血漿ドナーについて vCJD 感染リスクの主なソースは、1980 年以降に英国、フランス、またはその他のヨーロッパ諸国に旅行および/または居住していた間の食物を介する暴露である。ドナー排除基準が 1999 年から実施されて vCJD に暴露された人によってドネーションが行われるリスクは低下したが、排除されなかった人もおり、潜在的には vCJD 原因物質を含んでいる血漿がドネーションされる可能性がある。しかし、本評価モデルでは、vCJD に汚染された血漿プールができる可能性は低いことが示唆されている。
ヒト pdFVIII 製剤の製造工程は vCJD 原因物質が存在していたとしても、それを低減させるものと思われるが、製造ステップを経由してどの程度低減されるかは正確には分かっていない。製造における TSE 原因物質のクリアランスは製剤によって異なるものと考えられるが、これまでのところ標準化された研究で測定されたことはなく、それが行われていればより意味のある直接比較ができていたであろう。現在得られている実験研究の結果に基づけば、pdFVIII 製剤では製造工程での vCJD 原因物質の低減が 4 log10(すなわち 10,000 分の 1)となるものと推定されている。製造工程での低減を 4～6 log10 と仮定すると、本評価モデルでは、pdFVIII 製剤を用いて重症血友病 A の治療を受けた患者の 1 年あたりの潜在的リスクは、vCJD の発生率として高い推定値を用い、製剤使用量が多い場合には 15,000 人年に 1 回

使用上の注意記載状況・
その他参考事項等

代表としてテタノブリン IH 静注 250 単位の記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病(vCJD)等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

DRAFT

A 2010 Update of the Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US

October 6, 2010

Center for Biologics Evaluation and Research
US Food and Drug Administration

156

CONTRIBUTORS

Center for Biologics Evaluation and Research
Office of Biostatistics and Epidemiology
Steven Anderson
Hong Yang
Richard Forshee

Office of Blood Research and Review
Jay Epstein
Mark Weinstein
David Asher
Dorothy Scott

157

TABLE OF CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

EXECUTIVE SUMMARY

A February 2009 report from the Health Protection Agency of the United Kingdom (UK) of a probable case of pre-clinical variant Creutzfeldt-Jakob Disease (vCJD) infection in a man over 70 years of age with hemophilia and recent information on broader genomic susceptibility to vCJD of human population prompted the U.S. Food and Drug Administration (FDA) to re-examine the potential vCJD risk for recipients of US-sourced pdFVIII. The man, who was in his 70s at death, had been treated 11 years earlier with UK-sourced plasma-derived Factor VIII (pdFVIII) from a "vCJD-implicated" lot, i.e., a lot of pdFVIII manufactured from pooled plasma containing at least one donation from a person who later died of confirmed or probable vCJD.

FDA presented an update of its risk assessment "Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US "(October 15, 2006) at the June 12, 2009 Meeting of TSEAC. The FDA began updating the risk assessment in 2009 based on new accumulating scientific information. This document is the completed 2010 update of the FDA risk assessment documents and model of potential vCJD risks and the use of human plasma-derived factor VIII manufactured under United States (US) license from plasma collected in the US.

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease attributed to human infection with the agent of bovine spongiform encephalopathy (BSE) and is most often transmitted by the consumption of beef products from infected cattle. Cases of vCJD were first reported in humans in the U.K. in 1996 – and as of June 2010, 221 cases have been reported worldwide, with 174 cases in the U.K. Since December 2003, there have also been four reports in the United Kingdom (U.K.) of probable variant Creutzfeldt-Jakob disease (vCJD) transmission by red blood cell transfusions. The donors were healthy at the time of donation, but later developed vCJD. Of the four red blood cell recipients who probably became infected with the vCJD agent after transfusion, three developed vCJD and died from the disease. The third died of an unrelated illness. U.K. authorities have notified physicians in the U.K. and their patients who received plasma derivatives made from plasma from U.K. donors about the potential for risk of vCJD from these products. These products included coagulation factors VIII, IX, and XI, as well as antithrombin III, and intravenous immune globulins.

This document "A 2010 Update of the Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US" quantitatively estimates the probability and level of exposure to the vCJD agent and the possible risk of vCJD infection in patients with severe hemophilia A (HA) and von Willebrand disease (vWD) patients with severe

disease who have used human plasma-derived Factor VIII (pdFVIII) product manufactured from US plasma. Because BSE occurs at an extremely low level in US cattle (2 native born cows and 1 cow imported from Canada), the risk of plasma donors acquiring vCJD by consuming domestically produced beef is thought to be very low. Because of concerns about potential exposure to the BSE agent in US blood donors who traveled to or lived in the UK and other at risk European countries, FDA implemented donor deferral policies beginning in 1999. The policies are believed likely to reduce the possible risk from blood donors potentially exposed to BSE agent by ~ 90%. However, it is possible that a small number of non-deferred US donors may have been exposed to the BSE agent during extended travel or residence in the UK, France or other European countries and may be at risk for vCJD. Some of these donors may have been unknowingly infected with vCJD through eating beef from BSE-infected cattle and then contributed donations to plasma pools used to manufacture pdFVIII in the US.

The FDA risk assessment utilizes a computer-based simulation model that evaluates successively the impact on vCJD risk of individual processes used in the production of human pdFVIII starting with plasma donation, extending through manufacturing steps, and finally, addressing utilization by various patient subpopulations. Risk for these products was estimated for the baseline year of 2002 but the results and conclusions also are likely to reflect the current vCJD risk for recipients of pdFVIII. A few major elements of the model greatly influence vCJD risk. The most influential of these are manufacturing processes, which may reduce or eliminate the amount of vCJD agent in the final product. The amount of product used by patients in different clinical scenarios also has a significant impact on risk. Additionally, the risk estimate is significantly affected by the prevalence of vCJD in the United Kingdom population, which is used to estimate vCJD prevalence in US donors who resided in or traveled to the UK and other countries of Europe. The risk assessment model estimates the potential for vCJD exposure and the potential risk of vCJD infection for patients receiving pdFVIII from plasma collected in the US and the accompanying uncertainty of these estimates. Because scientific data on the level of exposure to vCJD agent and the likelihood of certain human health outcomes, such as infection and illness, are lacking, the estimates generated may not be accurate. As a result of these and other large uncertainties, it is not possible to provide a precise estimate of the vCJD risk to patients potentially exposed to the agent through plasma-derived products.

Patients with hemophilia A (HA) have an inherited, recessive, sex-linked bleeding disorder that affects approximately 14,000 individuals in the United States (Soucie et al 1998). FDA estimated that there are approximately 1,800 patients in the US with severe disease who use plasma-derived products. The blood of affected individuals contains functionally abnormal or abnormally low concentrations of FVIII. FVIII is a glycoprotein circulating in blood plasma that is part of the blood coagulation pathway and is critical for the normal clotting of blood. In the case of severe disease, FVIII is <1% of normal. Among severely affected persons, spontaneous bleeding or bleeding at the site of an injury or within a joint is common and can lead to severe disability or death without treatment. The complications of HA can be prevented by appropriate clinical management and treatment with pdFVIII or recombinant FVIII products.

Patients with severe vWD (Type 3) have an inherited, non-sex linked bleeding disorder associated with abnormal platelet adhesion caused by deficiency in von Willebrand Factor (vWF) activity. FDA estimated that there are approximately 250 patients in the US with severe vWD who use plasma-derived products. Mucosal bleeding is common in patients with vWD due to the platelet

adhesion disorder. In some cases there may be a deficiency in FVIII coagulant activity (anti-hemophilic factor) as well. Patients with severe vWD can experience persistent bleeding into joints resulting in pain, degeneration of joints, swelling and loss of range of motion similar to patients with HA. Mild forms of vWD are often treated successfully with desmopressin but more severe forms of the disease usually require treatment with coagulation factor concentrates that contain both vWF and FVIII. Patients who need vWF must use plasma-derived sources of FVIII which contain vWF. No recombinant vWF is currently available.

Results from the Model

An important, yet also highly uncertain parameter in driving the risk assessment results is the estimate used for vCJD prevalence in the UK. The prevalence of vCJD in the UK population was estimated in the model using two different approaches. The first approach to estimating vCJD prevalence in the UK was from a study based on epidemiological modeling that was derived using actual reported vCJD cases in the UK combined with an estimate of future vCJD cases (Clarke and Ghani, 2005). Several factors used in epidemiologic modeling approaches are difficult to quantify and add uncertainty to the final estimated number of future vCJD cases. These factors include: the intensity of human exposure to the BSE agent, incubation period, time of infection, and whether illness will develop in individuals who are not homozygous for methionine at codon 129 of PrP. All cases of vCJD to date have occurred in individuals who are homozygous for methionine at this location. Our calculations, based on the Clarke and Ghani study (2005) and diagnosed cases in 2002 and 2003, yielded a prevalence estimate of approximately 4.5 vCJD cases per million in the UK.

Running the model with this vCJD case prevalence estimate (~4.5 per million) produces an estimate suggesting that, on average, there was a 0.03% likelihood that a plasma pool, which then undergoes manufacturing, will contain at least one donation from an individual whose blood contains the vCJD agent. Therefore, on average, more than 99% of the time the model predicts the product as administered will contain no vCJD agent and this is reflected in the (0 – 0) values for the 5th and 95th percentiles shown for the lower prevalence estimate results in Table I.A. (below).

However, it is possible that the prevalence of vCJD in the UK is higher than that estimated above. This could happen if there are people infected who never develop the disease (but can still spread the infection) or if some individuals take extremely long to become ill. Therefore, a second approach to estimating vCJD infection prevalence was used based on a relatively small tissue surveillance study by Hilton, *et al* (2004), which tested stored tonsil and appendix tissues from the UK for accumulation of abnormal prion protein. It yielded a much higher prevalence estimate of 1 in 4,225 (237 infections per million). This study was not controlled using tissues from a non-BSE exposed population and false positive findings cannot be ruled out. It is also not known whether this staining of appendiceal tissues is a reliable marker for vCJD pre-clinical infection or for an individual's capability to transmit the infection through blood donation. However, while unconfirmed, the findings from this study provide a higher prevalence estimate that may be relevant to transfusion risk and therefore should also be considered. Use of these data as the basis for a vCJD infection prevalence estimate which is then used in the model produces a significantly higher estimate suggesting that, on average, if it were correct, there could be a 2.3% likelihood that a plasma pool, which then undergoes manufacturing, may contain at least one donation from an individual whose blood contains the vCJD agent.

Estimated annual potential vCJD risk associated with human pdFVIII used to treat severe Hemophilia A

Results from the model indicate that it is possible that a donor unknowingly infected with vCJD may have donated plasma used in the manufacture of pdFVIII in the US. Output from the model using the LOWER UK vCJD Case Prevalence estimate (~4.5 in 1 million) indicated that, on average, there is a 0.03% (5th - 95th perc: 0 % - 0 %) likelihood that a plasma pool may contain at least one donation from an individual with the vCJD agent in their blood. Readers may notice that the 5th and 95th percentile intervals for all of the model outputs are from 0 to 0, meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. This means that given the range of predicted answers at least ninety five percent of the time the model estimates the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. Again, actual model predictions indicated that, at the lower prevalence, 0.03% of the time the exposure to vCJD may be greater than zero. When the model was run using the higher UK vCJD prevalence estimate (1 in 4,225) to derive an estimate for vCJD prevalence in US plasma donors, the FDA model predicted that, on average, there is an approximately 2.3% (5th - 95th perc: 0 % - 8.2 %) likelihood that a plasma pool will contain at least one donation from an individual with the vCJD agent in their blood. For either set of results, the model assumes that if vCJD agent were present, the amount in a plasma pool would likely be reduced or possibly eliminated by processing steps used during the manufacture of pdFVIII product.

Individuals with HA vary in their degree of FVIII deficiency. For simplicity, the model results and this executive summary specifically address potential vCJD exposure and risk for persons with severe HA. FDA estimates that among the total population of 14,000 HA patients in the United States, approximately 1,800 (Table I.A.) have severe disease and use pdFVIII products. FDA obtained data on FVIII utilization from the Centers for Disease Control and Prevention (CDC). The data were generated as part of a collaborative effort between CDC and six states in a study conducted from 1993 –1998. Treatment regimens for HA are administered either as prophylaxis to prevent the occurrence of bleeding episodes or on an episodic basis to control bleeding when it occurs. Additionally, inhibitors may be treated with very high doses of pdFVIII to induce immune tolerance. Assuming these patients are treated with a pdFVIII product that has a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent, Table I.A. displays model outcomes for patients treated using either prophylaxis or episodic treatment, and with respect to their inhibitor status.

Table I.A. Model Results for all Severe Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 log_{10} Manufacture Process Reduction of vCJD Agent: *Predicted mean potential per person annual vCJD risk using two different UK vCJD prevalence estimates.*

				4 - 6 Log$_{10}$ Reduction Factor (LRF)	
				Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)	*Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)*
Treatment Regimen	Inhibitor Status	Est. Total Number patients in US	Mean quantity FVIII used per person per year [a] [b] (5[th] - 95[th] perc)	Mean potential vCJD risk per person per year [a] [b] (5[th] - 95[th] perc)	Mean potential vCJD risk per person per year [a] [b] (5[th] - 95[th] perc)
Prophylaxis	No Inhibitor	578	157,949 IU[a,d] (21,000 , 382,000)	1 in 4.0 million (0-0)[c]	1 in 63,000 (0- 1 in 13,000)
	With Inhibitor – No Immune Tolerance	63	190,523 IU[a,d] (27,000, 448,000)	1 in 3.4 million (0-0)[c]	1 in 53,000 (0- 1 in 11,000)
	With Inhibitor – With Immune Tolerance	62	558,700 IU[a,d] (33,000, 1,593,000)	1 in 1.1 million (0-0)[c]	1 in 18,000 (0- 1 in 3,700)
Episodic	No Inhibitor	946	85,270 IU[a,d] (46,000, 245,000)	1 in 7.1 million (0-0)[c]	1 in 115,000 (0- 1 in 24,000)
	With Inhibitor	151	160,458 IU[a,d] (5,000, 489,000)	1 in 4.0 million (0-0)[c]	1 in 61,000 (0- 1 in 13,000)

[a] Some numbers on quantity of product used that also appear in the 2006 FDA Risk Assessment have been rounded for simplification in the 2010 Updated FDA Risk Assessment

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[c] Risk estimates generated by the model should fall within the interval defined by the 5[th]- 95[th] perc (percentiles) 90% of the time

[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5[th] and 95[th] percentile interval of 0 and 0, respectively, the model estimates that for at least 95% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 95% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

The risk estimate for the entire severe HA population of 1,800 in the US who use pdFVIII, obtained by summing the total annual exposure and vCJD risk, is shown in Table I.B. Variant CJD risk for US donors with a history of travel to the UK, France or other countries in Europe since 1980 is further adjusted to account for donor age, country, duration and year of travel. Using the lower UK prevalence estimate as a starting point, the model estimates that the total patient population may be exposed to a potential population-based vCJD risk of 1 case observed in 2,600 years of treatment. If the higher vCJD prevalence estimate is used, the model estimates that the total patient population may be exposed to a potential population-based vCJD risk of 1 case observed in 41 years of treatment.

Table I.B. Model Results for Mean Total Population-based Potential vCJD Risk for all Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 log_{10} Manufacture Process Reduction of vCJD Agent: Risk estimates were calculated for patients with severe disease, using two different UK vCJD prevalence estimates.

			4 - 6 Log$_{10}$ Reduction Factor (LRF)	
			Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)	*Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)*
	Est. Total Number severe HA patients in US	Mean Total quantity FVIII used by all patients per year	Mean population –based potential vCJD risk [a] [b] (5[th] - 95[th] perc)	Mean population –based potential vCJD risk [a] [b] (5[th] - 95[th] perc)
Mean Total cumulative annual exposure and population risk	1,800	243 million IU[c]	1 in 2,600 years (0-0)[d]	1 in 41 years (0 - 1 in 8)

[a] Mean population-based potential annual vCJD risk – the risk of potential vCJD infection for the entire population of 1,800 based on animal model dose-response information.

[b] Risk estimates generated by the model should fall within the interval defined by the 5[th]- 95[th] perc (percentiles) 90% of the time.

[c] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[d] For a 5[th] and 95[th] percentile interval of 0 and 0, respectively, the model estimates that for at least 95% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 95% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

Estimated annual potential vCJD Risk Associated with Human pdFVIII used to Treat Severe von Willebrand disease (vWD)

Individuals with vWD have varying severities of disease; those with Type 3 disease have the severest form of the disease. This executive summary specifically addresses potential vCJD exposure and risk for persons with severe vWD (Type 3) who are assumed to use larger amounts of pdFVIII product and thus, may be at higher risk. FDA estimates that approximately 250 vWD patients have severe vWD disease in the United States and use human pdFVIII products to control

their disease (Table II.A.). Results from the risk assessment model for young vWD patients and adult vWD patients treated with pdFVIII product that is assumed to have a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent are shown in Table II.A. Generally results from the model are expressed for patients with vWD for two groups, either Prophylaxis or Episodic treatment. FDA obtained data on FVIII utilization from the Centers for Disease Control and Prevention (CDC). The data were generated as part of a collaborative effort between CDC and six states; the study was conducted from 1993 –1998. Annual usage of product by vWD patients was estimated based on an assumption that this patient class largely uses Humate-P®. Totaling the model results for the LOWER vCJD Case Prevalence estimate of ~4.5 per million reveals that the 250 severe vWD patients in the US (Table II.B.) are predicted to have an average potential vCJD infection risk for the population of 1 infection in 23,000 years. At the HIGHER vCJD Infection Prevalence estimate, the average potential vCJD infection risk for this population is 1 infection in 360 years.

Table II.A. Model Results for von Willebrand Disease (vWD) Patients[a] with Severe Disease: Predicted Potential Annual vCJD Risk:

- Assuming a reduction from manufacturing of 4-6 $\log_{10}$, and
- Two different UK vCJD prevalence estimates.

				4 - 6 Log_{10} Reduction Factor (LRF)	
				Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)	*Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)*
		Est. Total Number patients in US	Mean quantity product used per person per year (5th - 95th perc)	Mean vCJD risk per person per year[b] (5th - 95th perc)	Mean vCJD risk per person per year[b] (5th - 95th perc)
YOUNG vWD (≤ 15 yrs of age)	*Prophylaxis*	39	165,713 IU[d] (9900, 454300)[e]	1 in 3.8 million (0-0)[c]	1 in 59,000 (0 - 1 in 12,000)
	Episodic	60	11,045 IU[d] (1020, 34350)[e]	1 in 56 million (0-0)[c]	1 in 830,000 (0 - 1 in 210,000)
ADULT vWD (> 15 yrs of age)	*Prophylaxis*	73	186,880 IU[d] (17000, 540000)[e]	1 in 3.4 million (0-0)[c]	1 in 63,000 (0 - 1 in 11,000)
	Episodic	78	86,923 IU[d] (2200, 240000)[e]	1 in 7.1 million (0-0)[c]	1 in 110,000 (0 - 1 in 23,000)

[a] Some numbers on quantity of product used that also appear in the 2006 FDA Risk Assessment have been rounded for simplification in the 2010 Updated FDA Risk Assessment

Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or episodic treatment with Humate P only and no record of inhibitor.

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[c] Risk estimates generated by the model should fall within the interval defined by the 5th- 95th perc (percentiles) 90% of the time.

[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 95% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 95% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

Table II.B. Von Willebrand Disease (vWD) patients[a] with Severe Disease: Predicted Total Population-based Potential vCJD Risk:

- *Assuming a reduction from manufacturing of 4-6 $\log_{10}$, and*
- *Two different UK vCJD prevalence estimates.*

			4 - 6 Log_{10} Reduction Factor (LRF)	
			Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)	*Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)*
	Est. Total Number severe vWD patients in US	Mean Total quantity FVIII used by all patients per year	Mean population –based Potential vCJD risk[b] (5th - 95th perc)	Mean population –based Potential vCJD risk[b] (5th - 95th perc)
Mean total annual exposure and population risk	250	27.6 million IU[d,#]	1 in 23,000 years (0 - 0)[c]	1 in 360 years (0 - 1 in 74)

[a] Mean Total quantity FVIII used by all patients was incorrectly reported as 29.9 million IU in the FDA 2006 risk assessment, the value of 27.5 million IU is the correct value

Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of Hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or episodic treatment with Humate P only and no record of inhibitor.

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[c] Risk estimates generated by the model should fall within the interval defined by the 5th- 95th perc (percentiles) 90% of the time.

[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 95% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 95% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

Conclusions

Results from the FDA pdFVIII risk assessment model suggest that the risk of vCJD infection from US manufactured pdFVIII generally appears likely to be very low, but may not be zero. For US plasma donors, the major source of vCJD risk is dietary exposure during travel and/or residency in the UK, France, or other countries in Europe since 1980. Although donor deferral criteria in place since 1999 have reduced the risk of donation by exposed persons, some are not deferred and potentially may donate plasma that contains the vCJD agent. However, the model suggests that the likelihood of a vCJD contaminated plasma pool is low.

Manufacturing processes for human pdFVIII products likely reduce the quantity of vCJD agent, if present, but the level of reduction through manufacturing steps is not precisely known. Clearance of TSE agents in manufacturing appears to vary among products, but has not been measured in standardized studies which might allow more meaningful direct comparisons. Based on currently available experimental studies, it is estimated that pdFVIII products potentially have 4 $\log_{10}$ (or 10,000 fold) or greater manufacturing process reduction of the vCJD agent. Assuming a 4-6 $\log_{10}$ manufacturing process reduction, the model predicts that the potential risk per person per year for patients with severe HA using pdFVIII ranges from1 in 15,000 for the higher vCJD prevalence estimate and high product usage to 1 in 9.4 million for the lower vCJD prevalence estimate and low product usage. Due to the wide range of methods used for currently available clearance studies, the results themselves, and gaps in information, it is not possible at this time to determine with any certainty if a specific product may be less or more safe than another.

Although results of the model suggest exposure to vCJD agent is possible, and there is a potential risk of infection that is likely to be very low, it is not possible for the model to provide a *precise* estimate of the vCJD risk in general, or of the actual risk to individual patients. Although the actual risk is highly uncertain, the risk assessment model indicates that the most important factors affecting risk are the clearance of the vCJD agent though manufacturing steps, how much product individuals used, and the vCJD prevalence in the UK donor population.

Results from our model suggest that the actual risk of vCJD infection from pdFVIII is likely to be very small. The absence of cases in the US does not rule out the possibility of exposure that could potentially result in illness in some recipients at some future point in time.

RISK ASSESSMENT

I. INTRODUCTION

In February 2009 the Health Protection Agency of the United Kingdom (UK) reported a probable case of pre-clinical variant Creutzfeldt-Jakob Disease (vCJD) infection in a man over 70 years of age with hemophilia (http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733818681). Post-mortem examination of the brain found no neuropathological changes suggestive of vCJD, however, examination of the spleen revealed abnormal accumulation of prion protein (PrP^{res}) typical of vCJD. The man, who was in his 70s at death, had been treated 11 years earlier with UK-sourced plasma-derived Factor VIII (pdFVIII) from a "vCJD-implicated" lot, i.e., a lot of pdFVIII manufactured from pooled plasma containing at least one donation from a person who later died of confirmed or probable vCJD.

The recent vCJD infection case of the hemophilia patient and newly emerged information on broader genomic susceptibility to vCJD of human population prompted the U.S. Food and Drug Administration (FDA) to re-examine the potential vCJD risk for recipients of US-sourced pdFVIII. FDA presented a previous version of a risk assessment model at the December 15, 2006 meeting of the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) for vCJD risk associated with patients with a severe form of hemophilia A (HA) or von Willebrand disease (type-3 vWD) who have used pdFVIII product manufactured in US-licensed facilities. The document of the risk assessment "*Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US*" was posted on the FDA website. The FDA began updating the risk assessment in 2009 based on newly accumulated scientific information. The updates of the risk assessment were presented at the June 12, 2009 meeting of TSEAC. This document provides a 2010 update of the FDA risk assessment of potential vCJD risks and the use of human plasma-derived factor VIII manufactured under United States (US) license from plasma collected in the US.

This document quantitatively estimates the probability and level of exposure to the vCJD agent and the possible risk of vCJD infection in patients with severe hemophilia A (HA) and von Willebrand disease (vWD) patients with severe (Type 3) disease who have used human pdFVIII product manufactured in the US. Because BSE occurs at an extremely low level in US cattle (2 native born cows and 1 cow imported from Canada), the risk of plasma donors acquiring vCJD by consuming domestically produced beef is thought to be very low and this aspect was not incorporated into the 2010 update. Because of concerns about potential exposure to the BSE agent in US blood donors who traveled to or lived in the UK and other at risk European countries, FDA implemented donor deferral policies beginning in 1999. The policies are believed likely to reduce the possible risk from blood donors potentially exposed to BSE agent by ~ 90%. However, it is possible that a small number of non-deferred US donors may still have been exposed to the BSE agent during extended travel or residence in the UK, France or other countries of Europe and may be at risk for vCJD. Some of these donors may have been unknowingly infected with vCJD

through eating beef from BSE-infected cattle and then contributed donations to plasma pools used to manufacture pdFVIII in the US.

Scope of the risk assessment

The scope of this FDA risk assessment evaluates the annual potential exposure to the vCJD agent and risk of vCJD infection through human plasma-derived Factor VIII (pdFVIII) product collected in the US. Risk for these products was estimated for the baseline year of 2002, when FDA current guidance for donor deferral for vCJD risk was published, but the results and conclusions also are likely to reflect the current vCJD risk for recipients of pdFVIII. The FDA risk assessment specifically addresses pdFVIII used to treat patients with severe HA and severe vWD.

The FDA risk assessment utilizes a computer-based simulation model that evaluates successively the impact on vCJD risk of individual processes used in the production of human pdFVIII starting with plasma donation, extending through manufacturing steps, and finally, addressing utilization by various patient subpopulations. A few major elements of the model greatly influence vCJD risk. The most influential are manufacturing processes, which may reduce or eliminate the amount of vCJD agent in the final product. The amount of product used by patients in different clinical scenarios also has a significant impact on risk. Additionally, the prevalence of vCJD in the United Kingdom population, which is used to estimate vCJD prevalence in US donors who resided in or traveled to the UK and other countries of Europe, has a significant effect on the risk estimate.

The risk assessment model estimates the potential for vCJD exposure and the potential risk of vCJD infection for patients receiving pdFVIII from plasma collected in the US and the accompanying uncertainty of these estimates. Because scientific data on the level of exposure to vCJD agent and the likelihood of certain human health outcomes, such as infection and illness, are lacking, the estimates generated may not be accurate. As a result of these and other large uncertainties, it is not possible to provide a precise estimate of the vCJD risk to patients potentially exposed to the agent through plasma-derived products.

Background

Variant Creutzfeldt-Jakob Disease and potential risk associated with human plasma-derived product

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease attributed to human infection with the agent of bovine spongiform encephalopathy (BSE) and is most often transmitted by the consumption of beef products from infected cattle. Cases of vCJD were first reported in humans in the UK in 1996 – and as of June 2010, 221 cases have been reported worldwide, with 174 cases in the UK. Since December 2003, there have also been four reports in the United Kingdom (UK) of probable variant Creutzfeldt-Jakob disease (vCJD) transmission by red blood cell transfusions. The donors were healthy at the time of donation, but later developed vCJD. Of the four red blood cell recipients who probably became infected with the vCJD agent after

transfusion, three developed vCJD and died from the disease, one died of an unrelated illness. The probable transmission of vCJD via red blood cell transfusions raised the possibility that plasma derivatives might also pose a risk of vCJD transmission. In 2004, UK authorities notified physicians in the UK and their patients who received plasma derivatives made from plasma from UK donors about the potential for risk of vCJD from these products. These products included coagulation factors VIII, IX, and XI, as well as antithrombin III, and intravenous immune globulins.

Because only 3 cases of BSE (2 that originated in the US, 1 in Canada) have been reported in the US, the US vCJD risk from domestic beef is thought to be very low. However, some US residents (including blood and plasma donors) traveled to the UK, France and other countries in Europe since 1980 and may have been exposed to the BSE agent, and some of these donors may unknowingly be infected with vCJD. The UK had the largest epidemic of BSE among its cattle population and the largest human epidemic of vCJD, which as of June, 2010, reported 174 cases. The UK instituted strong food chain control measures to prevent the entry of high risk cattle tissues into its food supply in 1996; so risk after that time likely decreased considerably. France is considered to rank second in the world for risk for vCJD at this time, albeit at a much lower level than the UK, but higher than many other countries in Europe. As of July 2010 France has reported 25 cases of confirmed or probable vCJD (www.invs.sante.fr/display/?doc=publications/mcj/donnees_mcj.html). Current US blood and plasma donation policies defer donors with a history of travel or residence to: the UK for a period of three months or longer (1980—1996); France, for a period of five years or longer (1980 – present); and other countries in Europe (blood donation only) for 5 years or longer (1980 – present). The CJD geographic donor deferral policy likely removes most of the vCJD risk; however, there may be residual risk in the US donor population for persons who do not meet criteria for donor deferral, or who meet those criteria, but fail to be deferred due to limitations of the donor screening process.

In 1999, prior to the identification of transfusion-transmitted vCJD, FDA recognized a potential though unknown risk of transmitting vCJD by contaminated blood products. Therefore, consistent with advice from TSEAC, FDA recommended precautionary deferrals of blood and plasma donors who had traveled or lived for six months or longer in the UK from the presumed start of the BSE outbreak in the UK in 1980 until the end of 1996, when the UK had fully implemented a full range of measures to protect animal feed and human food from contamination with the infectious agent causing BSE. In January 2002, FDA recommended enhancing the vCJD geographical donor deferral policy by reducing the time that an otherwise suitable blood donor might have spent in the UK from six to three months. FDA also recommended deferring donors who had spent five or more years in France or cumulatively in any European country listed by the USDA as either having had BSE or having a significant risk of BSE. FDA added certain other measures to reduce potential risk, such as deferring any donor with a history of blood transfusion in the UK after 1979 (http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/ucm095138.htm; http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/ucm095143. htm). Taken together, these steps were estimated to have excluded donors representing slightly more than 90% of the potential vCJD risk while deferring about 7% of otherwise suitable donors. Since 2002, TSEAC has several times reviewed FDA vCJD/CJD blood donor deferral policies, most recently advising FDA to recommend deferral of blood donors transfused in France since 1980. FDA has recently issued revised guidance containing such recommendations (FDA 2010).

Because BSE has been detected in so few US cattle (only three reported cases: two in US-born cattle and one in a cow imported from Canada [http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=197033]), and because none of the three cases of vCJD recognized in the US appears likely to have resulted from exposure here (two cases in long-time UK residents and a third in a recent immigrant from Saudi Arabia), the risk that US plasma donors might have acquired vCJD infection from US beef is thought to be extremely low. (Because the likelihood of exposure of US donors to the BSE agent in US beef products was judged to be so much lower than likelihood of exposure in UK, its estimated contribution to overall risk seems negligible and—while not ignored in developing FDA Risk Assessments—was not included in the model summarized here.) However, it is possible that a few US donors might have been exposed to the BSE agent during travel or residence in the UK, France, or certain other countries of Europe; such donors are at an uncertain but increased risk for vCJD. A subset of such vCJD-infected donors might have contributed to plasma pools used to manufacture pdFVIII in the US. The FDA-recommended donor deferral policy probably eliminates most of the risk associated with vCJD-infected individuals; however, there could be residual risk from eligible donors who were nonetheless infected during brief stays in foreign countries (Yamada 2006) or from donors who should have been deferred by the screening process, but, for an unknown reason, were not.

Hemophilia A, von Willebrand disease and Factor VIII

Patients with HA have an inherited, recessive, sex-linked bleeding disorder that affects approximately 14,000 individuals in the United States (Soucie et al 1998). FDA estimated that there are approximately 1,800 patients in the US with severe disease who use plasma-derived products. The blood of affected individuals contains functionally abnormal or abnormally low concentrations of FVIII. FVIII is a protein in blood plasma that is part of the blood coagulation pathway and is critical for the normal clotting of blood. In the case of severe disease, FVIII is less than one percent (1%) of normal. Among severely affected persons, spontaneous bleeding or bleeding at the site of an injury or a joint is common and can lead to severe disability or death without treatment. The complications of HA can be prevented by appropriate clinical management and treatment with pdFVIII or recombinant FVIII products.

Patients with vWD have an inherited, non-sex linked bleeding disorder associated with abnormal platelet adhesion caused by deficiency in von Willebrand Factor (vWF) activity. FDA estimated that there are approximately 250 patients in the US with severe vWD who use plasma-derived products. Mucosal bleeding is common in patients with vWD due to the platelet adhesion disorder. In some cases there may be a deficiency in FVIII as well. Patients with severe vWD can experience persistent bleeding into joints resulting in pain, degeneration of joints, swelling and loss of range of motion similar to patients with HA. Mild forms of vWD are often treated successfully with desmopressin but more severe forms of the disease usually necessitate treatment with coagulation factor concentrates that contain both vWF and FVIII. Patients who need vWF must use plasma-derived sources of FVIII which contain vWF.

FVIII from human plasma is manufactured in a number of different ways. FVIII manufactured from human plasma is purified by fractionation of the protein from large plasma pools containing thousands of donations of plasma. Because thousands of donations are used to assemble the plasma pools used in the manufacturing of pdFVIII, there is a possibility that a donation from a vCJD

infected individual may be present in a large plasma pool used to manufacture pdFVIII. In turn, that may lead to exposure of product recipients to the vCJD agent and a risk of infection. Relatively recent advances in pdFVIII production technology have likely reduced potential exposure to the vCJD agent. However, further evaluation is necessary to more precisely determine the levels of vCJD clearance afforded by the manufacturing processes for each human pdFVIII product.

There are two approaches for the clinical treatment and control of HA and vWD using pdFVIII: (1) episodic treatment and (2) prophylaxis. Episodic treatment involves the administration of FVIII in response to bleeding episodes resulting from trauma or during and after surgery. Prophylaxis treatment for HA requires administration of clotting factor concentrates on a regularly scheduled basis necessary to maintain a minimal level of FVIII (common acceptable trough level is 2-5% of baseline level) to prevent bleeding episodes. In view of the demonstrated benefits of prophylaxis, the Medical and Scientific Advisory Council (MASAC) recommends that prophylaxis starting at an early age be considered as an optimal therapy for individuals with severe HA (MASAC 2001). Prophylaxis treatment requires higher doses of FVIII than episodic treatment (Linden, Kolakoski *et al* 2003; Globe, Curtis *et al* 2004) and thus presents a potentially higher risk of vCJD to the patients than episodic treatment when human pdFVIII is used. Also, some HA patients develop antibodies to FVIII, called inhibitors, that limit the effectiveness of FVIII used in treatment. Inhibitors can develop with the use of either recombinant FVIII or pdFVIII products. In some cases the development of inhibitors is treated with immune tolerance therapy in which large doses of one million or more units of pdFVIII may be administered. Because of the large doses of pdFVIII used, immune tolerance therapy can pose a potential risk for vCJD exposure if vCJD agent were present in the pdFVIII product. As a simplifying assumption in the model we assumed that in a given year a patient received either exclusively prophylaxis treatment or episodic treatment, but not both.

Risk Assessment Framework

This risk assessment generally follows the four step paradigm described by the National Research Council (NRC, 1983) and consists of: (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization. The hazard identification portion of the risk assessment provides an in-depth overview and analysis of all data and information sources to establish a causal relationship between the hazard and adverse effects on humans. The hazard characterization component (also known as dose-response) relates the information in the exposure assessment, which determines the dose, to the probability of adverse consequence(s) such as infection, illness, etc., expected at a given dose at the individual, subpopulation, or population level. Exposure assessment evaluates the routes of exposure to a hazard, the probability that exposure occurs and the amount (dose) of a hazardous agent to which a person or population may be exposed. Risk Characterization integrates the information from the hazard identification, hazard characterization and exposure assessment sections to characterize the probability and consequences of risk for individuals and populations.

II. HAZARD IDENTIFICATION

The hazard identification portion of the risk assessment provides an in-depth overview and analysis of information from laboratory studies, epidemiological studies, the scientific literature, government reports and other credible or peer-reviewed sources of data that establish a causal relationship between the hazard and adverse effects on humans. In this risk assessment, the vCJD agent is the hazard, and potential exposure can occur in individuals who use plasma-derived products that may have been manufactured from plasma that may have contained a donation(s) from a vCJD-infected individual. The probable transmission of vCJD to four recipients of red blood cell products donated by donors later diagnosed with vCJD in the UK had raised concern that vCJD might be transmitted via plasma-derived products. The most recent reported vCJD infection of a hemophilia A patient made the theoretic risk a more probable risk.

Human vCJD was first reported in the United Kingdom in 1996 (Will *et al* 1996). As of June 2010 over 221 cases, 174 of them in the UK, have been reported worldwide. Both vCJD and BSE belong to a class of fatal neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs). There is strong evidence and general agreement that vCJD results from infection of humans, most probably via dietary exposure, with bovine spongiform encephalopathy (BSE) agent present in contaminated beef (Knight 2004). The leading theory is that the transmissible infectious agent is a prion, or proteinaceous infectious agent, that is an altered but pathogenic form of the PrP protein that is normally present in cells. The altered PrP, herein referred to as PrP^{TSE}, consistent with terminology recommended by the World Health Organization, is highly stable and resistant to degradation by high heat and chemical treatments commonly used to denature infectious agents in the manufacture of plasma derivatives. The incubation period for TSEs is long. The mean incubation period of BSE in cattle is approximately 4.5 years. In humans, vCJD acquired through dietary exposure is thought to incubate approximately 15 years or longer, and individuals become symptomatic only in the last few months of the disease, making early detection very difficult. Confirmation of vCJD requires postmortem examination of brain tissue to confirm diagnosis, but prion protein has been detected in tonsil and appendix tissue of asymptomatic individuals as long as two years prior to the onset of symptoms. There are currently no validated tests available to detect the disease in its early stages of infection or to detect the presence of TSE agents in blood.

Transmission of TSEs through transfusion of blood products in animal models

Transmission of different TSE agents through the transfusion of blood or blood products has been demonstrated in animal models on multiple occasions. At least four studies reported transmission via blood transfusion in the same animal species: sheep experimentally infected with BSE (Houston *et al* 2000), sheep naturally infected with scrapie (Hunter *et al* 2002), hamsters with scrapie (Rohwer 2004), and mice with a human TSE (Brown *et al* 1999). Brown, Rohwer, Taylor (Taylor *et al* 2000) and others have attempted to estimate the amounts of intracerebral (i.c.) infectivity present in blood, which generally fell between 2 and 20 i.c. ID_{50}/ml. A recent study of scrapie-infected hamsters concluded that approximately 58% of the infectivity present in whole blood was associated with plasma (Gregori *et al* 2004). The model uses this more conservative estimate in the published literature and assumes that 58% of infectivity is associated with plasma.

Transmission of vCJD in the United Kingdom via blood, blood products and plasma-derived products

Secondary transmission of vCJD has likely occurred on several occasions for transfusion of blood and blood products; and plasma-derived products. As previously mentioned, the UK Health Protection Agency (2009) reported a probable case of pre-clinical vCJD infection in a man over 70 years of age with hemophilia in February 2009. Post-mortem examination of the brain found no neuropathological changes suggestive of vCJD, however, examination of the spleen revealed abnormal accumulation of prion protein (PrP^{TSE}) typical of vCJD. This was the first report of discovery of abnormal vCJD prion protein in a patient with hemophilia. To date, no hemophilia or bleeding disorder patients have been diagnosed with or died from clinical vCJD.

As of June 2010 four cases of transfusion-transmitted vCJD have been identified in the UK. The first case was announced in December 2003; the UK government announced that vCJD had likely been transmitted to a 69 year-old patient via blood transfusion. The patient had received non-leukoreduced red blood cells in 1996 from a donor who died three years later of vCJD. A second case was announced in July 2004 and occurred in a patient who died of a ruptured aortic aneurysm without clinical evidence of vCJD, but postmortem testing detected PrP^{TSE} in spleen tissue and cervical lymph node. In February 2006 a third case of probable transfusion transmitted vCJD was reported in the UK in a 31 year-old male; the patient had received a transfusion eight years earlier from a donor who died of vCJD 20 months after donation. In January 2007, the fourth probable transfusion-transmitted vCJD case had been reported; the patient was diagnosed about nine years after receiving a blood transfusion from the same blood donor who was also associated with one of the previously identified cases. None of the donors were known to have had vCJD at the time of donation.

It is possible that dietary exposure may have been responsible for some or all of the cases that were reported after red blood cell or plasma-derived product transfusions; however, given the circumstances, the probabilities that either a single, or, particularly, five such events are not associated with transfusion are small. The combined probability that the first two transfusion cases, identified in two elderly patients in a small cohort of transfusion recipients—in an age group underrepresented among vCJD cases—both acquired infection from food is remote. As Llewelyn *et al* (2004) pointed out in their publication discussing the first presumed blood cell transfusion-transmission case "the age of the patient was well beyond that of most vCJD cases, and the chance of observing a case of vCJD in a recipient in the absence of transfusion transmitted infection is about 1 in 15,000 to 1 in 30,000."

Potential vCJD risk for travelers with a history of extended travel or residence in the UK, France, and other countries in Europe and reduction of risk via donor deferral

Public health control measures, such as surveillance, culling of sick animals, or banning of specified risk materials, and others have been instituted in many European countries, particularly in those with indigenous cases of confirmed BSE, in order to prevent potentially BSE-infected tissues from entering the human food supply. Since 1996, the UK has instituted some of the most stringent of these control measures, including a program that excludes all animals older than 30

months of age and prevents high risk tissue from slaughtered animals from entering the human food and animal feed supplies. In June 2000, the European Union Commission on Food Safety and Animal Welfare strengthened the European Union's BSE control measures by requiring all member states to remove specified risk materials from animal feed and human food chains. As of October 1, 2000 such bans had already been instituted in most member states.

US travelers to and residents of the UK, France and other countries in Europe during the period of BSE pandemic may have been exposed to the BSE agent through dietary sources and are possibly at increased risk of vCJD. However, the risk can not be determined precisely due to factors such as the great uncertainty about incubation period of the disease, the sensitivities of each country's surveillance for BSE and vCJD, the compliance with and effectiveness of public health measures instituted in each country to prevent BSE contamination of human food, and the trade and export of cattle products from one country that are consumed elsewhere.

In the UK, the current risk of acquiring vCJD from eating beef and beef products appears to be extremely small, perhaps about 1 case per 10 billion servings (CDC, 2005). In the other countries of the world, the current risk, if it exists at all, would not likely be any higher than that in the UK if BSE-related. The implementation of animal and public health control measures has caused the prevalence of BSE to decline. The US blood donor deferral criteria currently in effect focuses on the time (cumulatively 3 months or more) that a person lived in the UK from 1980 through 1996, whereas for the rest of Europe the criteria focuses on the time (cumulatively 5 years or more) that a person lived in these countries from 1980 through the present. This deferral policy likely reduces the risk of vCJD transmission via blood and plasma donations from potential infected donors.

Three cases of vCJD in US residents who were likely infected outside the US

In 2002, the first case of vCJD was reported in the United States in a 22-year-old woman who was living in Florida and is believed to have become infected with vCJD during extended residence in the UK. The patient was born in Great Britain in 1979 and immigrated to the United States in 1992. In early November 2001, the patient was evaluated for depression and memory loss. In late January 2002, the patient was transported to the United Kingdom where her condition worsened. The diagnosis of vCJD was confirmed by western blot and immunohistochemical analysis. The patient died in June 2004, approximately 32 months after illness onset (Belay *et al* 2005).

A second case of vCJD was diagnosed by the UK National Creutzfeldt-Jakob Disease Surveillance Unit in November 2005 in a 30-year old man who resided in Texas during the period 2001-2005 (CDC 2006). The onset of symptoms occurred in early 2005 while the man was in Texas. He returned to the UK and died of the disease in early 2006. A postmortem examination confirmed the diagnosis of vCJD.

The third patient was born and raised in Saudi Arabia and has lived in the United States since late 2005. The patient occasionally stayed in the United States for up to 3 months at a time since 2001 and there was a shorter visit in 1989. The patient's onset of symptoms occurred in Spring 2006. The patient has no history of receipt of blood, a past neurosurgical procedure, or residing in or visiting countries of Europe. Based on the patient's history, this case likely attributed to consumption of BSE-contaminated cattle products in Saudi Arabia.

Surveillance studies to detect CJD and vCJD in patients with hemophilia

Studies in the United States

Because of the large number of blood products used, persons with hemophilia might be expected to be at risk of developing transfusion-related vCJD or classical Creutzfeldt-Jakob disease (CJD). However, a study conducted by the US Centers for Disease Control and Prevention (CDC) (Evatt *et al* 1998) examined the brains of 24 decedents with a history of bleeding disorders and dementia and found no evidence of CJD in any of the cases.

Another study conducted by the CDC and the Hemophilia Treatment Center identified no cases of clinical diagnosis of CJD among over 12,000 HA patients who have been assessed since 1996. (Evatt *et al* 1998)

Studies in the United Kingdom

A study in the UK (reference: Lee *et al* 1998) conducted post mortem histological examination of the brains of 33 hemophilia patients who were treated with coagulant factor concentrates spanning the years from 1962 – 1995 and observed no evidence of vCJD.

In summary, the experimental and epidemiological evidence indicates the risk of transmission of vCJD via blood transfusion or plasma-derived products is no longer theoretical but a real possibility. Transmission of vCJD via transfusion of red blood cell products (Llewelyn *et al* 2004) and a plasma-derived product (http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733818681) have likely occurred.

III. HAZARD CHARACTERIZATION

The hazard characterization component (also known as dose-response) relates the information in the exposure assessment, which determines the dose, to the adverse consequence(s) such as infection, illness, etc., at the individual, subpopulation, or population level. Determining dose-response relationships can be difficult to accomplish because data are frequently limited, especially exposure and outcome data for humans. Other factors such as characteristics of the hazard (e.g. strain, chemical make-up, etc.), route of introduction, genetics of exposed individuals, influence the dose-response relationship but are often difficult to characterize. Often in lieu of human data, animal data are used and appropriately extrapolated as best as is possible to estimate the dose-response relationship for humans.

Another challenge is estimating the probability of infection when the exposure to TSEs is small and/or occurs repeatedly over a period of time. It is unknown whether for TSE diseases there is a minimal amount of the agent (presumably the prion protein PrP^{TSE}) or threshold that is needed to initiate infection in an individual. This phenomenon has been observed with many other pathogens such as viruses or bacteria, for which infection requires exposure to at least one, and often more, units of the infectious agent. Furthermore, it is not known whether the effects of small multiple exposures over a period of time are cumulative and may result in the possibility of infection and

disease equivalent to a single, larger exposure (e.g., via intracerebral injection in laboratory animals). Some risk assessments have made assumptions concerning the exposure and dose for TSE agent that leads to infection. For instance, the Det Norske Veritas (Feb 2003) blood products risk assessment assumes that exposure to infectivity, quantified in ID_{50} units, is cumulative over the period of one year. Based on advice from the TSEAC (2005), and consistent with suggestive data from studies of TSE agents in animal models (Diringer *et al* 1998, Jacquemot, *et al* 2005), FDA also assumes that exposure to vCJD ID_{50} is cumulative over a one year period. The ID_{50} is the common metric used to quantify the infectivity of TSEs. One ID_{50} is defined as the amount of infectious material or tissue that is necessary to initiate infection in 50% of the treated population. The route of exposure to TSE infectious material influences the efficiency of transmission of the disease. Based on advice provided to FDA by the FDA Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) (October 31, 2005) the model assumes that transmission via the intravenous (i.v.) route is between 1 and 10 times less efficient than the transmission via the intracranial (i.c.) route.

In estimating the dose-response relationship for TSEs one could use a strict interpretation of the ID_{50} and assume a linear relationship between exposure and infection. In the pdFVIII model FDA assumed there was a linear relationship between the exposure dose of vCJD agent and the probability of infection. The ID_{50} relationship used in the model was based on infectious TSE units estimated from rodent model studies (Brown 1998, 1999; Rowher 2004). We further assumed there was no threshold or minimum dose necessary to initiate infection, that is, exposure to even low quantities of vCJD agent has a probability of initiating infection in an individual, albeit the probability of infection would likely be low at low levels of exposure. The model further assumes that in such a case exposure to 1 ID_{50} would suggest a 50% probability of infection, exposure to 0.1 ID_{50} would suggest a 5% probability of infection, and so on. However, given the lack of information and high degree of uncertainty on the dose-response relationship because of the limited data available for TSE agents, it is plausible that low level exposures, even on a chronic basis, may not attain a threshold or minimum quantity of agent necessary to initiate infection in humans. Again, FDA makes a conservative assumption that low-level exposure(s) over the period of one year to any quantity of vCJD agent could potentially lead to infection and that there is not a minimum dose necessary to initiate infection.

The FDA assumes persons with *PRNP*-MV and VV genotypes are all equally susceptible to vCJD infection as MM genotype and that they might also progress to develop clinically symtomatic vCJD. The MM, MV and VV genotypes are thought to comprise approximately 40%, 50% and 10% of the population, respectively. The FDA updated the risk assessment for potential vCJD infection for recipients of US pdFVIII and presented the assessment at the June 2009 Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) meeting and incorporated the assumption of susceptibility of all genotypes into this 2010 risk assessment model.

There are considerable uncertainties in determining the correct form for the vCJD-human dose-response model. For instance, the nature of the dose-response line, its slope, or whether it is more accurately described using a dose-response curve is uncertain because animal data are so limited and human data are not available. The FDA risk assessment estimates the potential individual risk of infection and assumes that a linear interpretation of the rodent model accurately reflects the pathology and progression of vCJD infection and disease in humans, but it may not. Furthermore,

exposure to the vCJD agent may not necessarily lead to infection, and vCJD infection may not necessarily produce symptomatic vCJD disease or illness in an individual or population contributing considerable uncertainty to estimating vCJD risks.

IV. EXPOSURE ASSESSMENT

Exposure assessment evaluates the routes of exposure to a hazard, the probability that exposure occurs and the amount (dose) of a hazardous agent to which a person or population may be exposed. This exposure assessment specifically addresses the probability of exposure and, if present, the quantity of vCJD agent that may potentially be present in plasma-derived FVIII products manufactured in the United States. The administration of pdFVIII and, thus, the route of exposure, is intravenous.

Plasma pools consisting of 6,000 or more donations collected from US plasma donors are used as the starting material from which a number of plasma-derived products are purified, including pdFVIII, which is addressed in this assessment. Because of the relatively large number of donations per plasma pool, there is a small probability that even in the United States some of the pools may contain a donation from a donor who may unknowingly be infected with vCJD, but who does not meet criteria for donor deferral, or who meets those criteria but fails to be deferred due to the limitations of the screening process.

Potential vCJD risk for use of US pdFVIII products may be expected to vary, to some degree, from year to year since 1980. In this risk assessment, the potential vCJD risk associated with pdFVIII products was estimated for the baseline year of 2002, but the results and conclusions are likely to reflect the current risk.

This section of the document provides a general description of modeling approaches, rationale, input data, assumptions and results of the model. Additional technical details on the model and calculation are provided in Appendix A in sections under A-IV. The section titles and numbers used in this document are consistent with those used in Appendix and model spreadsheets.

Overview of Model

Module 1 – Estimation of the Prevalence of vCJD in the UK. This module estimated the vCJD prevalence in the UK used in our model as the basis for estimating vCJD prevalence in US plasma donors. The model assumes that the major source of potential vCJD in the US would likely be associated with plasma donors with a history of travel and residency in the UK, France or other countries in Europe since 1980 and may have had dietary exposure to the BSE agent during their stay. Two different data sources were used to estimate UK vCJD prevalence:

- An epidemiological modeling-based estimate for UK vCJD case prevalence: generated based on epidemiological modeling of clinical cases (Clarke and Ghani 2005), and adjusted to include the MV and VV genotypes as subpopulations that are equally susceptible to vCJD infection as MM genotype and that might also progress

to develop clinically overt vCJD. The mean estimate is approximately ~4.5 cases per million persons.

- A tissue surveillance-based estimate for UK vCJD infection prevalence: generated using data from a tissue surveillance study (Hilton *et al* 2004). The mean estimate is 1 case per 4,225. Most of tissue samples examined were from patients at the age group of 20-29 years old.

Prevalence of asymptomatic vCJD in UK in 2002 was estimated using the above data and adjusted for age and susceptibility of population groups.

Module 2 –vCJD Prevalence in US Plasma Donors and Plasma Pools. This module estimates the number of US plasma donors that may potentially be infected with vCJD, the percentage or number of plasma pools containing vCJD agent, and the amount of vCJD infectivity in individual pools. The model assumed that the major source of vCJD in the US would likely be associated with plasma donors who have a history of travel and residency in the UK, France or other countries in Europe since 1980 who may have had dietary exposure to the BSE agent during their stay. This module uses blood donor survey data to determine US plasma donors potentially at risk for vCJD, including those with a history of:

- Dietary exposure to BSE-contaminated beef during long term travel or residence in the UK (1980-1996), France and other countries in Europe (since 1980), or during Military service when posted on or residing near military facilities in Europe; and
- Transfusion with blood collected in Europe, or Euroblood.

vCJD prevalence in US plasma donors was estimated based on vCJD prevalence in the UK, number of US plasma donors who have history of travel or residence in BSE countries and relative exposure risk of those at-risk US plasma donors compared to UK residents. US plasma donors who were potentially at risk for vCJD were characterized by:

- Age
- *PRNP* genotypes
- Country, year and duration of travel or residence

Other factors were also included in the calculation of vCJD prevalence of US plasma donors and plasma pools:

- Effectiveness of donor deferral policies
- The time period during the course of disease when blood of an infected person contain vCJD infectious agent
- Type of plasma pool (source or recovered), number of donors per plasma pool, and
- Age specific rate and frequency of plasma donation,

The amount of infectivity in an infected plasma pool was calculated based on the estimated amount of infectivity in a unit of plasma donated by an infected person, the size of plasma pool, and the likely number of infected donations per plasma pool.

Module 3 - pdFVIII Manufacturing and Processing. This portion of the model calculated the quantity of vCJD agent in pdFVIII products made from an infected plasma pool based on:

- Initial quantity of infectivity (i.v. ID_{50}) present in the infected plasma pool
- Reduction in the quantity of potential vCJD agent during manufacture, and
- Total yield and number of international units (IU) of pdFVIII produced from plasma pool.

Considering the uncertainty in the degree of infectivity clearance that can be achieved during various pdFVIII manufacturing processes, this risk assessment models two levels of potential clearance in infectivity, 4-6 $\log_{10}$ and 7-9 $\log_{10}$.

Module 4 - Utilization of pdFVIII by Hemophilia A (HA) and von Willebrand Disease (vWD) Patients. The potential exposure of an individual HA patient or vWD patient to the vCJD agent through use of pdFVIII was estimated in the model based on:

- The total quantity of pdFVIII used per year, and
- The estimated potential quantity of vCJD agent predicted in the pdFVIII product.

The quantity of pdFVIII utilized by an individual HA patient is dependent on the severity of the disease and the treatment regimen and was estimated using data from a Centers for Disease Control and Prevention and Prevention (CDC) sponsored study in 6 states from 1993-1998. This risk assessment provides outputs that estimate annual exposure for several patient subpopulations with:

Severe HA disease for persons in the following clinical treatment groups:

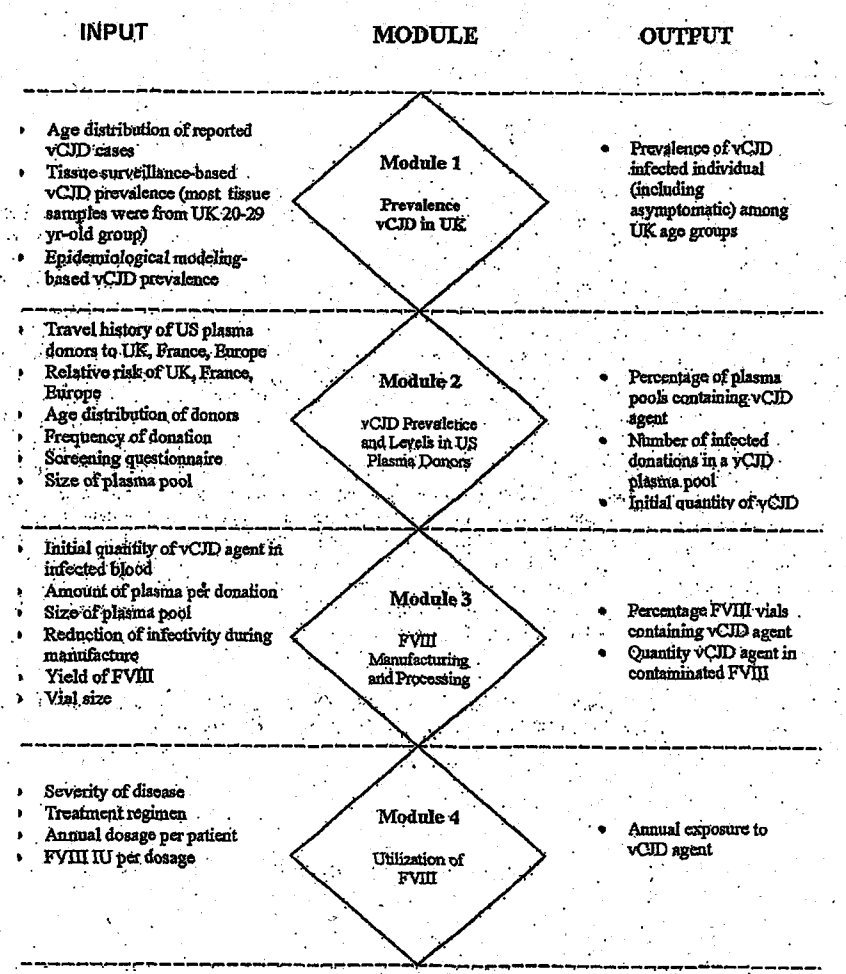
- Prophylaxis
- Prophylaxis plus inhibitor
- Prophylaxis plus inhibitor and immune tolerance
- Episodic
- Episodic plus inhibitor

vWD for adult (≥15 yrs of age) and young (≤15 yrs of age) persons, including those in either clinical treatment group:

- Prophylaxis
- Episodic.

Most of the model inputs are statistical distributions representing the variability and uncertainty associated with the input variables. In general, we used a point estimate if no information was available that could be used to quantify the variability and uncertainty; a uniform distribution, consisting of a minimum and maximum value, when there was only enough information to define a range; a triangular distribution, consisting of a minimum, most likely, and maximum value, when there was enough information to define a range and a most likely value. We used other more sophisticated parametric distributions when there was enough data with which we could fit a statistical distribution. In other cases, we used point estimate for sets of correlated input variables such as donation rates by individual age group and percentage travel by destination. Applying statistical distributions to these variables would greatly complicate the model and likely require several extra days to compute the results and we believe point estimates give a reasonable representation of the input variables. However, we acknowledge that using point estimates may underestimate the uncertainty associated with the input variables.

Figure 1. Model of Exposure Assessment



IV. A. Estimation of vCJD Prevalence in the United Kingdom (Module 1)

This module estimates the vCJD prevalence in the UK population by age and genotype. The UK vCJD prevalence was used as the basis for calculation of vCJD prevalence of US plasma donors in the following module of the model.

The potential prevalence of vCJD in the UK was and continues to be dynamic and changes throughout time as people are exposed to the BSE agent, infected with vCJD, develop the disease and eventually die. Variant CJD exposure and infections in the UK population likely occurred in proportion to the UK BSE epidemic which peaked in 1992. The first human vCJD cases were referred to UK public health authorities in 1994. The number of cases per year in the UK reached a maximum of 28 in the year 2000, and since then has been continuously declining with 3 confirmed or probable cases identified in year 2009. This section of the risk assessment estimated the vCJD prevalence in the UK in 2002, but again, assumes the estimated risk would be valid for current day in the year 2010 .The FDA model assumes that the major source of potential vCJD in the US would likely be associated with plasma donors with a history of travel and residency in the UK, France or other countries in Europe since 1980 and who may have had dietary exposure to the BSE agent during their stay. The potential vCJD prevalence in US plasma donors with a history of travel to BSE countries since 1980 was estimated based on the UK vCJD prevalence. For the prevalence among US donors the UK vCJD prevalence was adjusted based on the time spent in the UK, the year of travel and age of the donor. The number of asymptomatic vCJD cases in the UK is difficult to estimate because of the long incubation period of the disease and a lack of a validated test that can detect them. The prevalence of asymptomatic vCJD infections in the UK in 2002 was estimated in the FDA model using two different approaches based on two different data sources. The prevalence of vCJD in the UK is difficult to estimate because of the long incubation period of the disease and a lack of a validated test that can detect infection in its asymptomatic stages. A check panel is set up on the worksheet, "Model Control", which allows us to switch UK vCJD prevalence estimates from one to another. The discrepancy between the two estimates reflects the limitation on the current knowledge of the disease.

IV. A. 1. UK Asymptomatic vCJD Infections Estimated using Epidemiological Modeling Results (Clarke and Ghani 2005) and Adjusted for All Three Genotypes

IV.A.1. a. Estimation of the Number of Asymptomatic vCJD Infections in the UK in 2002

The first approach used to estimate UK vCJD prevalence in the FDA model relied largely on epidemiological modeling results (Clarke and Ghani 2005) that estimated a mean 70 future vCJD cases (90% CI: 10-190 cases) in the UK for the years 2004 – 2080. Since the FDA model estimates the baseline vCJD infection risk for pdFVIII product used in the year 2002, we assumed the potential risk for US donors should be calculated based on a UK vCJD prevalence that included all current vCJD cases and potentially incubating vCJD infections in the year 2002.

In the 2006 version of the model we added 32 total cases diagnosed in years 2002-2003 and the estimated 70 vCJD cases for years 2004-2008 (Clarke and Ghani 2005) to estimate the number of cases in the UK for the years 2002 – 2080. We assumed that all clinical cases predicted to occur after 2002 were incubating in 2002, thus, representing the number of vCJD infections among the total UK population in 2002. This prediction did not account for potential asymptomatic vCJD infections from PRNP-129 MV and VV genotypes, because all reported clinical vCJD cases had been in persons with the PRNP-129 MM genotype.

As mentioned earlier, recent findings suggest that it is now more reasonable to assume that the entire general UK population is at risk for vCJD infection, and this assumption has been incorporated throughout the FDA 2009 updated draft risk assessment presented at the June 2009 TSEAC meeting. Our 2010 risk assessment also assumes all genotypes to be equally susceptible to vCJD infection, and vCJD infections among PRNP-129 MV and VV genotypes might eventually progress to develop clinically overt vCJD. Therefore, predicted vCJD clinical cases for the whole population of three genotypes from 2002 to 2080 was derived by multiplying predicted number of cases for MM genotype with a factor of 2.5 (times that size of total population compared to the size of MM sub-population). Therefore, the FDA model estimated an average of 255 cases ((32+70) x 2.5=255) of asymptomatic vCJD infections for the year 2002 wth a 5th percentile of 105 cases ((32+10) x 2.5=105) and 95th percentile of 555 cases ((32+190) x 2.5=555). The results of the input information and calculations for the number of vCJD cases in the UK in 2002 are summarized in Table 4.1. Assuming the population of the UK in 1997 is approximately 58 million, the prevalence of vCJD (United Kingdom Office for National Statistics, 1997) would be a mean of approximately 4.5 vCJD infections per million population (255 potential vCJD cases / 58 million).

Table 4.1. FDA Model Estimation of UK vCJD Cases for Years 2002 – 2080.

	Diagnosed vCJD cases in the UK-MM genotype (Health Protection Agency, 2006)			**Estimation of future UK vCJD cases-MM genotype** (Clark and Ghani 2005)	**FDA model: Estimation of UK vCJD cases for years 2002 - 2080-All three genotypes**
Year(s)	**2002**	**2003**	**Total**	**2004 - 2080**	**2002 - 2080**
Number of vCJD cases	16	16	32	70 (10 – 190)	255 (105 – 555) Mean =(32+70)x2.5=255 5th=(32+10)x2.5=105 95th=(32+190)x2.5=555

There are some limitations associated with estimates of future vCJD cases and vCJD incidence in the UK generated by epidemiological modeling based on the current reported vCJD cases. Many of the published models of future vCJD cases or vCJD incidence in the UK, including Clarke and Ghani (2005) and Cooper and Bird (2003), use simplifying assumptions in generating their predictions. Although these simplifying assumptions are a necessary part of vCJD case estimation efforts, they contribute considerable uncertainty to the final case estimates. Generally, the types of assumptions used to estimate vCJD cases fall into four general areas. First, the models must estimate the number of clinical and pre-clinical BSE-infected cattle slaughtered in the UK to

estimate the intensity of human exposure to the BSE agent. Second, they assume a level of effectiveness of the 1989 Specified Ban on Offals, which was assumed to reduce the quantity of infectious BSE agent in the food supply, thereby reducing human exposure in the UK. Third, the models generate an appropriate mathematical representation (or statistical distribution) for the incubation period, which is represented by many using a unimodal statistical distribution. There may be constraints on the incubation period used in the model (e.g., the vCJD incubation period of all individuals in the population would not exceed 40 years, etc.). Fourth, many of the modeling approaches incorporate age-specific dependencies that influence exposure, susceptibility to the disease, and incubation period. Depending on the assumptions used, estimates of future cases of vCJD have varied considerably. Past estimates of vCJD cases from epidemiological models predicted from 250 to 440 future cases under certain assumptions (d'Aignaux et al 2001). As actual reported vCJD cases peaked in 2000 and have since been declining, predicted estimates of future cases have decreased (Boelle et al 2003; Clarke and Ghani 2005, Cooper and Bird, 2003).

There are additional uncertainties in predicting future vCJD cases that might arise from individuals with different genetic backgrounds and susceptibilities in the UK population. However, because no cases of clinical vCJD have been identified in individuals with non-MM genotypes, it is still uncertain whether these individuals will in fact develop or transmit clinical disease. Therefore, any estimation of the incubation period for potential cases with the non-MM genotype would rely heavily on assumptions, which adds considerable uncertainty to any estimate of the size or number of cases in a possible secondary wave of vCJD cases that might occur in non-MM individuals.

Assumptions used in the model:

- All genotypes are equally susceptible to vCJD infection, and vCJD infections among PRNP-129 MV and VV genotypes might eventually progress to develop clinically overt vCJD.
- All vCJD cases that occur after 2002 are incubating in year 2002

IV.A.1. b. Age Distribution of Asymptomatic vCJD for All Three Genotypes

The number of asymptomatic vCJD cases in the UK derived from epidemiological modeling results above is the average number of cases for entire population. In order to extrapolate the prevalence to other age groups, this section of the model calculated the age distribution of UK subpopulation who are infected with vCJD and asymptomatic. The age distribution of individuals who are infected with vCJD and asymptomatic was derived from the data on the number of persons diagnosed with vCJD from different age groups, and the assumptions on incubation period of the disease. The distribution of age at time of initial infection is calculated by left shifting the distribution of age at diagnosis by 15 years, which is the estimated average incubation period for the MM genotype. The model assumed that this distribution of age at time of initial infection is applied to all three genotypes. The distribution of age at time of diagnosis for *PRNP*-129 MV and VV genotypes was generated by right shifting the distribution of age at time of diagnosis for MM by an extra incubation period needed for MV and VV compared to MM genotype. The model used a gamma distribution with mean of 15 years, 5th and 95th percentile of 5 and 35 years to represent the incubation period for persons with the MM genotype. The estimation of incubation periods for people with MV and VV genotypes remains complicated and more uncertain than for persons with MM genotype, because so for there has been no clinical cases or deaths from vCJD reported from

non-MM genotypes. So it is not possible to precisely estimate the duration of the incubation period of vCJD in non-MM persons. Given this considerable uncertainty, we made simplifying assumptions that the incubation period for non-MM is 20-year longer than MM represented by a Gamma distribution with mean of 35 years, 5th percentile of 25 years, and a 95th percentile of 55 years. The high value of 55 years (95th percentile) was estimated based on the maximum incubation period for kuru (Collinge 2006). The values of incubation period for MV and VV genotypes are randomly drawn from this distribution.

For any given age category, the probability that an individual is infected and asymptomatic can be calculated by multiplying the cumulative probability that they are infected (from the distribution of age at time of initial infection) by the cumulative probability that they have not been diagnosed (from the distribution of age at time of diagnosis). These probabilities are then normalized to sum to one to the age distribution of individuals who are infected and asymptomatic.

Assumptions used in the model:

- The distribution of age at initial infection is the same for all genotypes
- Genotypes, MM, MV and VV represent 40%, 50% and 10%, respectively, of the total donor population

IV.A.1.c. Prevalence of Asymptomatic vCJD in the UK by Age and Genotype

Combing the data derived from the previous two sections, this section of the model estimated the prevalence of asymptomatic vCJD in each UK age subpopulations with specific genotype.

IV.A.2. UK vCJD Prevalence derived from a Tissue Surveillance study (Hilton *et al* 2004)

IV.A.2.a. UK Asymptomatic vCJD Prevalence of 20-30 years Age Group

We used a second approach for estimating UK vCJD prevalence drawing on results from a tissue surveillance study that tested lymphoreticular tissue samples (tonsils and appendices) for prion protein accumulation. The study was a retrospective survey of stored tonsil and appendix tissues surgically removed from UK patients in 1995 and subsequent years. The authors identified appendix samples from 3 patients as positive for lymphoreticular accumulation of prion protein out of a total of 12,674 patient samples tested (Hilton *et al* 2004). No tonsil biopsies showed such findings. The significance of the detection of prion protein in the appendix is not certain, and it is not known whether this test is a reliable marker for either vCJD pre-clinical infection or the ultimate development of disease. Nor is it known whether or not such detection is a marker for an individual's potential capability to transmit infection through blood donation. However, while unconfirmed, the findings from this study provide a higher prevalence estimate and therefore should also be considered. Results from the tissue surveillance study are summarized in Table 4.2. Assuming the sensitivity and specificity of the testing method is 100%, this translates roughly to a vCJD prevalence of 237 cases per million (95% CI: 49 – 692 cases per million) for all age groups.

The authors (Hilton et al 2005) indicated that approximately 60% of the samples tested (from 7,600 patients) came from patients 20-29 years of age. The 3 positive samples were also from this age group. After adjustment correcting for sampling bias we calculated a vCJD prevalence of approximately 400 cases per million for which we assumed a 95% CI of 100-1200 cases per million for the 20-29 year old age group (see appendix for detailed calculations).

Table 4.2. Summary of Surveillance Testing of Tonsil and Appendix Tissues in the UK.

Reference	Ages of population examined	Years tissue taken	Number of positives	Total samples examined	Rate per million (95% CI)
Hilton DA, et al. 2004	10 – 60+ yrs (60% of patients were 20-29 yrs)	1995 - 1999	3 Appendices	14,964 Appendices 1,739 Tonsils 4,029 excluded	237/million (49–692 per million)

There are some possible limitations of using the Hilton *et al* tissue surveillance study in estimating vCJD prevalence. In their tissue survey, Hilton *et al* stressed that there were uncertainties and suggested caution in attempting a prevalence estimate for infection or a prediction of future vCJD cases in the UK based on detection by immunohistochemical staining of lymphoreticular accumulation of prion protein in three of 12,674 adequate tissue samples studied. First, the prevalence of infection might have been underestimated because the stage of vCJD infection during which the appendix first accumulates detectable amounts of abnormal prion protein is not known and because the accumulations might not be uniformly distributed throughout the tissue. Second, the study design did not permit an estimate of specificity of the method or an independent confirmation of results because it did not examine a large number of similarly obtained appendices from a non-BSE-epidemic country. Therefore, it is possible that the results might have been false positives leading to an overestimation of prevalence. In their paper the authors stated: "Although immunohistochemical accumulation of PrP in lymphoreticular tissues has not been demonstrated in any disease other than vCJD, the significance of the positive samples in this study is not certain. In one case, the immunohistochemical pattern of immunoreactivity resembled that seen in appendix tissue from pre-clinical and autopsied cases of vCJD, but in the other two cases, a more finely granular pattern of staining was present in relation to follicular dendritic cells, raising the possibility that these may be false positives. However, we have been unable to demonstrate PrP immunoreactivity in a range of other disorders including other human prion diseases, neoplastic disease, or a range of inflammatory conditions."

Module 2 IV.B-IV.D. Potential vCJD Risk for US Plasma Donors and Plasma Pools

This module estimates the annual numbers of US plasma donors who were at vCJD risk, plasma donors who might be infected and contain vCJD infectious agents at the time of donation, and plasma pools used to manufacture pdFVIII that might potentially contain a donation from an

infected plasma donor. This module also estimates the potential quantity of vCJD agent that might be present in a positive.

The largest source of potential vCJD risk in US plasma donors is presumably associated with donors who traveled to or resided for extended periods of time in the UK, France and other countries of Europe since 1980. These donors might be exposed to the BSE agent in contaminated beef products and infected with vCJD during travel and residence abroad. Other populations in the US at potential risk for vCJD include US military deployed for extended periods of time in the UK or other countries of Europe and individuals in the US who received blood collected in Europe ("Euroblood"). The prevalence of BSE in the US cattle population is very low and therefore there is a very low probability that domestic dietary exposure to the BSE agent would give rise to human vCJD cases. Because of this very low prevalence, risk via US domestic dietary exposure was assumed to be negligible in the model.

IV. B. Estimation of vCJD Prevalence in US Plasma Donors and Plasma Pools

IV.B.1. Annual Number of Plasma Donors

This section of the model calculates the annual number of donors who might have contributed plasma used to manufacture plasma-derived FVIII. The calculation was based on the market data for FVIII products, estimated yield of FVIII per unit plasma, donation rate and amount of plasma per donation. The donors were grouped by age and type of donation (source or recovered plasma).

Age is an important factor in estimating potential vCJD risk for US plasma donors. In the US the majority of donors are less than 40 years of age, and since vCJD primarily affects younger persons (median age of 28 yrs for clinical vCJD) The donor population is at particular risk for vCJD. Infected donors, who are asymptomatic at the time of donation, may unknowingly transmit the the infection to recipients (Table 4.3). The FDA model is organized by age groups of 18 and 19 yr olds, 10-14, 15-29, etc. (by five yr age groups to age 69) as "bins". In each of these bins, donors will be further categorized by type of donation (source or recovered), country of travel, duration and year of travel for specific vCJD prevalence (or relative risk).

Two different types of plasma are used in the manufacture of pdFVIII. Source Plasma is collected through plasmapheresis, a process that separates red blood cells from plasma and returns red blood cells to the donor. Recovered plasma is prepared from whole blood units collected from blood donors. Source Plasma accounts for approximately 80% of the total plasma collected annually in the United States, and recovered plasma accounts for the remaining 20%. Source Plasma pools are usually smaller and contain larger volume donations (an average of 700 milliliters) from fewer donors than recovered plasma pools (average volume of a donation is ~200 milliliters). Larger pool size increases the chance that a plasma pool may contain a donation from an infected donor. However, blood deferral policies instituted beginning in 1999 are believed to have reduced the risk of vCJD donations by more than 90%. In addition, because Source Plasma donors are allowed to donate more frequently, and give more plasma per donation, there is a greater chance that if a vCJD infected donor were in the Source Plasma donor pool they might contribute multiple donations to a single plasma pool or donate to multiple pools. Additionally, Source Plasma donors are usually younger than blood (recovered plasma) donors (see percentages of donors for Source

and recovered plasma donors by age group in Table 4.3). Because of their younger age demographic, Source Plasma donors are likely to be more susceptible to vCJD infection. Because of the unique characteristics and potential differences in risk for Source and recovered plasma donations and plasma pools, the FDA risk assessment modeled Source and recovered plasma donors and plasma pools separately, and considered the factors that may result in different risk for pdFVIII product made from each of the two types of plasma. Besides, Source Plasma donors are thought to travel less, so presumably their vCJD risk may be somewhat lower than that of blood donors. However, because travel data for source plasma donors is not available, FDA risk assessment used travel data of blood (recovered plasma) donors for source plasma donors, which may lead to slight overestimate of the risk for source plasma donors.

Table 4.3. Reported vCJD cases in the UK and Percentage of US Source Plasma and Blood (recovered plasma) Donors by Age Group

Age group	<10	10-14	15-17	18-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	>70
Reported vCJD cases in UK (through 2003)[a] (%)	0	5 (3.4%)	27 (18.4%)		32 (21.8%)	30 (20.4%)	22 (14.9%)	13 (8.8%)	5 (3.4%)	3 (2%)	5 (3.4%)	0 (0%)	5 (3.4%)		
Age distribution of US Source Plasma donors (%)[b]	0	0	0	12%	29.3%	14.1%	14.1%	9.6%	9.6%	5.8%	5.8%	0%	0%	0%	0%
Age distribution of US Blood (Recovered plasma) donors[c]	0	0	0	5%	13%	8%	10%	12%	13%	12%	11%	7%	4%	5%	0%

[a] Hilton *et al.* 2004

[b] Plasma Protein Therapeutics Association (Jan 07, 2005). Where data were organized in broader age group we allocated donor equally among smaller 5 year age groups

[c] Data provided to FDA by Westat in 2002

IV.B.2. Annual Number of Plasma Donors Potentially Infected and Whose Blood May Contain Infectivity

The purpose of this section of the model is to estimate the prevalence of vCJD in US donors and the probability that a plasma pool may contain a donation (donations) from a vCJD infected donor with infectious agent in their blood at the time of donation.

The largest source of potential vCJD risk in US plasma donors is presumably associated with donors who traveled to or resided for extended periods of time in the UK, France and other countries of Europe since 1980. These donors might be exposed to the BSE agent in contaminated beef products and infected with vCJD during travel and residence abroad. Other populations in the US at potential risk for vCJD include US military deployed for extended periods of time in the UK or other countries of Europe and individuals in the US who received blood collected in Europe ("Euroblood"). The prevalence of BSE in the US cattle population is very low and therefore there is a very low probability that domestic dietary exposure to the BSE agent would give rise to human

vCJD cases. Because of this very low prevalence, risk via US domestic dietary exposure was assumed to be negligible in the model. The numbers of US plasma donors who might have been infected and contain vCJD infectivity in blood are calculated separately in the worksheets, "IV.B.2.a. Travel-UK", "IV.B.2.b. Travel-FR", "IV.B.2.c. Travel-EU", "IV.B.2.d. Military", "IV.B.2.e. Euroblood" for different exposure sources; then summarized and summed to yield an annual total of US plasma donors who might have infected and contain vCJD infectivity in blood in worksheet "Model-IV-Exposure Assessment".

The percentages of blood donors with a history of travel or residency in the UK, France and other European countries, who are military members who resided in bases in UK and elsewhere in Europe during 1980-1996, and who are recipients of "Euroblood" were obtained from 1980-1996 Blood Donor Travel Survey conducted by American Red Cross (TSEAC 2000). The percentage of at-risk donors was calculated by destination (e.g., the UK, France or other European countries) and duration of travel, further adjusted for year of travel. In the 2009 version of FDA's model, the percentage of at-risk donors is further adjusted for different *PRNP*-129 genotypes. Travel data used in this risk assessment may not accurately represent the travel pattern of source plasma donors, who are likely younger and travel less frequently than the blood donors. However, no travel data for source plasma donors is available.

The model estimated the annual number of plasma donors who might have been exposed to vCJD and infected during travel or residence in BSE countries. For US donors with a history of travel to the UK the vCJD prevalence was derived from the vCJD UK prevalence and adjusted for duration of time that donors spent in the UK, and the years of travels. The magnitude of vCJD risk is assumed to be proportional to the accumulated time spent in the UK and correlated with the magnitude of BSE epidemics at the year of travel. Calculation of the potential vCJD risk for donors who traveled to France was estimated relative to the risk for travel to the UK (or the relative risk) based on the amount of beef that France imported from UK, the number of domestically acquired vCJD cases in France, and other factors. The relative risk of vCJD in France was assumed to be 0.05 times that for the UK. Applying similar criteria for other countries in Europe their relative risk was assumed to be 0.015 times that of the UK. Risk was calculated in the model by multiplying the UK vCJD prevalence by either 0.05 for vCJD prevalence of France or 0.015 for vCJD prevalence of other European countries. The risk for US plasma donors is further adjusted to account for factors such as the duration of time that donor spent in the BSE countries, and the year of travel.

Then, the model derives an estimate of the number of infected donors who may actually have vCJD infectious agent in blood at the time of donation. The model assumes that infectious vCJD agent most likely present in blood during the later 75% of the incubation period (minimum=50%, maximum=90%) and was represented by a triangular distribution with values of (50%, 75%, 90%). This assumption was based on the results from recent findings from studies in animal models (Brown 2007).

The model further incorporates the risk reduction effect of FDA donor deferral policies, implemented beginning in 1999 and last revised in 2002. Current donor deferral policy defers donors who:

- Traveled to or resided in the United Kingdom from 1980 – 1996 for ≥ 3 months
- Traveled to or resided in France - since 1980 for ≥ 5 years

- Traveled to or resided in other countries in Europe – since 1980 for ≥ 5 years (does not include source plasma donors)
- Were US Military personnel or their dependents – deployed in UK or other countries in Europe since 1980
- Were Euroblood recipients in US – that received blood collected from donors in Europe

This geographic deferral policy removes donors with a history of extended travel or residence in the UK and other countries in Europe since 1980. It is believed that these policies are likely to reduce the possible vCJD risk from plasma donors by 85- 90% and this range was represented in the model using a uniform distribution (0.85, 0.99). It is impossible to estimate the efficiency of donor deferral directly based on the number of donors who present for donation at a blood center and are deferred on site, because a substantial, unknown number of potential donors likely self-defer and do not present to donate. The efficiency of donor deferral used in this model was extrapolated from the efficiency for other transfusion transmitted diseases, such as HIV, HBV and HCV etc, based on high risk behaviors such as intravenous drug use and others. This extrapolation is thought to reasonably approximate the expected deferral rate, however it may not accurately reflect the efficiency of donor deferral for vCJD risk and represents a source of uncertainty. For example, social stigma and lower social acceptability of high risk sexual behaviors and intravenous drug use, which are associated with higher rates of HIV, HBV and HCV, might discourage some potential donors from responding truthfully to screening questions on the donor questionnaire. Accordingly, the rate of 'true' response to screening questions for potential donors with these behaviors, and thus, efficiency of donor deferal might be lower than for questions concerning travel history that may trigger geographic deferral for vCJD risk.

There is a small chance that infected donors might still donate for two reasons. First, donor screening based on questionnaire is inefficient and subject to bias. Some donors with a history of an extended period of travel or residence in BSE countries may not be indentified by questionnaire screening because of recall failure, recall errors, or other reasons. Other sources of bias such as missing data, misunderstanding or mis-comprehension of questions or false reporting can introduce further inaccuracies in screening. Second, some donors may have been infected while on a short stay in a BSE country that does not meet the guidance deferral criteria, and thus, would not be deferred from donating plasma.

Assumptions used in the model:

- The relative risk for the UK , France and the other European countries is 1, 0.05 and 0.015, respectively
- The risk of vCJD exposure is cumulative, proportional to the duration of stay or time spent in the BSE countries, for instance a person who lived in the UK for one year has one-fifth the risk of a donor who spent five years
- The risk of vCJD exposure is correlated with the magnitude of the BSE epidemic at the time of travel or stay
- The vCJD agent is assumed to appear in the blood of infected persons most likely after 75% of the incubation period of the disease has elapsed, with a range between 50% to 90% elapse of the incubation period.
- Efficiency of donor questionnaire to identify at-risk donors ranges from 85% to 99%.

IV.B. 2.a. US plasma donors with History of Travel to the UK: Annual Number Potentially Infected and Whose Blood may Contain vCJD Agent

Generally, because of the higher prevalence of BSE in the UK in the late 1980s and early-to-mid-1990s and the higher occurrence of vCJD in the UK human population (currently 174 cases as of June 2010), US donors who traveled to the UK from 1980 through 1996 are likely at higher risk for vCJD infection than donors who traveled to other European countries in the same time period. This model uses the concept of relative risk to estimate the vCJD risk (and prevalence) for a donor population – a value of 1 is used for the UK and this is equal to the vCJD prevalence. Relative risk is used to compare the risk of other regions to that of the UK and is estimated based on factors such as amount of contaminated feed, percentage of meat from the UK, number of cases of BSE, vCJD, etc.

The potential vCJD risk faced by US plasma donors exposed to vCJD during travel or residence in the UK (since 1980) is assumed in the model to be proportional to the time a donor spent in the UK (or France or other countries in Europe), and also a function of the age of the donor, and year of travel. Duration of travel is an indicator of possible exposure and we assumed that the probability of exposure was proportional to the time spent in the UK from 1980 - 1996. The longer the duration of travel, the higher the risk of human exposure to the BSE agent. The magnitude of possible exposure to the BSE agent is also influenced by the specific year of travel. The risk is the highest when travel took place during the peak of BSE epidemic in 1992. The FDA risk assessment grouped plasma donors based on age, duration and year of travel, genotype, and estimated the number of donors, probability of an individual being infected, probability of infected individual containing vCJD infectious agent in the blood and potential number of donors infected and whose blood may contain vCJD agent at the time of donation for each group.

IV. B. 2. b. US Plasma Donors with a History of Travel to France: Annual Number Potentially Infected and Whose Blood may Contain vCJD Agent

Donors who traveled to France are potentially at risk but that risk is likely significantly lower than that for travel to the UK. France likely imported BSE-contaminated feed materials in the 1980s and 1990s and approximately 5% of its beef was supplied by the UK at the time of its BSE epidemic. To date, France has reported 25 cases of vCJD (www.invs.sante.fr/display/?doc=publications/mci/donnees_mci.html) supporting the notion that there may be vCJD infection risk for US donors who may have traveled to or resided in France since 1980. France is assumed to have a relative risk of 0.05, since they received about 5% of their beef and feed supply from the UK and also have fewer domestically-acquired vCJD cases. Therefore, the risk (prevalence) for vCJD for travel or residence in France was assumed to be 0.05 times that for travel to the UK.

FDA guidance (2002) indicates that for donors with a history of travel to France "we now recommend deferral of blood and plasma donors with a history of 5 or more years of cumulative residence or travel in France since 1980."

IV.B. 2. c. US Plasma donors with history of travel to other Countries in Europe: Annual Number Potentially Infected and Whose Blood may Contain vCJD Agent

Donors who traveled to other countries in Europe (other than the UK or France) are potentially at risk of vCJD but that risk is likely significantly lower than that for travel to the UK or France. Other countries in Europe likely imported BSE-contaminated feed materials in the 1980s and 1990s and approximately 1.5% of their beef may have been imported from the UK at the time of its BSE epidemic. The potential for BSE exposure to donors who traveled to or resided in other countries in Europe is possible. Hence, there may be a vCJD infection risk for blood donors who may have traveled to or resided in a European country (other than the UK and France) for periods greater than 5 years since 1980. The risk (prevalence) for vCJD for travel or residence in France was assumed to be 0.015 times that for travel to the UK. The current US vCJD geographic deferral policy defers blood donors with a history of travel or residence in a country in Europe (other than the UK and France) for 5 years or more since 1980. Source Plasma donors who resided in a country in Europe (other than the UK and France) are not deferred from donation. Because Source Plasma donors are not deferred from donation, their risk was not estimated by the model. Therefore, this portion of the model only estimates potential vCJD risk for US recovered plasma donors who traveled to countries in Europe (other than the UK) since 1980.

FDA guidance (2002) indicates that for donors with a history of travel to other countries in Europe (other than the UK and France) "the current recommendation is to exclude from transfusion use, blood and blood components from donors with a history of 5 or more years of residence or travel in Europe outside of the UK". Furthermore, for donors with a history of travel to other countries in Europe (other than the UK and France) the FDA guidance (FDA 2002) states "...we do not recommend that you defer Source plasma donors who have lived or traveled in Europe for 5 or more years".

The FDA risk assessment model reflects FDA guidance for vCJD deferral of Source Plasma and recovered plasma donors. Because the guidance recommendations for each type of plasma were different the model estimated the potential vCJD risk as follows:

- Recovered plasma donors – the FDA risk assessment calculated the potential vCJD risk because deferral was recommended for donors with a history of 5 or more years of residence or travel to countries in Europe (other than the UK)

- Source Plasma donors - the FDA risk assessment did not calculate the potential vCJD risk because deferral was not recommended for donors with a history of 5 or more years of residence or travel to countries in Europe (other than the UK and France).

The term "other countries in Europe" as used in this portion of the risk assessment is defined as all countries in Europe (other than the UK and France).

IV. B. 2. d. US plasma Donors Deployed by the Military in the UK or Other Countries in Europe: Annual Number Potentially Infected and Whose Blood may Contain vCJD Agent

For the purposes of this risk assessment we assumed that military personnel or dependents who have been deployed to US military bases in the UK, France and other European countries during the period from 1980 through 1996, might have been exposed to the BSE agent and infected through consumption of BSE contaminated beef procured for use on US military bases from the UK.

Exposure via UK beef likely varied but the model assumes that up to 35% of beef consumed on military bases in Europe came from the UK. The model assumes that approximately 2% of US blood and plasma donors may have been military, military family or their dependents posted to US military bases in the UK or elsewhere in Europe from 1980 through 1996 (TSEAC, 2002). It was further assumed that the average deployment period was 2 years.

The FDA risk assessment model incorporates information from current guidance for geographic donor deferrals for vCJD (FDA 2002) in estimating potential vCJD risk for donors with a history of travel to countries where BSE has occurred. The FDA guidance (2002) indicates that for donors with a history of service on US military bases in Europe "we recommend that you should indefinitely defer current and former US military personnel, civilian military personnel, and their dependents who were stationed at European bases for 6 months or more during the time periods outlined (in the document)". FDA has recently issued revised guidance containing such recommendations (FDA 2010).

IV. B. 2. e. Annual Number of US Plasma Donors who have been Euroblood Recipients

Euroblood is whole blood that was collected at several different collection centers in Europe and shipped to and used by transfusion centers in the United States. The practice was stopped in 2002 with the implementation of geographic vCJD deferrals. The blood was used largely in the New York City metropolitan area and possibly in other areas on the east coast of the US
The model assumed that a total of 1.2% of US blood donors may have received Euroblood (TSEAC, 2002). To our knowledge there are no specific data available for plasma donors, therefore, data for blood donors was used in this risk assessment.

Assumption used in the model: All infected Euroblood recipients have vCJD agent present in their blood and plasma.

IV. B. 2. f. Total Number All Plasma Donors who may Potentially be Infected with vCJD and the vCJD Agent may be Present Through All Sources of Exposure

This portion of the model sums the total number of all potential US donors that may have been infected with vCJD from different exposure sources. The model estimates the total number of all plasma donors who may be infected with vCJD during extended residence, travel or military service in the UK, France, or other countries of Europe. Potential vCJD risk is also estimated for

donors that may have received Euroblood. Furthermore, the model estimates the number of total US donors potentially infected with vCJD and in whose plasma the vCJD agent may be present.

IV. B. 3. Annual Number of All US Plasma Donors Potentially Infected and Whose Blood may Contain vCJD Agent and Who May Not be Deferred by Questionnaire Screening

No validated test is currently available to detect the presence of vCJD agent in blood or plasma. The donor questionnaire, administered to all blood donors, can be used to screen donors for potential vCJD risk based on travel history, specifically involving extended travel to the UK, France or other countries in Europe where BSE was known to occur. In 1999 the FDA implemented a donor deferral policy aimed at reducing the potential risk of donations from those potentially exposed to the BSE agent during extended travel to the UK, France and other countries of Europe. Current policies (FDA 2002) defer blood and plasma donors:

- diagnosed with vCJD or other forms of CJD
- at increased risk for CJD, e.g. the donors have received a dura mater transplant, or human pituitary-derived growth hormone; the donors have blood relatives diagnosed with CJD
- with a history of a 3-month or longer travel/residency period in the UK between 1980-96
- with a history of a 5-year or longer travel/residency period in France since 1980
- current or former US military personnel, civilian military personnel, and their dependents resided in Northern Europe for 6 months or more between 1980-90, or resided in military bases elsewhere in Europe for 6 months or more from 1980 to 1996
- received a transfusion of blood or blood components in the UK since 1980
- injected bovine insulin since 1980 unless it is confirmed that injected bovine insulin was not made after 1980 from UK cattle, and
- whole blood donors with a 5-year or longer travel/residency period in Europe (other than the UK) since 1980

Deferral of donors with a history of travel to BSE countries is an effective tool for eliminating a significant portion of potential vCJD risk in US donors. The model incorporates information on the effectiveness of US deferral policies in reducing potential vCJD risk and potential vCJD prevalence in the US donor population.

Assumption about variable: Based on advice from the TSEAC at the October 31, 2005 meeting, the FDA model assumed 85-99% of potential vCJD infected donors would have been deferred prior to donation.

Assumption about variable: Model includes potential recovered plasma donors with vCJD agent present in blood and plasma (prionemic) that have long term travel history to the UK (≥3 mo), France (≥ 5 yrs), and Europe (≥5 yrs); and history of military deployment, military dependent or related travel or residence in Europe.

There is a possibility that some individuals who traveled to the UK, France, and other countries in Europe since 1980 stayed for periods of time that were shorter than the deferral period, were exposed to BSE agent, and were infected with vCJD. These individuals represent a source of

residual risk – or the remaining donor vCJD risk after interventions (in this case donor deferral policies) are applied. The section below addresses the calculation of residual risk for non-deferred at risk donors that traveled for periods of time that were shorter than recommended guidelines. The total number of all US plasma donors potentially infected with vCJD with agent present in blood and plasma that may not be deferred by questionnaire screening was determined by summing the estimates generated for both Source and recovered plasma donors that may not be deferred by current screening procedures.

Model Results for Module 2: vCJD Risk of US Plasma Donors

The FDA FVIII risk assessment model uses the concept of relative risk to semi-quantitatively estimate the vCJD risk for US plasma donors with a history of travel to the UK, France and other countries of Europe since 1980. Relative risk is the vCJD risk in a population relative to the UK vCJD relative risk of 1 (or 100%), which is equal to the prevalence of vCJD in the UK. Elements used in the model to calculate vCJD risk for travelers include travel destination (UK, France or other countries of Europe), duration of travel, specific year of travel, and age of donor. The estimated vCJD risk for all potential routes was summed to generate the total mean predicted number of potential vCJD-infected plasma donors in the US. Because of current policies, a blood or plasma donor potentially infected with vCJD has a high probability (85% - 99% chance) of being deferred from donation.

The predicted mean number per year of potential vCJD-infected donors and the number of potential vCJD donors who are likely not deferred from donation and donate to plasma pools used to manufacture pdFVIII are shown in Table 4.4 (below). The estimated mean number of US donors who potentially donated plasma containing infectious agent is approximately 0.02 donors per year based on calculations using a vCJD case-based epidemiological model estimated prevalence of ~4.5 in 1,000,000 (Clarke and Ghani 2005), or a mean of approximately 1.23 donors per year using calculations based on a tissue sample surveillance study yielding a prevalence estimate of 1 in 4,225 (Hilton *et al* 2004) (Table 4.4).

Table 4.4 Model Results: Annual Number of US Plasma Donors Predicted by Model to be Potentially Infected with vCJD and Donate to Plasma Pools used to Manufacture pdFVIII. Results from model provided for two different UK vCJD prevalence estimates. In the table the mean value is shown above with the 5th and 95th percentiles in parentheses below. The total number of vCJD donors for each prevalence estimate has been rounded to nearest decimal place.

	Model Output for LOWER vCJD Case Prevalence estimate of ~4.5 in 1,000,000 based on Clark and Ghani (2005)		*Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)*	
	Mean number (5th - 95th perc)[a] US plasma donors with history of travel to:		Mean number (5th - 95th perc)[a] US plasma donors with history of travel to:	
	United Kingdom	*France, Europe, or Military Service*	United Kingdom	*France, Europe, or Military Service*
Total number vCJD donors for all US pdFVIII pools Prior to screening	*0.0707* (0 – 1)[b]	*0.0203* (0 – 0)[b]	*4.66* (0 – 11)	*1.39* (0 – 4)
Number vCJD donors NOT DEFERRED (ineffective screening)	*0.0050* (0 – 0)[b]	*0.00005* (0 – 0)[b]	*0.34* (0-2)	*0.003* (0 – 0)[b]
Number vCJD donors NOT DEFERRED (short-term travel <3 mos, UK: <5 yrs, FR and EU)	*0.0069* (0 – 0)[b]	0.0067 (0 – 0)[b]	*0.43* (0 – 2)	0.46 (0 – 2)[b]
Total number vCJD infected donors NOT DEFERRED Donate to pdFVIII Plasma Pools	*0.0186* (0 – 0)[b]		*1.23* (0 – 4)	

[a] Risk estimates generated by the model should fall within the interval defined by the 5th - 95th perc (percentiles) 90% of the time.

[b] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 95% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 95% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

IV. C. Estimation of Annual Number and Percentage of Plasma Pools Potentially Containing vCJD Agent

The annual number and percentages of source or recovered plasma pools, potentially containing vCJD agent used to manufacture pdFVIII in the US were estimated by the model. The starting material for manufacturing pdFVIII is a plasma pool containing donations from thousands of donors. In this section, model first estimates the probability that an infected plasma pool is present. Manufacturers provided information to FDA on the approximate range and average number of donations per plasma pool which was combined with information on market share to develop two aggregate statistical distributions, one each representing donations for source and for recovered plasma pools. The distributions were used to predict the number of donations per source or recovered plasma pool in the model. The majority of pdFVIII is manufactured from Source Plasma and the minority from recovered plasma. Source Plasma pools are usually smaller than recovered plasma pools. Plasma from fewer donors reduces the chance that a plasma pool may contain a donation from an infected donor. However, because Source Plasma donors are allowed to donate more frequently, and give more plasma per donation, there is a greater chance that if a vCJD infected donor were in the Source Plasma donor pool they might contribute multiple donations to a single plasma pool or donate to multiple pools.

The probability that an infected plasma pool occurs is calculated using a binomial distribution probability function with parameter n equal to number of donors per plasma pool and parameter p equal to the vCJD prevalence of plasma. The model estimates that the probability an infected plasma pool containing plasma from multiple infected donors is small (at the least 2 magnitudes lower than the probability an infected pool containing plasma from only one infected donor). Therefore, the model assumes only one infected donors may be present in a plasma pool, if present at all. The model assumes number of infected plasma pools is equal to the number of donors who are infected and with infectious agent in blood at the time of donation.

The model also used market data for pdFVIII product, combined with estimated yield of pdFVIII per liter plasma and information on the number of donations per pool by type (either source or recovered) to calculate annual volume of plasma and annual number of plasma pools used to produce pdFVIII products distributed in the US in 2002.

Results-Module 2: vCJD risk of US plasma pools

As a general comment, the number of donations per plasma pool influences the potential exposure risk for infrequent recipients of plasma derivatives. The use of fewer donations and smaller plasma pools during manufacturing would result in a lower percentage of plasma pools potentially containing vCJD agent and potentially expose a lower percentage of infrequent recipients to vCJD (if present). Frequent recipients of plasma-derived products would likely face a similar level of risk of potential vCJD exposure whether large or small numbers of donations per plasma pool are used in manufacturing.

LOWER UK vCJD Case Prevalence estimate of ~4.5 in 1,000,000 (based on Clarke and Ghani, 2005). The lower prevalence estimate used in the FDA model suggested that an average of 0.03% of all US plasma pools used to manufacture pdFVIII in the year 2002 potentially contained the vCJD agent (**Table 4.5**). In fact, on average >95% of the time plasma pools would be predicted not to contain a donation from a vCJD infected donor. Only an average of 0.10% recovered plasma pools are predicted by the model to contain a vCJD donation from a US donor in any given year. Of interest at the lower prevalence, the model predicts that the occurrence of a recovered plasma pool with a vCJD donation would be infrequent (as indicated by 5th and 95th percentile values of 0); suggesting that at least 95% of the time zero pools would contain the agent. Also at the lower prevalence, a vCJD donation in a Source Plasma pool would be predicted to be even more infrequent. Given the relatively small number of pools used annually in the United States (mean of 63) to produce pdFVIII the model predicts a positive pool to occur on average at a rate of 1 in 53 years.

HIGHER UK vCJD Infection Prevalence estimate of 1 in 4,225 (Hilton et al 2004). The higher prevalence estimate used in the FDA model suggested that an average of 2.30% of all US plasma pools used to manufacture pdFVIII in 2002 were predicted by the model to contain vCJD agent (**Table 4.5**). It should be noted that fewer recovered plasma pools than Source Plasma pools are used in the US annually to produce pdFVIII. Also, recovered plasma pools contain the largest number of plasma donations. Since recovered plasma pools contain many more donations than Source Plasma pools the likelihood that a recovered plasma pool may contain a donation from an individual potentially infected with vCJD is considerably higher than for a Source Plasma pool. Using the higher UK vCJD prevalence estimate, the model predicts that on average, 7.10% of recovered pools and 1.30% of Source Plasma pools potentially contain vCJD agent.

Table 4.5 Annual Percentage of US Plasma Pools Potentially Containing a vCJD Donation. Results from model include only those US plasma pools used annually to manufacture pdFVIII.

- Results provided for two different UK vCJD prevalence estimates.

	Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)		Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)	
	Source Mean (5^{th} - 95^{th} perc)[a]	Recovered Mean (5^{th} - 95^{th} perc)[a]	Source Mean (5^{th} - 95^{th}) perc)[a]	Recovered Mean (5^{th} - 95^{th} perc)[a]
Percent pools potentially containing vCJD agent	0.02% (0 – 0%)[b]	0.10% (0 – 0%)[b]	1.30% (0 – 5.60%)	7.10% (0 – 29.0%)
Mean percentage of pools potentially containing vCJD agent	0.03% (0 – .0%)[b]		2.3 % (0 – 8.2%)	

[a] Risk estimates generated by the model should fall within the interval defined by the 5^{th}- 95^{th} perc (percentiles) 90% of the time.
[b] For a 5^{th} and 95^{th} percentile interval of 0 and 0, respectively, the model estimates that for at least 95% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

IV. D. Estimation of the Quantity of vCJD agent in a Plasma Pool that Contains a Donation from a Donor Infected with vCJD

This section of the risk assessment estimates the quantity of vCJD agent in each vCJD plasma donation and pool that may be used to make pdFVIII. The quantity of infectious agent present in plasma pools may vary depending on the infectivity of each infected donation and the number of infected donations in the pool.

IV.D.1. Quantity of vCJD Agent Present in a Donation of a Donor Infected with vCJD

Whole blood collected from a vCJD-infected individual can vary from person to person in the quantity of infectivity it contains. Based on limited available data (see below), FDA believes that the quantity of infectivity present in blood from a vCJD infected individual in i.v. ID_{50} is likely represented by a distribution with the following characteristics: Minimum value = 0.1, 5^{th} percentile = 2, Most likely value = 10, 95^{th} percentile = 30, and Maximum value= 1,000 i.v. ID_{50}. Given the possible parameters, statistical distributions were fitted to the selected parameters using Best Fit part of the @Risk Professional software package (Palisade Corporation, New York).

200

Using the software we determined that a log normal statistical distribution of (2, 12, 30) i.c. ID_{50}/ml (5^{th} percentile, most likely, and 95^{th} percentile) with minimum and maximum of 0.1 and 1,000, respectively, provided the best fit.

Conclusions from several research groups arrive at somewhat similar estimates for the quantity of infectivity that might be present in the whole blood of mice and hamsters. Using a mouse model and human CJD Brown *et al* (1999) found a range from 0.5 to 15 mouse i.c. IU per ml which we assumed to be roughly equivalent to 1 to 30 i.c. ID_{50} (assuming a linear dose-response for infectivity). An infectious unit is the quantity of infectivity associated with a 100% probability of infection in recipients and roughly equates to two ID_{50} units (1 IU = 2 ID_{50}). Brown *et al* (1998, 1999) conducted experiments to determine the infectivity of buffy coat material and plasma but not red blood cells. Assuming that red blood cells retain approximately 25% of the infectivity of whole blood, then the infectivity present in whole blood could be estimated to be in the range of approximately 10 i.c. ID_{50} and 20 i.c. ID_{50} per ml. Cervenakova *et al* (2003) found levels as high as 20 – 30 infectious doses per ml (40-60 i.c. ID_{50} per ml) associated with buffy coat and plasma during incubating and symptomatic stages of the disease. Red blood cells were not found to be infectious. Transfusion of blood products using the hamster scrapie model by Rohwer suggests that addition of infectivity levels derived for individual blood components would generate a titer for whole blood of approximately 2 to 20 i.c. ID_{50}/ml. Summarizing the above literature it seems that the range of reported values for infectivity ranged from 0.5 to as high as 30 i.c. ID_{50} with the possibility that at times the infectivity present in blood may exceed this range. Attempts to identify vCJD infectivity titers in human blood have not been successful, but the assay sensitivity for vCJD *in vitro* and in animal models is limited (Bruce *et al* 2001 and Wadsworth *et al* 2001). Wadsworth *et al* estimated a limit of sensitivity of about 1,000 ID_{50}/ml by their assay meaning that infected blood containing less than 1,000 ID_{50} would not have elicited infection or disease in their animal model, hence infectivity would not have been detected (Wadsworth, 2001).

Assumption used in the model: *The model used a log normal statistical distribution to represent* the variability and uncertainty of the quantity of infectivity in blood. It was assumed that whole blood from an infected person potentially carries a minimum of 0.1 i.c. ID_{50} per ml, a 5^{th} percentile of 2 i.c. ID_{50} per ml, a medium of 12 i.c. ID_{50} per ml, a 95^{th} percentile of 30 i.c. ID_{50} per ml and a maximum of 1,000 i.c. ID_{50} per ml.

Studies in animal models have shown that greater than 50% of transmissible spongiform encephalopathy agent present in whole blood is associated with plasma. Experiments by Gregori *et al.* (2004) using a hamster – sheep scrapie model showed that approximately 58% of infectivity in whole blood is associated with plasma.

Assumption used in the model: *The model assumes that 58% of infectivity is associated with* plasma.

Studies with mouse-adapted scrapie agent suggest that the i.v. route of administration is approximately 10 times less efficient in causing infection than the intracerebral route (Kimberlin *et al* 1996). Brown *et al* (1999) used a mouse-adapted human TSE agent to show that i.v. injection of plasma was about seven times less efficient and i.v. injection of buffy coat approximately 5 times less efficient than were i.c. inoculations of the same materials in transmitting infection. Based on discussion and advice from the FDA Transmissible Spongiform Encephalopathies Advisory

201

Committee (TSEAC, 2005) the range of efficiency of the i.v. route (versus the i.c. route) was assumed in the model to range between the values of 0.1 and 1.

Assumption used in the model: Exposure to infectivity by the i.v. route is between 1 and 10 times less efficient at causing infection than introduction via the intracerebral route. The FDA risk assessment used a uniform distribution (0.1, 1) to represent of efficiency of transmission through i.v. route versus i.c. route. Using a value of 1 for the ratio of the lower bound of the efficiency is a conservative estimate and assumes that theoretically there would be no difference between the efficiency in initiating infection between the i.c. and i.v. routes.

IV.D. 2. Quantity of vCJD Agent in a Plasma Pool Containing a Donation from Donor Infected with vCJD

The quantity of vCJD agent present in a donation from a US donor infected with vCJD will be diluted out in a plasma pool among plasma from thousands of other donations. This section calculates the quantity of agent present in a plasma pool containing a donation that contained vCJD agent.

Assumption used in the model: We assumed only one infected donor per plasma pool, because based on the calculation in section IV. C. the prevalence of vCJD in the US is very low and the chance a pool involves multiple donations from vCJD infected donors is small.

Assumption used in the model: We assumed an average number of donations that individual donor would contribute to a Source plasma pool is -(b)(4)- units. The model used a Pert distribution with a minimum of -(b)4), most likely of -(b)(4), and maximum of -(b)(4)- to represent the uncertainty for this estimate. Individual infected recovered plasma donors most likely give only one donation to a pool.

MODULE 3 (IV.E)— CLEARANCE OF vCJD INFECTIVITY DURING MANUFACTURE OF pdFVIII

IV.E. Clearance of vCJD Infectivity during Manufacture of pdFVIII

The plasma separated from whole blood is a protein rich, straw-colored liquid that contains FVIII, a number of other clotting factors, immune globulins, serum albumin and a number of other proteins. Common viral inactivation procedures such as heating, solvent-detergent treatment, and UV irradiation have little effect on the quantity of TSE infectivity present in plasma and plasma derivatives, however, fractionation and purification of individual protein component such as FVIII may partially remove TSE infectivity in the protein component. The fractionation and purification steps include alcohol precipitation, size exclusion, affinity chromatography, etc., which may

remove vCJD infectivity are summarized in **Table 4.6.** (Lee et al. 2000; Stenland *et al.* 2002; Foster 2004; Foster, *et al.* 2004).

For a specific pdFVIII product, usually only one or two processing steps have been studied for potential reduction of infectivity. Experimental designs of these studies are not standardized; therefore, study results are not directly comparable. In order to achieve a high concentration of vCJD infectivity in initial materials, many studies used vCJD infected brain homogenate as spiking material, which may not mimic the physical form of infectious agent in the blood. Based on TSE clearance studies in the published literature and manufacturers' data available to the FDA, FDA staff believe that the plasma-derived products currently on the market employ manufacturing processes that achieve a clearance of vCJD agent of 4 $\log_{10}$ or greater in the final pdFVIII product.

Table 4.6 Reduction Factor (RF) of Fractionation Procedures

Fractionation Procedures	RF ($\log_{10} ID_{50}$)	References
Cryoseparation	0-1	(Foster *et al* 2000; Farrugia 2002; Lee *et al* 2002)
General fractionation	1-3	(Farrugia 2002; Lee *et al* 2002)
3% PEG	2-4	(Farrugia 2002)
11.5% PEG	1.7	(Foster *et al* 2000)
$Zn+Al(OH)_3$	2.7-3.5	(Foster *et al* 2000; Cervenakova *et al* 2002)
Ion exchange Membrane filtration	1	(Foster *et al* 2000)
Immunopurification	4.4-6.3	(Foster, Welch *et al* 2000; Cervenakova *et al* 2002)

IV.E. 1. Estimated Quantity of vCJD Agent per IU FVIII Product made from a Specific vCJD Plasma Pool

This section of the risk assessment models each infected plasma pool to estimate the potential reduction in infectivity for each pool during manufacturing processing, and to estimate the quantity of any remaining infectivity (i.v. ID_{50} s) in the pdFVIII product made from each pool. The quantity of vCJD agent in FVIII product made from different infected plasma pool may vary depending on the initial quantity of vCJD agent in the plasma pool, the infectivity clearance through manufacturing, and the yield of FVIII product per liter of plasma. Considering the uncertainty in the degree of clearance that can be achieved during various pdFVIII manufacturing processes, this risk assessment models two levels of potential clearance in infectivity, 4-6 $\log_{10}$ and 7-9 $\log_{10}$.

Assumption used in the model: yield of FVIII (including high purity and intermediated purity FVIII) most likely is 150 IU (International Unit) per liter plasma with minimum of 130 and

maximum of 270 IU per liter plasma, and represented by a pert distribution (130, 150, 270) (WFH, 2004).

IV.E. 2. Estimated Percentage of FVIII Vials that Contain vCJD Agent

This section calculated the percentage of FVIII vials that contain vCJD agent. The percentage of FVIII vials that contain vCJD agent is used in the later section of the model to determine the likelihood of an individual patient receiving vCJD vial(s) basing on amount of FVIII product used by an individual patient.

Assumption used in the model: the percentage of FVIII vials that contain vCJD agent is equal to the percentage of FVIII plasma pools that contain vCJD agent, which is calculated in section IV. C.

Results-module 3: Per Vial vCJD Infection Risk for US Manufactured pdFVIII

The mean potential risk of vCJD infection per 1,000 IU vial of US manufactured pdFVIII is shown in **Table 4.7**. The mean potential per vial vCJD risk per year is a function of two factors:

1) Percentage of pdFVIII vials containing vCJD agent and,
2) Quantity of agent (i.v. ID_{50}) present in each vial.

If vCJD agent were present in US plasma pools, the risk assessment model assumed that the quantity of agent was likely reduced by manufacturing processes used to produce purified pdFVIII. Based on currently available experimental studies, it is estimated that pdFVIII products potentially have 4 $\log_{10}$ (or 10,000 fold) or greater manufacturing process reduction of the vCJD agent. **Table 4.7** shows potential risks associated with products attaining a 4-6 $\log_{10}$ level of reduction during manufacture. Results are shown only for 1,000 IU vials but the model assumed that the final purified pdFVIII product was packaged with equal likelihood into vial sizes of 250, 500, 1,000 and 1,500 international units (IU).

Per vial vCJD risk: Results based on lower epidemiological model estimated prevalence of ~4.5 in 1,000,000 (Clarke and Ghani, 2005). The per vial risk provides an estimate of the potential vCJD infection risk for a 1,000 IU vial of pdFVIII product manufactured from plasma collected from US donors. The model generated estimates of per vial risk using the lower prevalence estimate *(based on Clarke and Ghani 2005)* and results are shown in **Table 4.7**. Based on the lower prevalence estimate the average percent of plasma pools containing the vCJD agent is estimated to be 0.03%. Assuming a clearance of 4-6 $\log_{10}$ the model estimates that the average quantity of i.v. ID_{50} per vial is 9.5×10^{-6} for vials produced from a contaminated pool. This result can be interpreted to mean that only 1 in 210,000 vials made from a contaminated pool would contain an infectious dose of vCJD. Combining these estimates yields an average risk per vial of 1 in 700 million. Alternatively, this could be taken to mean that a patient would need to infuse 700 million vials of product before accumulating one full infectious dose of vCJD.

At this lower prevalence estimate, there is a lower probability that plasma pools contain a donation from a donor potentially infected with vCJD, and a pdFVIII vial would be much less likely to contain vCJD agent. Readers may notice that the 5th and 95th percentile intervals for all of the model

outputs using the lower prevalence estimate (~4.5 per million) are from 0 to 0, meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. Greater than 95% of the time the model estimates the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. Again, the model predicts that, on average, 0.03% of the time the exposure to vCJD may be greater than zero. Results indicate that using FVIII made from recovered plasma is likely have greater risk than using FVIII made from source plasma because a pool of recovered plasma contains plasma from more donors.

Per vial vCJD risk: Results based on the Higher vCJD Infection Prevalence estimate of 1 in 4,225 (Hilton, et al 2004). The per vial risk provides an estimate of the potential vCJD infection risk for a 1,000 IU vial of pdFVIII product manufactured from plasma collected from US donors. The model generated estimates of per vial risk using the higher prevalence estimate (Hilton, et al 2004) and results are shown in **Table 4.7**. Using the higher prevalence estimate the average percent of plasma pools containing the vCJD agent is estimated to be 2.3%. Assuming a clearance of 4-6 $\log_{10}$ the model estimates that the average quantity of i.v. ID_{50} per vial is 9.2×10^{-6} for vials produced from a contaminated pool. This result can be interpreted to mean that only 1 in 217,000 vials made from a contaminated pool would contain an infectious dose of vCJD. Combining these estimates yields an average risk per vial of 1 in 9.4 million. Alternatively, this could be taken to mean that a patient would need to infuse 9.4 million vials of product before accumulating one full infectious dose of vCJD. Similar to the results based on the Lower vCJD Case Prevalence estimate these results indicate that using FVIII made from recovered plasma is likely have greater risk than using FVIII made from source plasma because a pool of recovered plasma contains plasma from more donors.

Table 4.7 Annual Predicted per Vial vCJD Infection Risk for US Manufactured pdFVIII from Model: • Results for 1,000 IU vial
• Assumed manufacturing process reduction of 4-6 $\log_{10}$, and
• Two different UK vCJD prevalence estimates.

	4 - 6 $\log_{10}$ Reduction Factor (LRF)					
	Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)			Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)		
Type of Plasma Pool	Percentage FVIII vials with vCJD agent (5^{th}, 95^{th} perc)[c]	Quantity iv ID_{50}[a] per vial[a] (5^{th}- 95^{th} perc)[c]	Mean potential per vial vCJD risk[b] (5^{th}- 95^{th} perc)[c]	Percentage FVIII vials with vCJD agent (5^{th}, 95^{th} perc)[c]	Quantity iv ID_{50}[a] per vial[a] (5^{th}- 95^{th} perc)[c]	Mean** potential per vial vCJD risk[b] (5^{th}- 95^{th} perc)[c]
Source	0.02% (0 – 0%)[d]	1.81×10^{-5} (6.65×10^{-7} – 6.89×10^{-5})	1 in 552 million (0, 0)[d]	1.30% (0 – 5.60%)	1.84×10^{-5} (8.26×10^{-7} – 6.73×10^{-5})	1 in 8.4 million (0, 1 in 531,000)
Recovered	0.10% (0 – 0%)[d]	1.55×10^{-4} (6.70×10^{-6} – 5.70×10^{-4})	1 in 1.3 billion (0, 0)[d]	7.10% (0 – 29%)	1.56×10^{-4} (8.65×10^{-6} – 5.42×10^{-4})	1 in 18.1 million (0,1 in 1.3 million)
Average of all vials	0.03 % (0 – 0%)[d]	9.50×10^{-6} (1.14×10^{-7} – 4.27×10^{-5})	1 in 620 million (0, 0)[d]	2.30% (0 – 8.2%)	9.23×10^{-6} (1.76×10^{-7} – 3.72×10^{-5})	1 in 9.4 million (0, 1 in 656,000)

[a] Mean i.v. ID_{50} in vials containing vCJD agent
[b] iv ID_{50} represents the probability that 50% of those exposed to 1 ID_{50} intravenously may become infected with vCJD.
Mean potential annual per vial vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Percentage vials with vCJD agent x mean quantity iv ID_{50} per year x 0.5 (50 % chance infection from ID_{50})
[c] Risk estimates generated by the model should fall within the interval defined by the 5^{th} - 95^{th} perc (percentiles) 90% of the time.
[d] For a 5^{th} and 95^{th} percentile interval of 0 and 0, respectively, the model estimates that for at least 95% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 95% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

Module 4 (IV. F): FVIII Utilization and Annual Exposure

FDA estimated that there are approximately 1,800 patients with severe hemophilia A (HA) disease and an estimated 250 patients with severe vWD in the US who use pdFVIII to manage their disease. Traditionally, HA patients were treated with factor concentrates only when bleeding

206

occurred, which is called episodic treatment. Patients are also treated using prophylactic therapy regimens that seek to prevent bleeding events through regular infusions of pdFVIII. Patients who need vWF must use plasma-derived sources of FVIII which contain vWF. No recombinant vWF is currently available.

IV. F. pdFVIII Utilization by HA and vWD Patients and Potential Exposure to the vCJD Agent

The potential exposure of an individual HA or vWD patient or patient population with severe disease to the vCJD agent through use of pdFVIII was estimated in the model based on the:

• total quantity of pdFVIII used per year
• probability of receiving infected pdFVIII product, and
• potential quantity of vCJD agent predicted in infected pdFVIII product.

IV. F. 1. Estimation of Annual Number vCJD Vials used by Individual Patient

The more a patient uses pdFVIII product the greater the chance they may receive an infected product. The quantity of pdFVIII utilized by an individual patient is dependent on the severity of the disease and the treatment regimen.

IV. F. 1. a. Estimation of pdFVIII Utilization by Patients with Severe Hemophilia A Disease

Plasma-derived FVIII utilization and the size of each of the severe HA clinical treatment subpopulations were estimated using data from a Centers for Disease Control and Prevention (CDC) sponsored study in 6 states from 1993-1998. This risk assessment provides outputs that estimate the annual exposure for several patient subpopulations with severe HA disease for patients in the following clinical treatment groups and patient subpopulation:

• Prophylaxis – No inhibitor
• Prophylaxis - With inhibitor
• Prophylaxis - With inhibitor and immune tolerance
• Episodic – No inhibitor
• Episodic - With inhibitor

Because patients with severe HA are likely to use higher quantities of pdFVIII product we reasoned that they would be at potentially greater risk, than those with moderate or mild hemophilia, if the vCJD agent were present in US manufactured product. A summary of the utilization data used for the model is provided in the table below. We 'fit' different parametric models to the actual patient product utilization data and chose the model which best approximated the overall pattern of product use by each specific patient subpopulation. The beta distribution provided the best fit to the utilization data for each patient subpopulation, and therefore, was chosen as the input distribution for the variable of "FVIII annual usage". Each distribution was

207

truncated by minimum and maximum FVIII usage for each of the patient clinical treatment subpopulations.

Table 4.8. Annual Usage of pdFVIII by Individual HA Patients with Severe Disease-data and Input Distribution

Treatment Regimen	Inhibitor Status	n	Input distribution (min, max)	Mean	5^{th}, 95^{th} percentiles
Prophylaxis	No Inhibitor	578	(300, 1200000)	157949	(21000, 382,000)
	With Inhibitor – No Immune Tolerance	63	(2000, 1000000	199523	(27000, 448000)
	With Inhibitor – With Immune Tolerance	62	(10000, 4000000)	558700	(33000, 1593000)
Episodic	No Inhibitor	946	(0, 1000000)	85270	(4600, 245000)
	With Inhibitor	161	(2000, 1000000)	160458	(5000, 489000)

IV. F. 1. a. FVIII Utilization in Patients with Severe von Willebrand disease

The CDC six state Hemophilia Surveillance System project conducted from 1993-1998 did not include patients with vWD. We assumed that vWD patients with severe disease would largely use Humate P product only for factor replacement treatment. A search of records in the Hemophilia Surveillance System project data revealed a total of 58 records that indicated Humate P had been used, among which, 8 records indicate patients had developed inhibitor, which are considered uncommon among vWD patients and were excluded from analysis. Among the 58 records, 35 were from Adults (≥15 yrs of age) and 23 records were from young persons (<15 yrs of age). Records for each age group were further grouped by clinical treatment using either a prophylaxis or episodic treatment regimen. Data were initially analyzed individually using the statistical package "JMP" (SAS Institute, Cary, NC) to generate descriptive statistics and statistical distribution(s) for each patient treatment group that best reflected the variation in pdFVIII

utilization. The Generalized Beta distribution was identified as the best fit to the pdFVIII utilization data (as determined by using the software Best Fit (Palisade Corp, NY) and was used as the input distribution for pdFVIII usage by individual vWD patients in the model. Graphical representations of the original data and the fitted Generalized Beta distributions are shown in Appendix C. Table 4.9 summarizes pdFVIII usage data from CDC sponsored study and the input distribution generated based on the data. FDA used data in the CDC and six state Hemophilia Surveillance System project conducted from 1993-1998 to estimate FVIII utilization by all vWD patients. The data represent only a sample of all possible vWD patients with severe disease in the US. FDA estimated that there were approximately 250 patients in the US with Type 3 vWD. To calculate the total number of patients in each age group and treatment regimen group we adjusted the 58 patient population to equal a total of 250 patients by multiplying the patient population in each group by a factor of 4.3 (250/58 = ~4.3). The utilization data for patients in each treatment regimen in the sample population were used in the risk assessment model to generate outputs for the annual exposure to vCJD for all vWD for Adult (>15 yrs of age) and Young (≤15 yrs of age) persons in the US among clinical treatment groups of prophylaxis and episodic. The FVIII utilization data were used to calculate the potential vCJD risk for vWD patients; these results are shown in Tables 5.2A and 5.2B.

Table 4.9. Annual Usage of pdFVIII by Individual Severe vWD Patient - Data and Input Distribution

Treatment Regimen	Input Distribution n	(min, max)	Mean	5^{th}, 95^{th} percentiles
Young (≤15 yrs of age)				
Prophylaxis	9	(9200, 504625)	165713	(9900, 454000)
Episodic	14	(1010, 41850)	11045	(1020, 34350)
Adult (>15 yrs of age)				
Prophylaxis	17	(15000, 772800)	186880	(17000, 540000)
Episodic	18	(1000, 293800)	86923	(2200, 240800)

IV. F. 2. Quantity of vCJD Agent in pdFVIII Vials

This section of the model randomly draws vCJD vials received by individual patient from different plasma pools. The amount of infectious agent in vCJD vials vary because of variation on initial

infectivity of plasma pool, degree of infectivity clearance in manufacture processing and yield of product.

Assumption used in the model: The pdFVIII vials a patient received are randomly drawn from different plasma pools

IV. F. 3. Estimation of Potential Annual Exposure

This section of the model calculates the final output of the model, the annual predicted exposure of individual patient (ID_{50} per person, year). It is the total amount of infectious agents from all vCJD vials received by the patent during a one year period.

Assumption used in the model: *Exposure to vCJD is accumulative during one year period*

V. RISK CHARACTERIZATION

The risk characterization section of the risk assessment integrates information from the hazard identification, hazard characterization and the exposure assessment components to arrive at estimates of the risks posed by a hazard.

In this risk assessment data for hazard characterization (dose-response) for humans are lacking, so we could not develop a human vCJD dose-response. The dose-response relationship provides information needed to use the exposure (dose) assessment results to estimate the probability of adverse responses including infection, illness or mortality based on assessment of exposure (dose) to the hazard. Many TSE models and risk assessments, including our model, use the ID_{50}, or amount of material that leads to infection in 50% of the population, as a semi-quantitative estimate of the amount of TSE agent. The ID_{50} has been derived from rodent animal models and may or may not approximate infection and occurrence of vCJD in humans. This lack of knowledge about the animal data and how they relate to actual human clinical vCJD outcomes adds considerable uncertainty to the risk estimates generated by the model. The FDA risk assessment interprets the ID_{50} as representing a linear dose-response relationship between exposure and the probability of infection. In such a case, exposure to 1 ID_{50} would suggest a 50% probability of infection, exposure to 0.1 ID_{50} would suggest a 5% probability of infection, and so on.

The final results of this risk assessment provide estimates of potential annual exposure and annual vCJD infection risk for patients with severe HA and for patients with severe vWD for pdFVIII manufactured from plasma collected in the US. The risk was estimated by applying the linear ID_{50} dose-response relationship, which provides a probability of vCJD infection in the two populations and various subpopulations within the two groups. Given the limited data available FDA believes that any extrapolation or interpretation has limited utility in actually estimating outcomes such as

infection and illness. Therefore, any estimate of the risk based on estimates of exposure to the vCJD agent through use of pdFVIII will be imprecise and extremely uncertain.

V.A. THE MODEL

This risk assessment and simulation model links the available scientific and epidemiological data together to mathematically approximate the processes (predicted presence of vCJD in UK population, manufacturing, reduction of vCJD agent, and patient utilization) leading to potential exposure of US patients to vCJD agent present in US-manufactured pdFVIII. A summary of the variables, parameters and equations used in the model were described in Section III. Exposure Assessment and a summary of the variables and equations, data, and assumptions used in the model are provided in Appendix A. The model was run using @Risk software package (Palisades Corp, NY) to conduct the Monte Carlo analysis. Simulations of up to 1 million iterations were run.

The risk assessment uses Monte Carlo simulation to randomly draw values from probability input distributions (which are statistical representations of input data) once per iteration; thousands of iterations are used to generate the model outputs as risk estimates. This simulation method is often used in situations when a model is complex, non-linear, or involves several uncertain parameters. The output generated is usually an aggregate distribution whose shape can be summarized using measures of central tendency (mean, median, mode) or with boundaries such as the 95% confidence interval (CI), the 5th and 95th percentiles or the range, bounded by the minimum and maximum values generated as part of the output. The strength of Monte Carlo analysis is that it generates resulting risk estimates as statistical distributions, which reflect the underlying uncertainty and variability of the original input data and parameters. We used visual graphic methods to verify that model estimates converged.

V. B. Model Results: Estimated Annual Potential Exposure to vCJD i.v. ID_{50} and Potential vCJD Risk through Human pdFVIII used to Treat Severe HA

Individuals with HA vary in their degree of FVIII deficiency. Although the clinical spectrum generally can range from severe, to moderate, and to mild disease, this assessment specifically addresses potential vCJD exposure and risk for persons with severe HA. Among an estimated 14,000 HA population in the United States, approximately 50% have severe disease and 25% of all HA patients use human pdFVIII products. FDA estimated that there are approximately 1,800 HA patients (Tables 5.1A. and 5.1B.) with severe disease in the US that use human pdFVIII products. Although the estimated risk is very low, it is possible that some patients using human pdFVIII may potentially be exposed to vCJD agent if present in US manufactured product.

Estimation of PdFVIII product utilization by patients with severe HA. FDA obtained data on human plasma-derived FVIII utilization from the CDC. Data in the study were collected as part of a collaborative effort between CDC and six states during the time period 1993 – 1998. A summary of study results for New York State are described in Linden, et al. (2003). The comprehensive study collected standardized patient demographic, clinical, treatment and outcome data. Patient medical records were obtained from treatment sites including: hemophilia treatment centers (HTCs), hospitals, clinics, physician's offices, home-care agencies, nursing homes, prison

infirmaries, and dispensers of factor concentrates. The data abstracted from medical records tabulated all factor concentrate utilization prescribed by quantity, type, purpose (e.g., prophylaxis, treatment of acute bleeds, or immune tolerance therapy) and total quantity used per calendar year.

The data on the quantity of pdFVIII product utilized annually were used to develop statistical distributions of product usage for patients by treatment group. The mean quantities of products utilized by HA patients on different treatment regimens are shown in **Table 5.1A. and 5.1B.** Approximately 1,100 records for patients utilizing pdFVIII were analyzed in this study. The percentage of each patient subpopulation in proportion to the total HA population in the CDC-Six State study was used to extrapolate the estimated number of total individuals in each patient subpopulation. From the study results, we estimated that there are a total of approximately 1,800 persons with severe HA in the US who use pdFVIII.

Results from the risk assessment model for patients with severe HA who are treated with pdFVIII product with a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent are shown in **Tables 5.1A. and 5.1B.** Generally results are expressed for patients in several different HA clinical treatment groups including:

- Prophylaxis
- Prophylaxis plus inhibitor
- Prophylaxis plus inhibitor and immune tolerance
- Episodic
- Episodic plus inhibitor

Potential exposure of severe HA patients to vCJD agent: Results based on lower epidemiological model estimated prevalence of ~4.5 in 1,000,000 (based on Clarke and Ghani, 2005). The model estimates that severe HA patients treated using a prophylaxis regimen, with inhibitor, with immune tolerance and treated with a pdFVIII product (with 4-6 $\log_{10}$ reduction of vCJD agent) have the highest pdFVIII usage of the groups we examined and potentially face the highest risk among HA patients. **Table 5.1A.** indicates that approximately 62 severe HA patients in a prophylaxis treatment regimen with inhibitor and immune tolerance use an average of 558,700 IU per person per year and are potentially exposed to an average of 1.80×10^{-6} i.v. ID_{50} per person per year; representing an average potential vCJD risk of 1 in 1.1 million per person per year. If all of the assumptions in the model are correct at this lower estimated prevalence, this risk may yield 1 vCJD infection in an average of approximately 18,000 years of treatment among severe HA patients who are in a prophylaxis treatment regimen with inhibitor and immune tolerance. As mentioned earlier the 5th and 95th percentile intervals for all of the model outputs using the lower prevalence estimate (~4.5 per million) in **Table 5.1A.** are from 0 to 0 meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. Greater than 95% of the time the model estimated the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. However, the model predicted that 0.03% of the time the exposure to vCJD agent may be greater than zero, and there is a possible but low risk of vCJD infection.

The risk for the entire population was calculated by summing the cumulative risk potential of vCJD exposure and risk (**Table 5.1B.**). Using the lower prevalence estimate, the model predicts that the approximately 1,800 severe HA patient population in the US uses a total of approximately

243 million IU pdFVIII and is exposed to an average of 7.79×10^{-4} i.v. ID_{50}. This total annual exposure for the entire severe HA population in the US is equivalent to a mean potential population-based vCJD risk of 1 in 2,600 years. At this expected level of risk, 1 vCJD infection would be predicted to occur in 2,600 years of treatment for the entire population of 1800 severe HA patients that use pdFVIII.

Potential exposure of severe HA patients to vCJD agent: Results based on higher surveillance prevalence estimate of 1 in 4,225 (Hilton, et al 2004). The model estimates that severe HA patients in a prophylaxis regimen, with inhibitor, with immune tolerance and treated with a pdFVIII product (with 4-6 $\log_{10}$ reduction of vCJD agent) potentially face the highest expected risk among HA patients. **Table 5.1A.** indicates that approximately 62 severe HA patients in a prophylaxis treatment regimen with inhibitor and immune tolerance use an average of 558,700 IU per person per year, and are potentially exposed to an average of 1.10×10^{-4} i.v. ID_{50} per person per year, using the higher prevalence estimate. This represents an average potential vCJD risk of 1 in 18,000 per person per year for the treatment group. If all of the assumptions used in the model are correct and considering the total number of 62 patients in this category (or population-based risk), this expected risk would yield 1 vCJD infection in 290 years of treatment among the patients under this category.

The risk for the entire severe HA population is calculated by summing the cumulative risk potential of vCJD exposure and risk from all individual patients under five categories (prophylaxis with no inhibitor, prophylaxis with inhibitor, prophylaxis with inhibitor and immune tolerance, episodic with no inhibitor and episodic with inhibitor) (**Table 5.1B.**). Using the higher surveillance estimate, the model predicts that the approximate total of 1,800 severe HA patient population in the US uses a total of approximately 243 million IU pdFVIII, and is exposed to an average of 4.9×10^{-2} i.v. ID_{50} per year. This total annual exposure for the entire severe HA population in the US is equivalent to a mean potential population-based vCJD risk of 1 in 41, i.e., 1 vCJD infection would be predicted to occur in 41 years of treatment in this 1800 severe HA patient population.