Guidance for Industry

Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
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Guidance for Industry

Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus

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I. INTRODUCTION

This guidance document provides recommendations for assessing blood donor suitability and blood product safety and maintaining blood and blood product availability in response to pandemic (H1N1) 2009 virus. It is intended for establishments that manufacture Whole Blood and blood components intended for use in transfusion and blood components intended for further manufacture, including recovered plasma, Source Plasma and Source Leukocytes. Within this guidance, "you" refers to blood establishments; "we" refers to FDA.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. BACKGROUND

A. Epidemiology and Pathogenesis

The 2009 H1N1 pandemic is caused by a novel influenza A virus of swine origin. On April 26, 2009, then Department of Health and Human Services (DHHS) Acting Secretary Charles E. Johnson, pursuant to section 319 of the Public Health Service Act, 42 U.S.C. § 247d, declared a public health emergency when a novel swine-origin 2009 influenza A (H1N1) virus was identified in California, Texas, Kansas, and New York. The pandemic influenza H1N1 virus has since spread quickly to all fifty states and globally. In June 2009, the World Health Organization (WHO) declared a Phase 6 Level of Pandemic Influenza Alert. This declaration was based upon a standard definition reflecting worldwide spread of the pandemic (H1N1) 2009 virus and the observed

efficiency of human to human transmission. Importantly, a declaration of a pandemic is independent of the severity of illness caused by the virus or the degree of infrastructure disruption. On July 24 2009, DHHS Secretary Kathleen Sebelius renewed DHHS' April 2009 determination that a public health emergency exists nationwide involving pandemic influenza H1N1 that has significant potential to affect national security.

From April 15, 2009 to July 24, 2009, states reported to the Centers for Disease Control and Prevention (CDC) a total of 43,771 confirmed and probable cases of novel influenza A (H1N1) infection. Of these cases reported, 5,011 people were hospitalized and 302 people died.[1,2] From August 30, 2009 to October 24, 2009, 25,985 hospitalizations and 2,916 deaths attributed to influenza and influenza-like illnesses have been reported in the United States (U.S.). CDC has developed a model to estimate the true number of cases in the U.S. The model took the number of cases reported by states and adjusted the figure to account for known sources of underestimation (e.g., not all people with pandemic influenza H1N1 seek medical care, and not all people who seek medical care have specimens collected by their health care providers). Using this approach, it is estimated that more than one million people became infected with novel influenza A (H1N1) between April and June 2009 in the U.S.[3]

The symptoms of human influenza disease caused by pandemic (H1N1) 2009 virus are similar to the symptoms of seasonal flu and include fever, cough, sore throat, runny or stuffy nose, body aches, headache, chills and fatigue. A significant number of people who have been infected with pandemic (H1N1) 2009 virus also have reported diarrhea and vomiting.[4]

The most severe outcomes have been reported among individuals with underlying health problems that are associated with high risk of influenza complications. Pandemic (H1N1) 2009 virus currently remains sensitive to oseltamivir (Tamiflu) and zanamivir (Relenza), though sporadic cases of resistance to oseltamivir have been reported. At this time, there is insufficient information to predict how severe the pandemic (H1N1) 2009 virus outbreak will be in terms of illness and death or infrastructure disruption, or how it will compare with seasonal influenza.

B. Potential Impact of the H1N1 Pandemic on Blood Product Safety and Availability

There is limited information available on pandemic (H1N1) 2009 virus viremia, especially during the asymptomatic period. No case of transfusion transmitted seasonal

[1] http://www.cdc.gov/h1n1flu/update.htm, (Accessed Nov. 2, 2009).
[2] CDC discontinued reporting of confirmed and probable cases of novel H1N1 infection on July 24, 2009. The most recent total numbers of hospitalizations and deaths due to H1N1 are available on the CDC website. http://www.cdc.gov/h1n1flu/update.htm, (Accessed Nov. 2, 2009).
[3] http://www.cdc.gov/h1n1flu/surveillanceqa.htm, (Accessed Nov. 2, 2009).
[4] http://www.cdc.gov/h1n1flu/sick.htm, (Accessed Nov. 2, 2009).

influenza has ever been reported in the U.S. or elsewhere, and, to date, no cases of transfusion transmitted pandemic influenza H1N1 have been reported. At this time, the pandemic (H1N1) 2009 virus has not been isolated from blood or serum of asymptomatic, infected individuals; however, studies are ongoing. Furthermore, the potential for transmission of pandemic influenza H1N1 through blood transfusion remains unknown.

In some previous studies, other Influenza A viruses were isolated from blood, and throat secretions or nasopharyngeal mucosa of children with clinical manifestations of influenza (Refs. 1-2). The virus was isolated from blood and throat washings of 1/29 healthy asymptomatic contacts who became ill 12 hours after the specimens were obtained (Ref. 3). From another study, virus isolation was reported from lungs, adrenals and meninges (from autopsy) which indicated that viremia must have been present (Ref. 4). In humans experimentally infected by nasal inoculation, viremia was observed in 4/15 subjects using sensitive culture methods. Symptoms occurred 2 days after initial viremia and one patient remained asymptomatic throughout the study period (22 days) (Ref. 5). However, other investigators were unable to detect viremia in 27 subjects using a similar virus strain and assay methods (Ref. 6).

The pandemic influenza H1N1 virus is a large lipid-enveloped virus. Validation studies performed by product manufacturers have shown that viruses with similar characteristics to the pandemic influenza H1N1 virus are effectively inactivated and/or removed during manufacturing of plasma derivatives.

Due to its known potential for rapid spread, pandemic (H1N1) 2009 virus has the potential to cause disruptions in the blood supply. A significant number of blood donors, blood establishment staff, and vendors of blood-related supplies (e.g., manufacturers of reagents and blood bags) could be affected as individuals become ill or need to care for ill family members. At the same time, during a widespread outbreak of disease caused by the pandemic (H1N1) 2009 virus, it is anticipated that the demand for blood and blood components may be reduced due to postponement of elective surgery, were that to become necessary in some affected healthcare settings.

In addition, the usual paradigm for ensuring blood availability in response to local disasters (i.e., hurricanes) may not be available under severe pandemic scenarios. In local disasters, interregional transfer of blood from unaffected to affected areas has been an effective strategy. However, in a more severe pandemic scenario, international, national, and regional outbreaks may occur simultaneously and a pandemic wave may last for months. Therefore, advanced planning is reasonable to prepare for the possible need to mitigate the effects of a more severe pandemic and to help ensure that blood is available in affected areas

Standard precautions for avoidance of contact with respiratory secretions may help to reduce the transmission of pandemic (H1N1) 2009 virus in blood and plasma collection establishments. The CDC has issued recommendations for infection control in the

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community[5], places of business[6], and in health care settings[7]. CDC also has issued "Interim Infection Control Guidance on 2009 H1N1 Influenza for Personnel at Blood and Plasma Collection Facilities."[8] We recognize the importance of the CDC recommendations for infection control in blood and plasma collection establishments.

III. RECOMMENDATIONS

FDA, in communication with DHHS Office of Public Health and Science, CDC, and the AABB Interorganizational Task Force on Pandemic Influenza and the Blood Supply, monitors blood availability closely. Similarly, we anticipate that you will maintain close communications with your hospital customers to anticipate demand for blood and blood components.

While shortages are not forecast at present, we are reminding you of regulatory pathways and providing regulatory clarification that may be helpful to you both in dealing with the current outbreak and in continuing to stay prepared.

We will continue to review any new scientific information about the potential risk of transfusion transmission of pandemic (H1N1) 2009 virus. We also will monitor closely the impact of the pandemic on blood availability. As our knowledge base grows, we may revise the recommendations in this guidance document as appropriate.

A. Training of Back-Up Personnel

Under 21 CFR 211.25 and 21 CFR 606.20, personnel performing critical functions in blood establishments must be adequate in number, educational background, training and experience, including professional training as necessary, or combination thereof, to assure competent performance of their assigned functions. Given the unknown extent of the disease caused by pandemic (H1N1) 2009 virus, we recommend that you have adequate back-up personnel, in the event of anticipatable personnel shortages. We further recommend that where possible, more than one back-up person should be trained for each critical function. Any such back-up personnel should be trained pursuant to your existing training program. We also recommend that as provided in your training program, you document this training and/or re-training.

[5] http://www.cdc.gov/h1n1flu/guidance/exclusion.htm, (Accessed Nov. 2, 2009).
[6] http://www.cdc.gov/h1n1flu/business/guidance, (Accessed Nov. 2, 2009).
[7] http://www.cdc.gov/h1n1flu/guidelines_infection_control.htm, (Accessed Nov. 2, 2009).
[8] http://www.cdc.gov/h1n1flu/guidance/blood_facilities.htm.

B. Blood Donor Suitability, Donor Deferral and Product Management

Blood Donor Suitability

In general, a donor medical history is obtained at the time of blood collection. However, under 21 CFR 640.3(a) and 21 CFR 640.63(a), the suitability of a donor as a source of Whole Blood or Source Plasma, must be made on the *day of collection* from the donor. These regulations do not explicitly define the term *day of collection.* Occasionally, donor's responses to the donor questions presented before collection are found to be incomplete upon review by the blood establishment. You may clarify a donor's response to the donor history questionnaire or obtain omitted responses to questions within 24 hours of the collection.

Blood Donor Deferral

- Under current FDA regulations, blood donors must be in good health, as indicated in part by normal temperature and free of acute respiratory diseases on the day of collection (21 CFR 640.3(a), (b)(1) and (4) and 21 CFR 640.63(a), (c)(1) and (7)).

- Available data do not currently support donor deferral for exposure to or contact with a person who has confirmed or probable pandemic (H1N1) 2009 influenza or influenza-like symptoms.

- To ensure donors are in good health on the day of donation as required under 21 CFR 640.3(b) and 21 CFR 640.63(c), donors with a confirmed or probable case of pandemic (H1N1) 2009 virus infection should be deferred until at least 24 hours after they are free of fever without the use of fever reducing medications[9] and they are otherwise asymptomatic.

- Available data do not support the deferral of donors following vaccination with live attenuated influenza vaccines (LAIV) or inactivated influenza vaccines against pandemic (H1N1) 2009 virus or for prophylactic use of the antiviral medications oseltamivir (Tamiflu) and zanamivir (Relenza). However, consistent with the recommendation above, donors taking antiviral medications for confirmed or probable pandemic (HIN1) 2009 virus infection should be deferred until at least 24 hours after they are free of fever without the use of fever reducing medications[10] and they are otherwise asymptomatic.

[9] A daily dose of pediatric aspirin (81 mg) is not considered fever-reducing medication.
[10] A daily dose of pediatric aspirin (81 mg) is not considered fever-reducing medication.

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Blood Product Management

The recommendations in this section apply to donations of Whole Blood and blood components intended for transfusion. This section does not apply to blood components intended for further manufacture (recovered plasma, Source Plasma, Source Leukocytes) since validation studies have shown that viruses with similar characteristics to pandemic (H1N1) 2009 virus are effectively inactivated and/or removed during manufacturing of plasma derivatives.

- Upon receipt of post donation information about a donor with confirmed or probable pandemic (H1N1) 2009 disease or influenza like illness within 48 hours after the donation, the Medical Director should evaluate the safety of the previously donated products consistent with existing Standard Operating Procedures (SOPs).

C. Changes to an Approved Application

As provided under 21 CFR 601.12(c)(5), we have determined that the following changes to an approved application for licensed blood establishments may be submitted as a "Supplement-Changes Being Effected".

- Use of a different outside test lab, provided the test lab is registered with FDA and has been performing donor testing.
- Implementation of self-administered donor history questionnaires, provided you follow the critical control points described in FDA's "Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires" (July 2003), and the submission contains the content recommended for all self-administered procedures and computer assisted interactive procedures outlined in the same guidance.

The recommendations set forth above supersede the recommendations in FDA's "Guidance for Industry: Changes to an Approved Application: Biological Products: Human Blood and Blood Components Intended for Transfusion or for Further Manufacture" (July 2001) at section IV.C and FDA's "Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires" (July 2003) at section IV.A, respectively (in both of these guidances, we previously had determined that these changes would require a "Supplement – Changes Being Effected in 30 Days").

IV. BIOLOGIC PRODUCT DEVIATION AND FATALITY REPORTING

Licensed manufacturers, unlicensed registered blood establishments, and transfusion services are subject to reporting requirements with respect to the reporting of product deviations under

21 CFR 606.171. Blood establishments are not expected to submit biological product deviation reports for post-donation information related to pandemic (H1N1) 2009 virus. If a complication of blood transfusion results in the fatality of a recipient, blood establishments must report the fatality to FDA as soon as possible (21 CFR 606.170(b)).

V. COLLECTION AND USE OF CONVALESCENT PLASMA

Plasma obtained after recovery from an acute infection (convalescent plasma) generally contains highly-specific antibodies directed at the infectious agent, and has theoretical potential to serve as a therapeutic product. In consideration that circumstances could arise where vaccines and antiviral drugs might not be sufficiently available, or where a patient is not responding to approved therapies, transfusion of convalescent plasma has been discussed as a possible empirical treatment during an influenza pandemic. (Ref. 7-8)

In July 2009, the WHO Blood Regulators Network issued a position paper[11] on the collection and use of convalescent plasma as an element in pandemic influenza planning. This paper recommends that scientific studies on the feasibility and medical effectiveness of the collection and use of convalescent plasma, and possibly fractionated immunoglobulins, should be explored through clinical trials. FDA encourages the development of new, safe and effective therapies for influenza. Because of its experimental nature, collection and administration of convalescent plasma should be conducted only under an Investigational New Drug Application. Blood establishments that intend to manufacture convalescent plasma should contact FDA to discuss their plans.

VI. IMPLEMENTATION

This guidance has been issued for comment purposes only.

[11] http://www.who.int/bloodproducts/brn/BRNPosition-ConvPlasma10July09.pdf, (Accessed Nov. 2, 2009).

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VII. REFERENCES

1. Naficy K., "Human Influenza Infection with Proved Viremia, Report of a Case". N Engl J Med. 1963. 31; 269:964-6.

2. Ritova VV, Schastnyi EI, Ratushkina LS, Shuster IY., "Investigation of the incidence of influenza A viraemia caused by virus strains circulating among children in 1968 – 1977". J Hyg Epidemiol Microbiol Immunol. 1979; 23(1):35-41.

3. Khakpour M, Saidi A, Naficy K., "Proved viraemia in Asian influenza (Hong Kong variant) during incubation period". British Medical Journal 1969:4, 208-209.

4. Roberts JT and Roberts GT., "Postsplenectomy sepsis due to influenzal viremia and pneumococcemia". Can Med Assoc J. 1976 September 4; 115(5): 435–437.

5. Stanley ED and Jackson GG., "Viremia in Asian Influenza". Trans Assoc Phys. 1966: 79: 376-7.

6. Alford RH, Kasel, JA, Gerone PJ, Knight V., "Human influenza resulting from aerosol inhalation". Proc. Soc. Exp. Biol. Med. 1966: 122:800-4.

7. Zhou B, Zhong N, Gong Y., "Treatment with convalescent plasma for influenza A (H5N1) infection". NEJM, 2007; 357(14):1450-1

8. Luke TC, Kilbane EM, Jackson JL, Hoffman SL., "Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment?" Ann Intern Med 2006; 145:599-609.

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研究報告の概要

多くの生物種に影響を及ぼしている致死的な神経組織障害である伝染性海綿状脳症（TSEs）において、病原性の重要因子は宿主にコードされている正常型プリオン蛋白（PrP^{C}）の異常型アイソフォーム（PrP^{Sc}）の蓄積である。PrP^{C} から PrP^{Sc} への変換についての正確な機序は理解されていないが、宿主 PrP^{C} 発現はプリオン伝播に効果的な感染性プリオンの産生の必要条件であることは明らかである。哺乳類の TSEs については多くの研究が実施されているが、魚における TSE の病原性についてほとんどわかっていない。
ここで私達は、BSE 感染ウシ又はスクレイピー感染ヒツジより調製された脳ホモジネートを経口投与されたヨーロッパヘダイは臨床症状を現わさなかったが、投与 2 年後に採取されたヨーロッパヘダイの脳は神経変性の徴候と抗タイ PrP 抗体が上昇し陽性に反応する沈着物の蓄積を示したことを提示する。
非感染動物由来の脳を投与されたコントロール群はそのような徴候を示さなかった。
注目すべきことに、TSE 感染脳ホモジネートよりも BSE 感染物質を投与された魚に多数のプロテアーゼ K 抵抗性の沈着物が急速かつ広範囲に現れた。これらのプラーク様凝集はコンゴ好染性と偏光下における複屈折を示し、アミロイド様成分と一致した。プリオン、特に BSE を投与された魚の脳における神経変性と異常な沈着物は公衆衛生上の潜在的リスクに関する懸念を増大させる。
魚の養殖はヒトや他の動物種に対する高タンパク栄養源を供給する経済的で重要な産業であり、感染性哺乳動物 PrP^{Sc} に汚染されている養殖魚におけるプリオン病発生の予測は気がかりであり、更なる評価が必要である。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリン 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
BSE 感染ウシ及びスクレイピー感染ヒツジの脳ホモジネートを経口投与されたヨーロッパヘダイの脳は神経変性と抗タイ PrP 抗体に反応する沈着物の蓄積を示し、公衆衛生上の潜在的リスクに関する懸念を増大させる可能性があるとする報告である。 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。

抗破傷風人免疫グロブリン

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Evaluation of the Possible Transmission of BSE and Scrapie to Gilthead Sea Bream (*Sparus aurata*)

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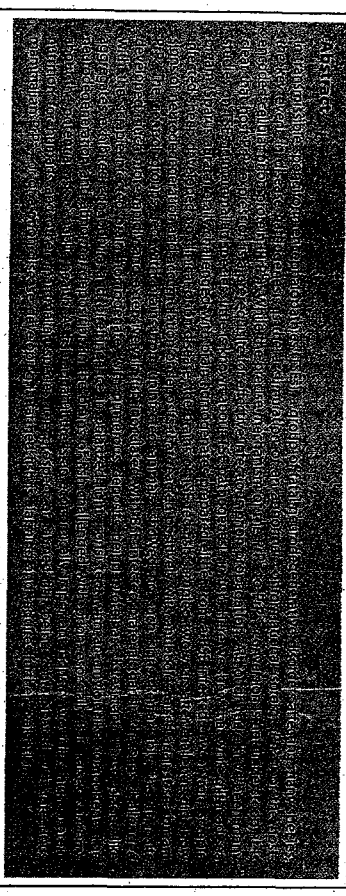

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Introduction

Transmissible spongiform encephalopathies or prion diseases are a group of fatal neurodegenerative disorders, including Creutzfeldt-Jakob disease (CJD), Fatal Familial Insomnia (FFI) and Gerstmann-Sträussler-Scheinker disease (GSS) in humans, scrapie in sheep and goats and bovine spongiform encephalopathy (BSE) in cattle [1].

The transmission of clinical prion diseases is limited by the so-called "species barrier" to conversion of endogenous host prion protein (PrP^{C}) to its abnormal, partially proteinase K-resistant conformational isoform, PrP^{Sc}. When high enough, this "barrier" can greatly impair or prevent potential interspecies transmissions, even under optimal conditions of dose and infection route. However, evidence of TSE replication without accompanying symptoms of clinical disease has prompted debate on the existence of asymptomatic infected individuals in an exposed population [2,3].

The identification of apparent PrP orthologues in lower vertebrates, including fish [4–16], raises the question of their susceptibility to prion diseases. While fish PrP-like sequences do not share high homology with their mammalian relatives (Table S1), they do contain several strongly conserved prion protein structural motifs [17]. Although mammalian to fish TSE transmission is considered unlikely [18], it is not certain that the species barrier would be high enough to prevent TSE transmission to fish.

The BSE epidemic has been linked to TSE-infected cattle feed [19] and the recognition of BSE in domestic cattle inevitably raised concerns about the potential risk to other ruminant and

non-ruminant livestock [20]. The European Commission's TSE risk-reducing measures include a total EU-wide ban on the use of all processed animal protein in livestock and aquaculture feeds. Any consideration of lifting this ban requires a scientific assessment of the TSE transmission risk through fishmeal. Another issue to be addressed is the rising concern that pigs, poultry or fish bred for human consumption and inadvertently fed with TSE-contaminated feed could eventually either develop clinical TSE or serve as reservoirs of infectivity without ever displaying clinical disease themselves. Such an assessment should consider the risk from TSE-contaminated feed being fed to farmed fish [18,21]. In aquaculture, a rapidly growing industry of economic importance in several EU countries, the farmed fish receive commercial feed containing 40–55% protein during the 12–20 months they generally spend in aquaculture facilities. Although remote, the possibility that some of this feed might be contaminated with mammalian prion cannot be excluded.

In the present work, we evaluated the potential transmission of TSEs to gilthead sea bream, a commercially important fish species. After force-feeding with multiple doses of brain homogenate prepared from either healthy or naturally BSE- or scrapie-infected cow or sheep, the fish were monitored for 2 years for evidence of disease development by clinical, histopathological and immunohistochemical criteria. None of the fish examined, showed symptoms of clinical disease. However, signs of neurodegeneration were often present and abnormal deposition was detected in the brains of both the scrapie-challenged and the BSE-challenged fish by 24 months post inoculation.

Results and Discussion

To evaluate the clinical state of the fish, we monitored control and TSE–challenged populations on a daily basis. Since locomotor deficits are often a major feature of the clinical presentation of prion diseases in a variety of hosts, we used the swimming behavior of the challenged fish as an indicator of their general activity and exploratory behavior. No clinical symptoms, including erratic swimming or behavioral abnormalities, were observed in any of the groups monitored. Although unusual in prion disease, a similar absence of clinical symptoms upon interspecies challenge has been reported for both the first passage of sheep scrapie and hamster prion transmission to mice [2]. In these cases, subsequent passage of brain material from the challenged individuals to additional mice did produce clinical disease, thereby demonstrating that asymptomatic animals can harbour high levels of infectious prions in their brains. Additionally, it is important to note that while certain experimentally or virally induced neurodegenerative effects do modify swimming parameters in fish, such as the swimming distance and orientation, the mean velocity, the turning angle and the equilibrium [22–24], this is not always the case. For instance, while both sea bass and sea bream can be infected with nodavirus, a naturally occurring piscine virus that causes brain lesions in both species, sea bream, in contrast to sea bass, show no clinical symptoms of disease [25,26].

To facilitate our evaluation we generated polyclonal antibodies against four different fish PrPs. The specificity of each antiserum was confirmed by both western blot (Fig. S1A) and immunohistochemistry (IHC) (Fig. S2A–C) with normal sea bream brain. Furthermore, anti-mammalian PrP antibodies (6H4, 12F10) did not stain sea bream brain, nor did our anti-fish PrP antisera recognize mammalian PrP^{Sc} (Fig. S1B). Moreover, absorption of our SaurPrP1 (*Sparus aurata* PrP1) antisera with recombinant sea bream PrP-1 protein [6], against which it was raised, resulted in a complete loss of its specific immunostaining in control fish (Fig. S2D).

Initially, in order to determine the distribution of normal endogenous PrP in the central nervous system of gilthead sea bream, we used our polyclonal antisera to perform a detailed immunohistochemical evaluation on brain sections from control fish. Regions displaying abundant PrP included the optic tectum (Fig. 1A), valvula cerebelli (Fig. 1B) and corpus cerebelli (Fig. 1C), while strong PrP-immunopositivity was generally observed in the nerve fibers (Fig. 1D). The most prominently stained regions of the optic tectum, homologue to the superior colliculus in mammals [22], were striatum fibrosum marginale (Fig. 1A and Fig. S2A, B) and striatum fibrosum profundum (Fig. S2A, B). Less intense labeling was observed in the striatum griseum centrale and striatum plexiform fibrosum externum layers of the optic tectum (Fig. 1A and Fig. S2A, B). Cerebellar PrP-immunopositivity was detected mainly in the molecular layer, between Purkinje cell dendrites, and in the granular layer, in the matrix surrounding the granule cells (Fig. 1C). The valvula cerebelli, a rostral protrusion of the cerebellum in the midbrain ventricle that has no counterpart in mammals, showed significant PrP-immunopositivity, similar to that observed in the molecular and granular layers of cerebellum (Fig. 1B and Fig. S2A, B). In cerebral regions, including thalamus, medulla oblongata, diencephalon and the lateral telencephalic pallium, proposed to be a homologue of the mammalian hippocampus [22], we observed intense labeling of fiber bundles (Fig. 1D), consisting primarily of dendritic and axonal prolongations in the neuropil. The same general PrP^{C} expression pattern was observed in challenged and control populations, with no variation detected over time. The remarkable similarity of the overall immunolabeling pattern obtained with our SaurPrP1 antiserum in sea bream brain to the PrP-immunostaining profile in the mammalian brain [27,28] provided further assurance of the specificity of our antibody for piscine PrP.

At the intracellular level, staining outlining the neuronal body was present in most of the neuronal populations observed, e.g. in the large neurons of the brainstem (Figure 1E). Axons displayed intense staining, while diffuse staining was observed inside some of these neuronal somata, suggesting a degree of PrP-immunopositivity within cell departments, e.g. the Golgi complex (Fig. 1E). These findings suggest that the intracellular localization of PrP in fish brain is comparable to the neuronal intracellular localization of mammalian PrP [27,29].

To test for pathology in selected peripheral tissues, we examined intestines and spleens from TSE-challenged fish, sampled at different timepoints. No lesions or any other abnormalities were revealed in comparison to the control individuals. Intestinal PrP-immunoreactivity was evident in the serosa, myenteric plexus and submucous plexus (Fig. 1F). At all timepoints, PrP-immunolabeling in spleen and intestinal tissue was similar in both TSE-challenged and control fish and revealed no PK-resistance and no lesions or any other abnormalities.

Detailed examination of brain sections revealed no histopathological evidence of disease in scrapie–challenged sea bream through 18 months post inoculation (p.i.). At 24 months, however, 2 out of 5 fish showed limited abnormal, PrP-immunoreactive, PK-sensitive, extracellular deposits (Table S3B) in the neuropil of brainstem, diencephalon, corpus cerebelli, valvula cerebelli, optic tectum and telencephalon (Fig. 2). Whilst the number of animals where plaques were found was too small to reach statistical significance, based on the high likelihood that the fish examined developed no plaques, we believe that the observation of aggregates in 2 out of 5 fish at the final time point could be considered as an important event of qualitative (and not

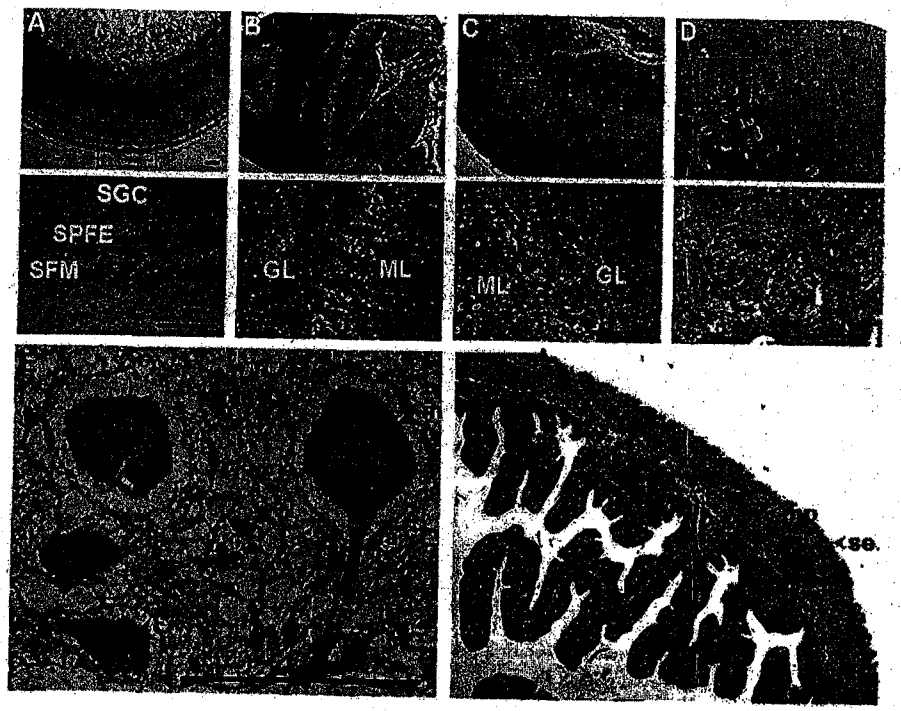

Figure 1. Normal PrP^{C} distribution in CNS and peripheral tissues. Sagittal, 4 μm-thick brain and intestine sections from control fish were treated with SaurPrP1 (1:2000 and 1:250, respectively) and normal endogenous PrP labeling in different anatomical regions was examined. **A**, Optic tectum; **B**, Valvula cerebelli; **C**, Corpus cerebelli; **D**, Nerve fibers in diencephalon; **E**, Neurons in brainstem; **F**, PrP-immunoreactive areas in the intestine. Rectangles indicate areas of magnification shown in the panel directly below. Arrowheads show positively stained regions. SGC, striatum griseum centrale; SPFE, striatum plexiforme et fibrosum externum; SFM, striatum fibrosum marginale; ML, molecular layer; GL, granular layer, m, plasma membrane; a, axon; c, cytoplasm. se, serosa; mp, myenteric plexus; sp, submucous plexus. Scale bars, 100 μm.
doi:10.1371/journal.pone.0006175.g001

quantitative) value (Text S1). No lesions were detected in the control fish force-fed normal sheep brain homogenate (Fig. S3 and Table S3B).

Plaque-like deposits were also observed in the brains of the BSE-challenged fish, beginning at earlier timepoints. Initially, at 8 months p.i., the majority of these aggregates were localized in brainstem, less in diencephalon and optic tectum, and even fewer in valvula cerebelli, cerebellum and telencephalon (Figure 3D–F). Just 10% of the deposits were PK-resistant and these had a mean diameter of 5 μm. Subsequently, we observed a general progression in their distribution, size, PK-resistance and morphological features. The incidence of the abnormal deposition was higher in fish sacrificed at earlier time points than at intermediate time points. However, the highest levels were measured at later time points, evocative of the phenomenon described in other prion cross-species transmission studies as an "eclipse" period [30].

Further analysis of the spatial and temporal progression revealed that with increasing time p.i., the deposition became more prominent in rostral brain regions, although caudal regions continued to be affected. By 24 months p.i., deposition in the brains of the BSE-challenged sea bream presented a striking picture, in which three out of five fish showed 500–800 deposits each, 70–85% of which were PK-resistant with a mean diameter of 30 μm. With regard to the remaining two fish, one displayed approximately 150 deposits, 93% of which were PK-resistant and the other showed only limited signs of abnormal aggregation. While deposits continued to be distributed throughout the brain at 24 months p.i., in the three highly affected fish the greatest increases in deposit numbers occurred in brainstem and diencephalon. The progression of the abnormal deposition is apparent in Fig. 3. and summarized in Figs. 4 and 5 and Tables S3A and S4.

In contrast to the BSE-challenged fish, no aggregates were detected at any time in the brains of the control fish fed with normal bovine brain homogenate (Fig. S4 and Table S3A). Notably, none of the brain tissues positive for abnormal deposition showed evidence of neuronal body degeneration. Finally, no residual mammalian PrP^{Sc} was detected using 12F10 and 6H4

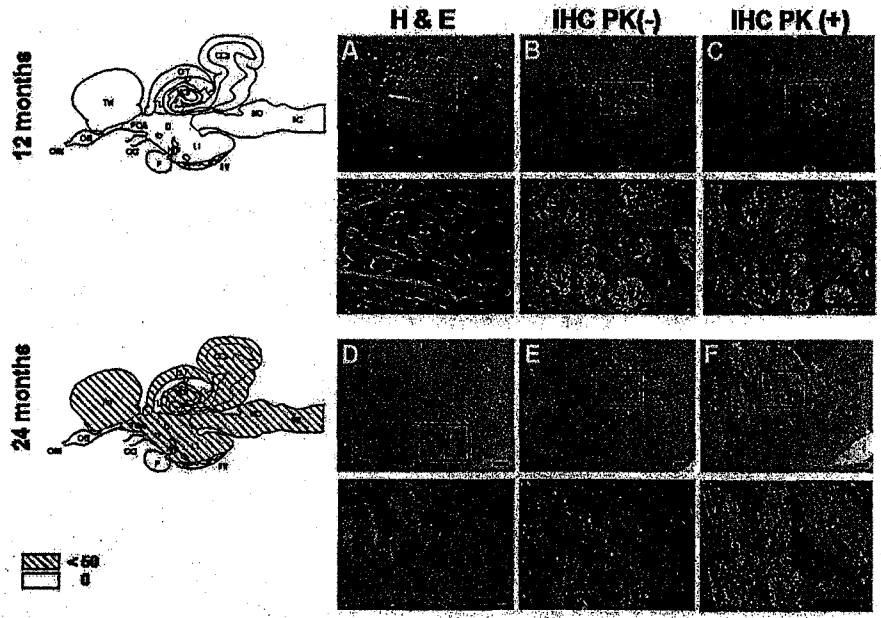

Figure 2. Progression of abnormal deposition in 2 scrapie-challenged fish. Sagittal brain sections from scrapie-challenged fish at 12 and 24 months p.i. were stained with H&E (**A, D**), or treated with SaurPrP1 (1:2000) without PK-digestion (**B, E**) and with PK-digestion (**C, F**). Images show diencephalon. The mean number of deposits (per section of fish containing deposits) observed in different brain regions without PK-treatment is indicated by the fill-type in the schematic drawings at the far left. CCe, corpus cerebelli; Di, diencephalon; Hyp, hypothalamus; LI, lobi inferioris; MO, medulla oblongata; OB, olfactory bulb; OC, optic chiasm; OIN, olfactory nerve; OT, optic tectum; P, pituitary; POA, preoptic area; SC, spinal cord; Tel, telencephalon; TL, torus longitudinalis; VCe, valvula cerebelli. The following areas were not examined: OB; OIN; OC; P. Rectangles indicate areas of magnification shown in the panels directly below. Arrowheads indicate the abnormal aggregates. Scale bars, 100 μm.
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monoclonal antibodies (data not shown). Overall, these data suggest that while both TSE strains resulted in similar abnormal brain pathology, the brains of BSE-challenged individuals were more rapidly and severely affected than those of scrapie-challenged fish. BSE, known to be a zoonotic TSE, may represent a thermodynamically favored PrP^{Sc} conformation that is permissive for PrP expressed in a wide range of mammalian species [31]. Despite this permissibility, however, attempts to orally transmit BSE to pigs and chickens have failed [32,33].

To characterize the nature of the deposition, we employed a variety of conventional staining techniques. Congo red-stained deposits in BSE-challenged sea bream brains at 24 months p.i. were congophilic (Figs. 6A and 7B) and birefringent under polarized light (Fig. 6B), suggesting an amyloid or amyloid-like component [34]. No Congo red birefringence was observed in either the control tissues or the scrapie-challenged fish brains (data not shown). While the plaque-like aggregates were prominent with hematoxyline and eosin (H&E) (Fig. 7A), Klüver-Barrera staining for myelin structures and von Kossa staining for calcium deposition both gave negative reactions (data not shown). Deposits were also PAS positive (Fig. 7C) but Alcian blue negative (Fig. 7E), indicating the presence of carbohydrates and the absence of acidic glycosaminoglycans, respectively. Finally, our four anti-fish PrP antisera positively labeled the deposits (see Fig. 7D for SaurPrP1), whereas the 12F10 and 6H4 antibodies did not (data not shown).

Two main types of plaque-like deposits were identified in the brains of the BSE-challenged fish: fibrous, diffusely stained aggregates (Fig. 8A, D, G, J), and those that were more amorphous and dense (Fig. 8B, E, H, K). At 8 months, small aggregates, generally in close proximity to neurofibrils, were detected, whereas the majority of the adjacent fiber bundles remained intact (Fig. 3D–F). At 10 and 12 months the first signs of neurodegeneration appeared as a primitive disorganization of dendrites and axons. By 16 and 18 months, the distention of neurites, mostly in grey matter, was exacerbated. The extensive deconstruction of microfilaments within the axons and the loss of their coherence, especially at 18 months, were detected histopathologically. Aggregates of dystrophic neurites were immunostained with SaurPrP1, exhibiting a diffuse PrP-immunolabeling with some marginal spicule-like projections (data not shown). At 24 months the diffusely immunolabeled aggregates of dystrophic neurites (Fig. 8A, D, G, J) coexisted with deposits that appeared more amorphous, condensed and flocculated and therefore were more intensely stained with all the techniques used (Fig. 8B, E, H, K).

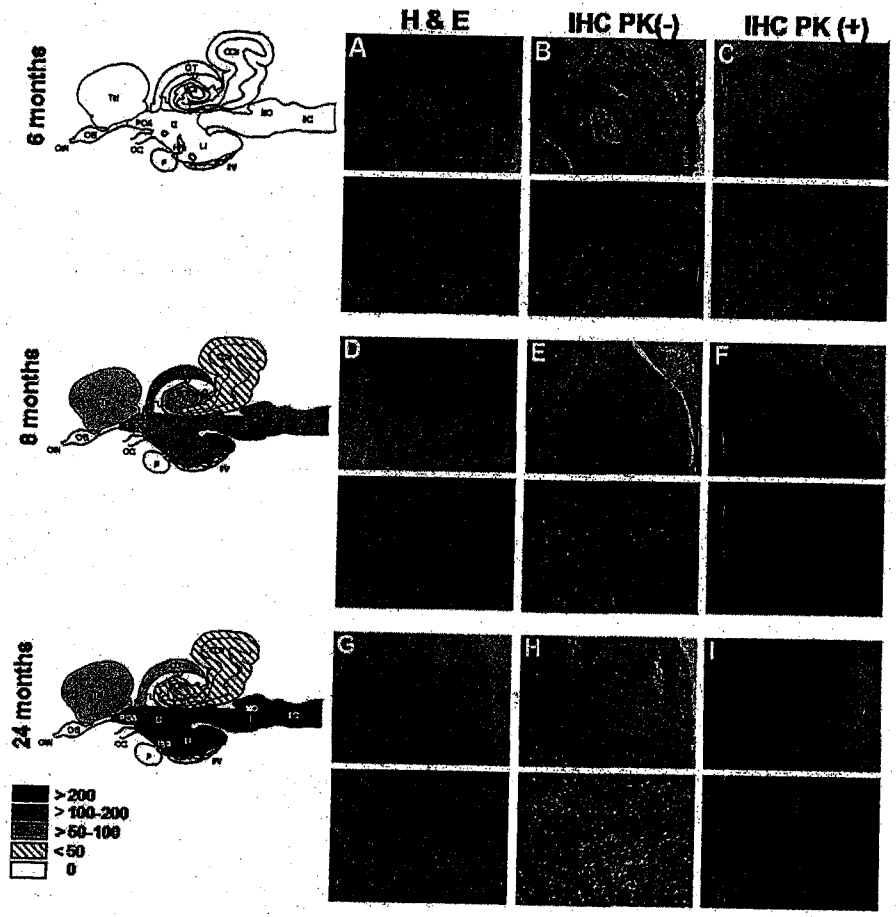

Figure 3. Progression of abnormal deposition in BSE-challenged fish. Sagittal brain sections from BSE-challenged fish taken at the indicated times p.i. were stained with H&E (**A, D, G**), or immunolabeled with SaurPrP1 (1:2000) without PK-digestion (**B, E, H**) and with PK-digestion (**C, F, I**). Images show diencephalon. The mean number of deposits (per section of fish containing deposits) observed in different brain regions without PK-treatment is indicated by the fill-type in the schematic drawings at the far left. Abbreviations as in Figure 2. The following areas were not examined: OB; OIN; OC; P. Rectangles indicate areas of magnification shown in the panels directly below. Arrowheads indicate the abnormal aggregates. Scale bars, 100 μm.
doi:10.1371/journal.pone.0006175.g003

Given the morphological progression of the abnormal deposition with time, it is tempting to hypothesize a scenario, in which the first type of aggregates (Fig. 8A, D, G, J) could have been the developmental ancestor of the second (Fig. 8B, E, H, K) and in which each may illustrate different stages of pathogenesis. The distention of axons and dendrites observed at 10 and 12 months p.i. reflects an initial neurodegenerative process in the brains of the BSE-challenged fish that may have been a very early reaction following exposure to the infectious agent. The complete destruction of the protective outer neurite layers, including the myelin sheath, followed by the disorganization of the microfilaments and microtubuli could have subsequently created the first morphological type of aggregates initially detected at 16 and 18 months. These "pre-mature" deposits mainly consist of dystrophic

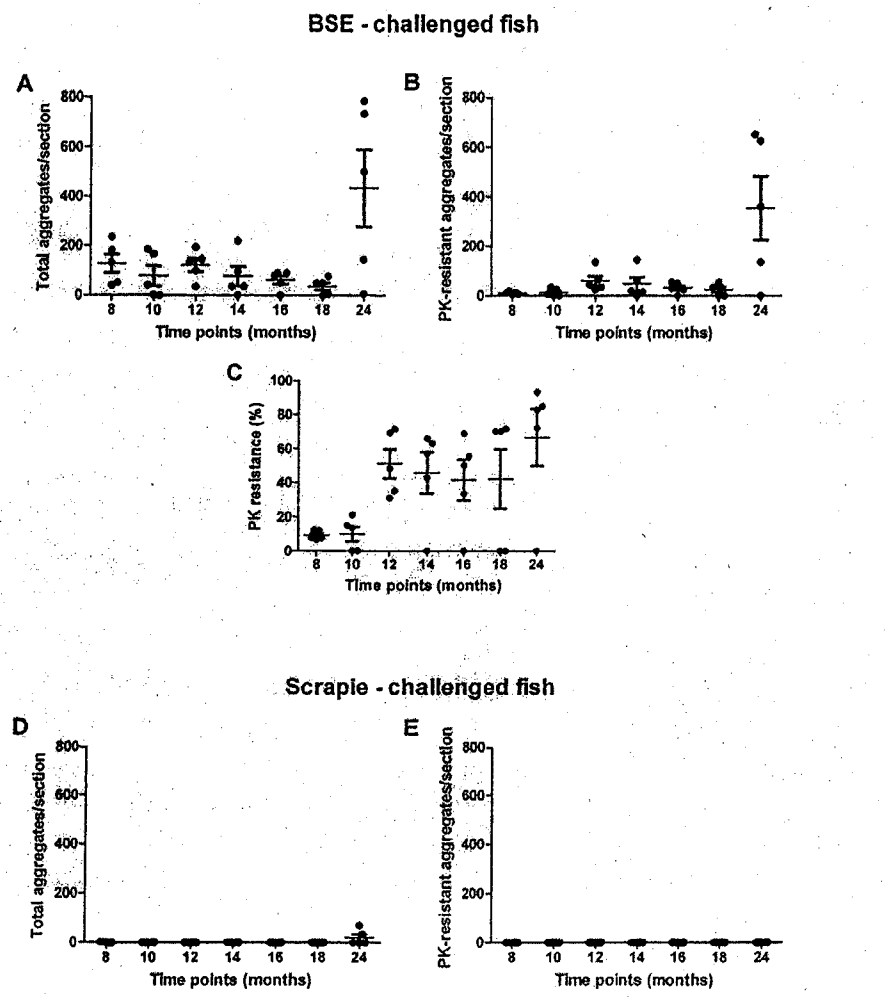

Figure 4. Abnormal deposits in the brains of the BSE- and the scrapie-challenged fish with reference to time. Each dot corresponds to the number of aggregates observed per brain section in each individual before PK-treatment (A, D) and after PK digestion (B, E), or to the percentage of PK-resistant aggregates in each BSE-challenged fish (C). Bars indicate the means and the standard error means (SEMs).
doi:10.1371/journal.pone.0006175.g004

neurites that have lost their coherence, with spicule-like projections, at their periphery (Fig. 8A, D, G, J). Given that aggregates were partially PK-resistant and showed affinity for Congo red by 24 months, the next step in the progression may have been the complete deconstruction of the fibers leading to the creation of a homogenous, flocculated, extracellular material that we describe as "mature" deposits (Fig. 8B, E, H, K), with increased PrP-immunopositivity, PK-resistance, congophilia and birefringence in polarized light [35]. Fish brains at 24 months post inoculation exhibit both types of abnormal aggregates, including intermediate

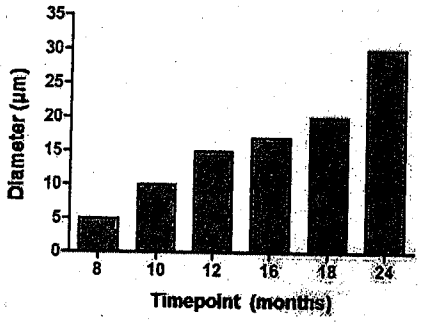

Figure 5. Progressive increase in the size of proteinase K resistant deposits in BSE-challenged fish. The mean diameter of the immunohistochemically detected (SaurPrP1) deposits after proteinase K-digestion is given with reference to time p.i.
doi:10.1371/journal.pone.0006175.g005

states (Fig. 8C, F, I, L) that cannot be easily classified into any of the previously described morphological categories.

The classical neuropathological hallmarks of prion diseases include neurodegeneration, spongiform change and gliosis, while PrP^{Sc} deposition is observed in the majority of TSEs. The lesion profile in the brains of the BSE-challenged fish shares both similarities and differences in comparison to this histological and immunohistochemical pattern of mammalian prion diseases. Specifically, the distribution of abnormal aggregates within the brains of 4 BSE-challenged fish at 24 months p.i. shared certain similarities with the PrP^{Sc} deposition pattern observed in TSE-affected mammalian brain. Notably, the abnormal deposits in sea bream brain were only detected in regions where neuronal parenchyma was present, a feature greatly resembling the location of mammalian prion deposition [36]. Cerebellum was extensively affected, with large fibrous aggregates in the molecular layer and a granule-like deposition profile in the granular layer, a pattern similar to that observed in mammalian TSEs [37]. The abnormal deposition in sea bream brain was also prominent both in the lateral nucleus of the ventral telencephalic area, a fish counterpart to the basal nucleus of Meynert in mammals [38], and the lateral telencephalic pallium, homologue to the mammalian hippocampus [22]. Both thalamus and diencephalon displayed numerous aggregates, most of which were interspersed within neuronal fibers. In striking contrast to the general neuropathological profile of mammalian TSEs, however, no vacuoles were observed in any regions of the fish brains examined. While spongiosis is a main characteristic in most prion diseases, it must be noted that in certain TSE subtypes there is little or no spongiform change. Such has been the case in patients suffering from FFI, an inherited human prion disease [39].

Evidence of neurodegeneration, although distinct from that commonly associated with mammalian prion disease, was apparent in many brain regions, primarily in places where the abnormal deposition was located adjacent to or within complexes of neurites (Fig. 8C, L). It is important to note, however, that no degeneration associated with neuronal somata was detected in any of the anatomical regions examined. The absence of classically defined neurodegeneration might be related to the ability of fish to produce new neurons continuously throughout their lifetime. It is known, for instance, that adult fish can regenerate damaged retinal tissue, optic axons and descending brainstem axons, leading to functional recovery [40,41]. In fact, this ability of adult fish for CNS regeneration has been postulated to explain the asymptomatic carrier state of halibut persistently infected with nodavirus

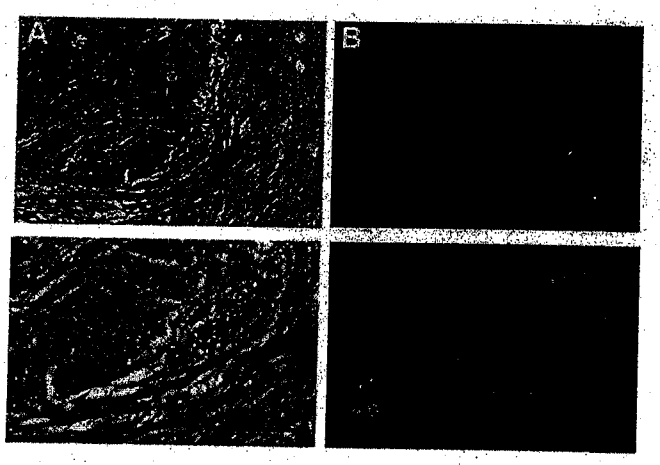

Figure 6. Congo red staining of deposits in the brain of BSE-challenged sea bream. A sagittal, 10 µm-brain section from a BSE-challenged individual, 24 months p.i., was stained with Congo red. A, Diencephalon with light microscopy; B, Same region in polarized light. Rectangles indicate areas of magnification shown in the panel directly below. Arrowheads indicate the abnormal aggregates. Scale bars, 100 µm.
doi:10.1371/journal.pone.0006175.g006

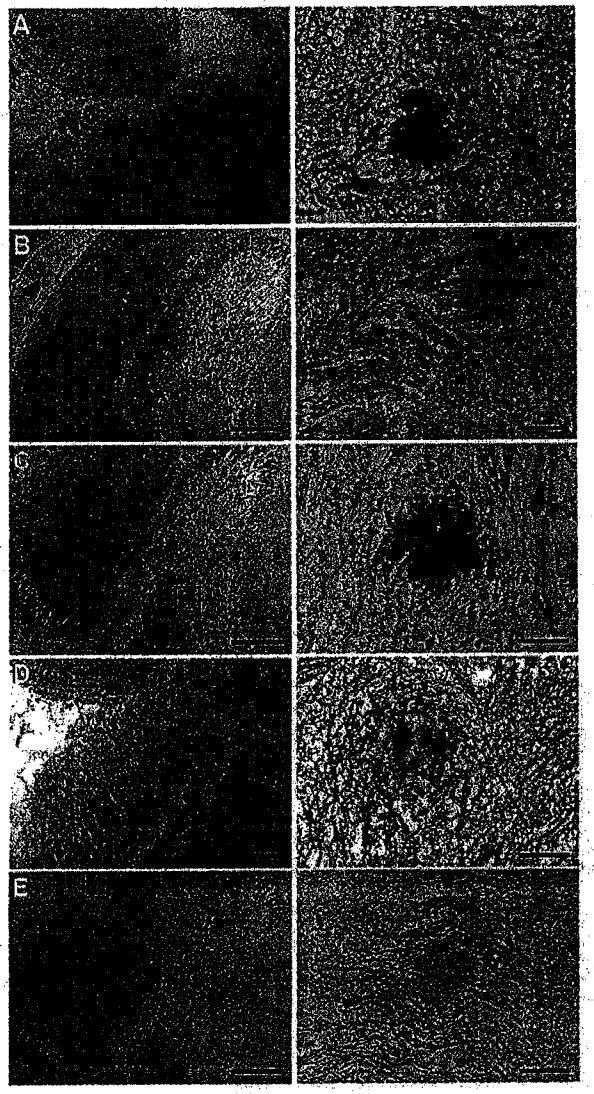

Figure 7. Staining of an aggregate in the brain of a BSE-challenged fish 24 months p.i. with different techniques. A, H&E; B, Congo red in normal light; C, PAS; D, IHC (SaurPrP1) after PK-digestion; E, Alcian blue. Rectangles in the left panel indicate areas of magnification shown in the right panel. Scale bars, 100 μm (left panel) or 10 μm (right panel).
doi:10.1371/journal.pone.0006175.g007

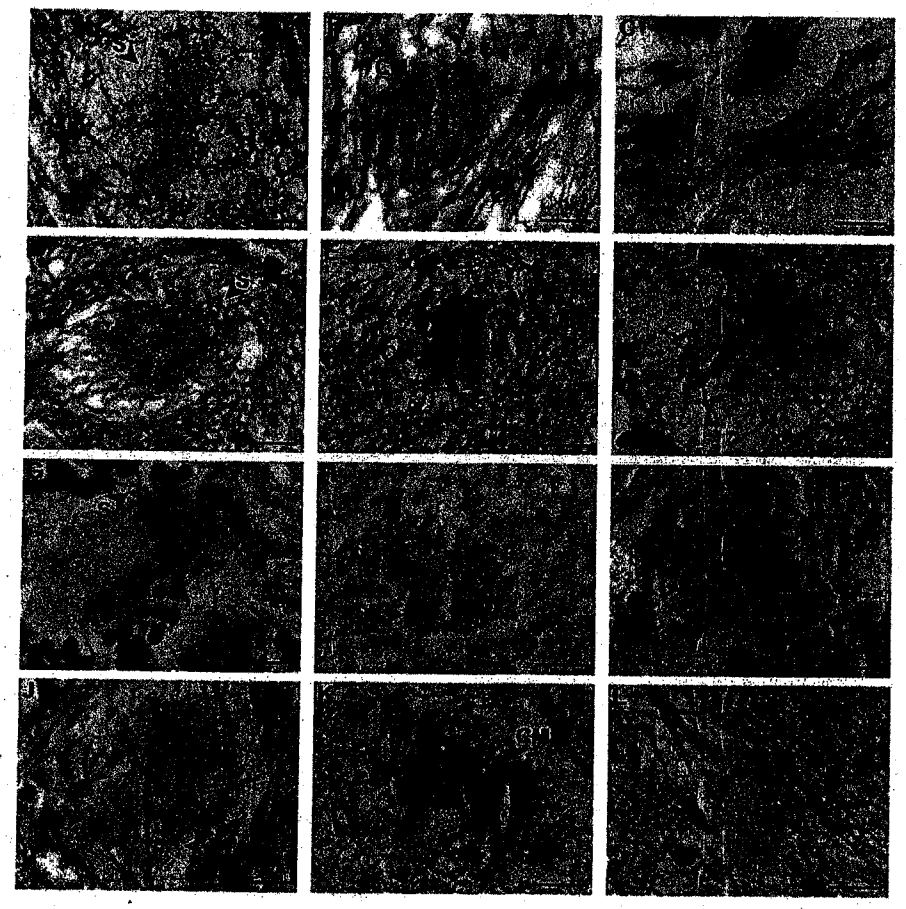

Figure 8. Morphology of the abnormal deposition in the brains of the BSE-challenged fish 24 months p.i.. Sagittal 4 μm-thick brain sections where stained with hematoxylin without a counterstain. pH variation renders the deposits visible by light microscopy. The panels on the left show diffuse "pre-mature" deposits in A, diencephalon; D, optic tectum; G, cerebellum; J, brainstem. The middle panels display examples of compact "mature" deposition in B, diencephalon; E, optic tectum; H, cerebellum; K, telencephalon. The panels at the right show intermediate stage-abnormal deposition within fiber bundles that disrupts synapses in C, diencephalon; F, optic tectum; I, valvula cerebelli; L, telencephalon. S, spicule-like projections; CM, condensed material. Scale bars, 10 μm.
doi:10.1371/journal.pone.0006175.g008

[42]. In the present study it may have contributed to the "eclipse-like" temporal appearance of the abnormal deposition, as well as to the lack of clinical symptoms in our BSE-challenged fish.

Despite the positive IHC results, western blotting failed to detect PK-resistant PrP isoforms in the TSE-challenged fish brains (Fig. S5), possibly because the whole brain homogenates used did not have a high enough concentration of PK-resistant PrP to allow detection. In fact, it is clear from the IHC results that even at 24 months p.i., the brain regions associated with the abnormal deposits constitute only a small percentage of the whole brain mass.

The results of this TSE transmission study with gilthead sea bream indicate the development of a CNS histopathology in the brains of the fish challenged with the TSE-inocula. This neuropathology displays characteristics resembling a novel fish amyloidosis more than a classical TSE. Specifically, while the fish

in our study showed no brain spongiosis and no clinical abnormalities, we did find numerous plaque-like deposits in the brains of a significant proportion of the BSE-challenged fish, especially. Although much of the PrP associated with these deposits is PK-sensitive, this should not be taken as an indicator of low potential infectivity, as instances of clinical prion disease, and even infectivity, associated with extremely low levels of detectable PK-resistant PrP have been reported [43–45].

In light of the serious ramifications that would follow an unequivocal demonstration of prion disease transmission to fish, it must be emphasized here that the abnormal deposition we observed in the brains of the TSE-challenged fish could possibly have resulted from pathogenic factors other than the prions they were fed. Despite the fact that no such naturally occurring, cross-species infections from mammals to fish have ever been reported [46], we cannot completely rule out this possibility. Thus, however unlikely, one must consider the possibility that the brains used to prepare the inocula for the TSE challenge were infected with an undetected virus or bacteria in addition to the scrapie or BSE present. Together, the time course of brain lesion appearance, i.e. months not days, the ability of the agent to survive the oral challenge route, the absence of brain histopathology in any of the control groups and the production of novel histological lesions in both the BSE- and the scrapie-challenged fish, in the absence of inflammation, however, make this possibility a remote one. A more plausible alternate explanation would be that the amyloidogenic nature of the TSE-inocula might have contributed to the development of a novel fish brain amyloidosis.

Infectivity and transmissibility are crucial issues that still need to be addressed. From a public health standpoint, the transmissibility of each prion strain and the relative ease with which it crosses species barriers, are its most significant characteristics. The spectrum of prionopathies, which has broadened in recent years, includes prion diseases that are not readily transmissible (*e.g.* some GSS cases), prion strains often associated with negligible clinical symptoms (*e.g.* the Nor98 scrapie strain), and even some without detectable PrP^{Sc} (*e.g.* PSPr) [44,47,48]. It is clear, then, that the evaluation and identification of both unusual prion diseases and prion diseases affecting unusual hosts is a complex task, requiring lengthy studies of pathogenesis, infectivity and transmissibility [49]. Until ongoing transmission studies using "bovinized" transgenic mice are completed, the possibility that the affected sea bream brain tissue might be infectious, must be taken seriously in any consideration to lift EU feed bans, especially those related to farmed fish.

Materials and Methods

Ethics Statement

All fish used in the experiments described in this work were treated in accordance with EU Council Directive 86/609/EEC for the protection of animals used for experimental and other scientific purposes.

Fish

Sixteen hundred gilthead sea bream of approximately 20 g weight were purchased from a commercial farm (Interfish, Greece). At the commercial farm, before purchase, fish were fed commercial pellets (Biomar), none of which contained protein sources derived from land animal products. After transportation to the laboratory (Fisheries Research Institute, Kavala, Greece), they were maintained at 18°C in temperature-controlled recirculating water tanks. After a two week adaptation period, the fish were divided into groups of 200 in separate tanks. The fish were allowed a further three weeks of acclimatization before experimental manipulations were initiated.

Preparation of inocula

For the force feeding of the fish, 10% (w/v) brain homogenates from scrapie-infected sheep, healthy control sheep, BSE-infected cow and healthy control cow were prepared in PBS (pH 7.4). For the sheep brain homogenates both cerebellum and brainstem from two animals were used (kindly provided by Dr. P. Toumazos, Veterinary Services, Cypriot Ministry of Agriculture), while the bovine brain homogenates were each prepared from the brainstem of a single animal. The BSE sample (RBSE 21028), taken in 1991 from a female Fresian two months after disease onset, was kindly provided by Dr. Ian Dexter, Pathology Department, Veterinary Laboratories Agency, Weybridge, UK. As healthy herdmate tissue was not available, the healthy control bovine brainstem was taken from a local Greek cow in 2002. All brain samples were stored at −80°C prior to use.

Challenge and maintenance

For inoculations, fish were removed from the tanks and mildly anaesthetized with 0.3% ethylene glycol monophenyl ether. Following anaesthesia, each fish was force-fed 100 µl brain homogenate. In total, 2 groups of 400 fish each, were each treated with scrapie-infected or control sheep brain homogenate and 2 groups of 200 fish each were each treated with BSE-infected or control bovine brain homogenate. For both the experimental and control groups, the force-feeding procedure was repeated fortnightly for a total of five treatments, so that the cumulative inoculum for each fish was 50 mg brain equivalents. Following the inoculation period all fish were kept on a maintenance diet with commercially available chow to prevent excessive growth and overcrowding during the multiyear study period. Data regarding maintenance of the fish, mortality due to technical and natural causes and sampling are shown in Table S2.

Clinical examination

Clinical evaluation of the fish in each tank was made on a daily basis, checking especially for any behavioral or swimming abnormalities.

Histopathological evaluation

Individuals from each group (5 TSE-treated and 5 controls) were sacrificed at regular selected time points post inoculation (3, 6, 8, 10, 12, 14, 16, 18, 24 months) and tissue samples, including brain, spleen and intestine, were taken. Tissues were fixed in buffered formalin (pH 7.4), embedded in paraffin wax and finally 4 µm-thick serial sections were subjected to conventional staining with a variety of staining techniques including H&E, PAS, Alcian blue, von Kossa and Klüver-Barrera. The resulting sections were examined histologically using light microscopy (Axioplan 2 Imaging System, Zeiss). Tissue pictures were taken using the Nikon Digital Sight DS-SMc visualizing system.

Congo red staining

For the identification of possible amyloid-like structures, 10 µm-thick brain sections were deparaffinized, stained with 0.5% Congo red (Merck, Darmstadt, Germany) alcohol solution for 15 minutes, destained in 0.2% KOH, subsequently counterstained with Mayer's hematoxyline, and after a short dehydration, they were finally cleared in xylene. The stained sections were observed microscopically under both normal and polarized light (Axiolab Carl Zeiss, rotatable analyzer +/−5°, 6×25, rotatable compensator Lambda, +/−5°, 6×25).

Generation of polyclonal antisera

The presumed mature sequences spanning residues 24–580 of zebrafish (*Danio rerio*) PrP-1 and residues 18–539 of zebrafish PrP-2 (sequence data provided by Dr. Edward Málaga-Trillo, Department of Biology, University of Konstanz), were each amplified from genomic DNA, whereas the mature sequence of gilthead sea bream (*Sparus aurata*) PrP-1 spanning residues 26–475 was cloned from plasmid DNA. All three were cloned into the pET21d DNA vector (Novagen, San Diego, CA) to produce recombinant proteins tagged with six histidine residues at the C-terminus. After sequence verification by double-stranded sequencing, the recombinant proteins were expressed in BL21 (DE3) *E.coli* (Stratagene, La Jolla, CA) with IPTG induction from single clone colonies. The recombinant proteins were purified under denaturing conditions from cell lysates on Ni-NTA agarose columns (Qiagen, Hilden, Germany) and then specifically eluted with imidazole. The polyclonal antisera ZebPrP1, ZebPrP2 and SaurPrP1 were raised against the purified zebrafish PrP-1, zebrafish PrP-2 and sea bream PrP-1 proteins respectively, by 3 successive subcutaneous inoculations of rabbits with 150 to 200 µg of recombinant protein at 4 weeks intervals. All pertinent sequence data are deposited in GenBank and the accession numbers are given at the end of the manuscript.

Affinity purification of SaurPrP1 polyclonal antiserum

2 aliquots containing 150 µg of gilthead sea bream recombinant PrP-1 protein each, were loaded onto 10% polyacrylamide gels and after SDS-PAGE, they were each electrotransferred to a nitrocellulose or a PVDF membrane. After electrophoresis the two membranes were stained with amido black staining solution (0.1%) and the protein-containing membrane pieces were finally excised. After a blocking step in 50 mM Tris HCl [pH 7.4], 150 mM NaCl, 0.05% Tween 20 (TBST) containing 5% milk, for 1 hr at RT, each membrane piece was probed with blocking buffer containing 500 µl of SaurPrP1 antiserum, at 4°C overnight. Following several washes, the IgGs that specifically bound to PrP-1, were finally eluted from the membranes with 0.2 M Glycine.HCl [pH 2.5], for 5 min at 4°C. Each eluate was neutralized with 2 M Tris HCl [pH 9.0], and then dialyzed overnight at 4°C in 50 mM Tris HCl [pH 7.4], 150 mM NaCl (TBS), using 100 mm-Spectra/Por molecularporous membrane tubing (Spectrum Medical Industries, Los Angeles, USA). Following dialysis, the purified IgGs were saturated in buffer containing 20 mM Tris HCl [pH 8.4], 150 mM NaCl, 5 mM EDTA, 1% gelatin, 0.1% BSA.

Depletion of recombinant gilthead sea bream PrP-1 – specific immunoglobulin fraction from SaurPrP1 polyclonal antiserum

SaurPrP1 polyclonal antibody was diluted in phosphate buffered saline (1:2000), containing 5% normal goat serum, 2.5% BSA and 0.05% Tween 20. The antibody was incubated with 0.6 mM of recombinant gilthead sea bream PrP-1 protein at 4°C overnight. The depleted antiserum was briefly centrifuged before use in all negative immunohistochemistry control experiments.

Immunohistochemistry

Four different polyclonal anti-PrP antibodies were used for the immunohistochemical detection of the endogenous PrP proteins of sea bream, namely ZebPrP1 (1:1000), ZebPrP2 (1:1000), SaurPrP1 (1:2000) and FuguPrP1 (1:500), the latter being raised by our group against PrP-1 protein of *Takifugu rubripes*. The commercially available monoclonal antibody, 12F10 (Cayman Chemical, Ann Arbor, MI), raised against amino acids 142–160 of human PrP, was used for the detection of residual mammalian PrP (1:200), since it also displays cross-reactivity with both ovine and bovine PrP. All paraffin sections were cut at 4 µm thickness. Depending on the prion protein of interest, PrP^{C} or PrP^{Sc}, two different pretreatment protocols were used. For PrP^{C} labeling, an antigen retrieval step was performed by boiling in citrate-buffered saline [pH 6.0] for 7 minutes before the staining procedure. For PrP^{Sc} detection, the sections were hydrated-autoclaved at 121°C for 30 minutes, then incubated for 5 minutes in 90% formic acid prior to an 8 minutes-incubation with proteinase K (Dako, Glostrup, Denmark) at RT. Sections were treated with appropriate biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA) and visualized using the avidin-biotin method-based Vectastain Elite ABC and the Diaminobezidine substrate kits (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. Negative controls for immunohistochemistry involved omitting the primary antibody. Staining with polyclonal (anti 14-3-3β, Santa Cruz, California, USA) and monoclonal antibodies (12F10, 6H4) raised against proteins of mammalian origin was also performed. The mouse anti-tubulin monoclonal antibody (Abcam, Cambridge, UK) and the monoclonal SAF84 antibody (raised against SAF preparation from infected hamster brain, assumed epitope 126–164) were used as well.

PrP^{Sc} enrichment and Western blot analysis

Western blot analysis of potentially enriched mammalian and teleost PrP^{Sc} was performed on brain homogenates from BSE-infected cows, scrapie-infected sheep, and TSE-challenged fish. Briefly, aliquots of 10% (w/v) brain homogenate were digested for 1 hr at 37°C with proteinase K at 25 µg/ml for sheep, 30 µg/ml for cow and 0.1–10 µg/ml for sea bream. PMSF (5 mM) was added to stop the protease digestion and PrP^{Sc} was precipitated with NaCl (10%) (w/v). The pellet was washed with 25 mM Tris HCl [pH 8.8] containing 0.05% sarkosyl and then resuspended in an appropriate volume of 2.5× O'Farrell buffer for gel electrophoresis.

For western blot analysis, untreated and proteinase K treated brain homogenates were analyzed by SDS-PAGE on 12% polyacrylamide gels and the separated proteins were then transferred onto PVDF membranes. After blocking with phosphate buffered saline containing 0.1% Tween 20 (PBST) and 5% milk, the immunoblots were probed with the fish-PrP specific polyclonal antisera, ZebPrP1 (1:10000), ZebPrP2 (1:35000), SaurPrP1 (1:20000), FuguPrP1 (1:20000), and the monoclonal antibody 6H4 (1:5000) (Prionics, Zurich, Switzerland) overnight at 4°C. After washing, they were incubated for 1 hr with either alkaline-phosphatase or horseradish-peroxidase conjugated secondary goat anti-rabbit or anti-mouse antibodies (Pierce, Rockford, IL) diluted 1:10000 in PBST. The blots were developed using the CDP-Star chemiluminescent substrate (NE Biolabs, Beverly, MA), or the ECL Western blotting Substrate (Pierce, Rockford, IL), depending on the secondary antibody and according to the manufacturer's instructions.

GenBank Accession Numbers

Danio rerio prion protein 1 coding sequence: AY438683
Danio rerio prion protein 1: AAS00159
Danio rerio prion protein 2 coding sequence: AY438684

Danio rerio prion protein 2: AAS00160
Sparus aurata prion protein 1 coding sequence: ABB90540

Supporting Information

Table S1 Percentage amino acid sequence homology between prion proteins of different species. Full sequences were aligned. The NCBI accession numbers of sequence data are: Homo sapien (human), AAC78725; Bos Taurus (cow), AAD19998; Ovis aries (sheep), CAE00188; Mus musculus (mouse), AAH06703; Mesocricetus auratus (hamster), AAA37092; Takifugu rubripes (fugu), AAN38988; Danio rerio (zebrafish) prion protein 1, AAS00159; Danio rerio prion protein 2, AAS00160; Sparus aurata (gilthead sea bream) prion protein 1, ABB90540. Sequence alignments were performed by ALIGN (version 2, Myers and Miller, CABIOS (1989) 4:11–17).
Found at: doi:10.1371/journal.pone.0006175.s001 (0.09 MB TIF)

Table S2 Cumulative record of the number of fish maintained post challenge. A, Fish inoculated with either scrapie- or normal ovine brain homogenate. B, Fish inoculated with either BSE- or normal bovine brain homogenate.
Found at: doi:10.1371/journal.pone.0006175.s002 (0.04 MB DOC)

Table S3 Cumulative record of brain tissue samples examined. A, BSE-challenged and bovine control fish samples. B, Srapie-challenged and ovine control fish samples.
Found at: doi:10.1371/journal.pone.0006175.s003 (0.14 MB DOC)

Table S4 Cytoanatomical analysis of brains from BSE-challenged fish sacrificed 24 months post challenge. The deposits have been classified into 2 morphological categories. F, fibrillary, >10 μm in diameter; NF, non fibrillary, circular<10 μm in diameter. Plus and minus symbols indicate the abundance of deposits: −, 0; +, 1–5; ++, 6–15; +++, 16–50, ++++, >50. NP, anatomical region not present in section; Mol L, molecular layer; Gran L, granular layer; WM, white matter; Cx, cortex; Ce, cerebellum; Vc, valvula cerebelli; Tel, telencephalon; Di, diencephalon; OT, optic tectum; Br. st., brain stem.
Found at: doi:10.1371/journal.pone.0006175.s004 (0.13 MB TIF)

Figure S1 Comparison of antibody specificities for the PrPs of gilthead sea bream, cow and sheep by western blot analysis. A, Five 0.4 mg brain equivalent-aliquots of gilthead sea bream brain homogenate were loaded onto a 12% SDS-PAGE gel. B, *Alternating* lanes of a 12% SDS-PAGE gel were loaded with 3 mg tissue equivalents of PrPSc-enriched (see Materials and Methods) bovine BSE brain homogenate (lanes 1, 3, 5, 7 & 9) and ovine scrapie brain homogenate (lanes 2, 4, 6, 8 & 10). The electrophoretically separated proteins were transferred to PVDF membranes that were cut into four sections (in B, each section included two adjacent lanes). Each section was stained with one of five primary antibodies: 6H4 (1:5000; lanes A1, B1, B2); FuguPrP1 (1:20000; lanes A2, B3, B4); ZebPrP1 (1:10000; lanes A3, B5, B6); ZebPrP2 (1:35000; lanes A4, B7, B8); SaurPrP1 (1:20000; lanes A5, B9, B10). After incubation with the appropriate alkaline-phosphatase-conjugated secondary antibody, the blots were developed with the CDP-Star reagent. The arrow heads indicate the positions of the molecular mass markers: A, 62 kDa and 47.5 kDa; B, 32.5 kDa.
Found at: doi:10.1371/journal.pone.0006175.s005 (0.28 MB TIF)

Figure S2 Antibody specificity in IHC. Sagittal, 4 μm-thick serial brain sections from control gilthead sea bream were treated immunohistochemically with four different primary antibodies, without proteinase K digestion. A, SaurPrP1 (1:2000); B, ZebPrP2 (1:2000); C, Pre-immune serum from the rabbit in which SaurPrP1 was raised (1:2000); D, PrP-specific immunoglobulin-depleted SaurPrP1 (1:2000). Arrowheads indicate the existence (A, B) or absence (C, D) of PrP-immunopositivity. SGP, striatum griseum periventriculare; SFP, striatum fibrosum profundum; SGC, striatum griseum centrale; SPFE, striatum plexiforme et fibrosum externum; SFM, striatum fibrosum marginale; ML, molecular layer of the valvula cerebelli; GL, granular layer of the valvula cerebelli. Scale bars, 100 μm.
Found at: doi:10.1371/journal.pone.0006175.s006 (8.23 MB TIF)

Figure S3 Temporal observation of the brains from control fish challenged with normal ovine brain homogenate. Sagittal brain sections taken at 12 and 24 months p.i. from fish challenged with normal ovine brain homogenate were stained with H&E (A, D), or immunolabeled with SaurPrP1 (1:2000) without PK-digestion (B, E) and with PK-digestion (C, F). Images show diencephalon. The mean number of deposits (per section of fish containing deposits) observed in different brain regions without PK-treatment is indicated by the fill-type in the schematic drawings at the far left. Abbreviations as in Figure 2 of the main manuscript. The following areas were not examined: OB; OIN; OC; P. Rectangles indicate areas of magnification shown in the panels directly below. Scale bars, 100 μm.
Found at: doi:10.1371/journal.pone.0006175.s007 (3.00 MB TIF)

Figure S4 Temporal observation of the brains from control fish challenged with normal bovine brain homogenate. Sagittal brain sections taken at the indicated timepoints p.i. from fish challenged with normal bovine brain homogenate were stained with H&E (A, D, G), or immunolabeled with SaurPrP1 (1:2000) without PK-digestion (B, E, H) and with PK-digestion (C, F, I). Images show diencephalon. The mean number of deposits (per section of fish containing deposits) observed in different brain regions without PK-treatment is indicated by the fill-type in the schematic drawings at the far left. Abbreviations as in Figure 2 of the main manuscript. The following areas were not examined: OB; OIN; OC; P. Rectangles indicate areas of magnification shown in the panels directly below. Scale bars, 100 μm.
Found at: doi:10.1371/journal.pone.0006175.s008 (4.48 MB TIF)

Figure S5 Sensitivity to proteinase K treatment of TSE-challenged sea bream brain tissues 24 months p.i.. After a short purification treatment, 0.4 mg brain equivalents from brain homogenates of either scrapie- (lanes 1, 3, 5 & 7) or BSE-challenged (lanes 2, 4, 6 & 8) fish, were digested with increasing proteinase K concentrations (0 μg/ml, lanes 1 & 2; 0.1 μg/ml, lanes 3 & 4; 1 μg/ml, lanes 5 & 6; 10 μg/ml, lanes 7 & 8) for 1 hr at 37°C. The samples were analyzed on a 12% SDS-PAGE gel, then electrotransferred onto a PVDF membrane and probed with SaurPrP1 polyclonal antibody (1:20000). After incubation with the appropriate secondary antibody, the immunoblots were finally developed with the ECL western blotting substrate. Arrowhead, 47.5 kDa.
Found at: doi:10.1371/journal.pone.0006175.s009 (0.27 MB TIF)

Text S1 Statistical analysis of data derived from the scrapie - challenged group.
Found at: doi:10.1371/journal.pone.0006175.s010 (0.02 MB DOC)

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This article is dedicated to Giorgos Arvanitidis, champion of the Olympus Marathon.

References

1. Prusiner SB (1998) Prions. Proc Natl Acad Sci U S A 95: 13363–13383.
2. Hill AF, Collinge J (2003) Subclinical prion infection in humans and animals. Br Med Bull 66: 161–170.
3. Thackray AM, Klein MA, Bujdoso R (2003) Subclinical prion disease induced by oral inoculation. J Virol 77: 7991–7998.
4. Christen B, Wuthrich K, Hornemann S (2008) Putative prion protein from Fugu (Takifugu rubripes). Febs J 275: 263–270.
5. Cotto E, Andre M, Forgue J, Fleury HJ, Babin PJ (2005) Molecular characterization, phylogenetic relationships, and developmental expression patterns of prion genes in zebrafish (Danio rerio). Febs J 272: 500–513.
6. Favre-Krey L, Theodoridou M, Boukouvala E, Panagiotidis CH, *Papadopoulos AI, et al. (2007)* Molecular characterization of a cDNA from the gilthead sea bream (Sparus aurata) encoding a fish prion protein. Comp Biochem Physiol B Biochem Mol Biol 147: 566–573.
7. Gibbs CJ Jr, Bolis CL (1997) Normal isoform of amyloid protein (PrP) in brains of spawning salmon. Mol Psychiatry 2: 146–147.
8. Liao M, Zhang Z, Yang G, Sun X, Zou G, et al. (2005) Cloning and characterization of prion protein coding genes of Japanese seabass (Lateolabrax japonicus) and Japanese flounder (Paralichthys olivaceus). Aquaculture 249: 47–53.
9. Maddison BC, Patel S, James RF, Conlon HE, Oidtmann B, et al. (2005) Generation and characterisation of monoclonal antibodies to Rainbow trout (Oncorhynchus mykiss) prion protein. J Immunol Methods 306: 202–210.
10. Miesbauer M, Bamme T, Riemer C, Oidtmann B, Winklhofer KF, et al. (2006) Prion protein-related proteins from zebrafish are complex glycosylated and contain a glycosylphosphatidylinositol anchor. Biochem Biophys Res Commun 341: 218–224.
11. Oidtmann B, Simon D, Holtkamp N, Hoffmann R, Baier M (2003) Identification of cDNAs from Japanese pufferfish (Fugu rubripes) and Atlantic salmon (Salmo salar) coding for homologues to tetrapod prion proteins. FEBS Lett 538: 96–100.
12. Rivera-Milla E, Oidtmann B, Panagiotidis CH, Baier M, Sklaviadis T, et al. (2006) Disparate evolution of prion protein domains and the distinct origin of Doppel- and prion-related loci revealed by fish-to-mammal comparisons. Faseb J 20: 317–319.
13. Rivera-Milla E, Stuermer CA, Malaga-Trillo E (2003) An evolutionary basis for scrapie disease: identification of a fish prion mRNA. Trends Genet 19: 72–75.
14. Simonic T, Duga S, Strumbo B, Asselta R, Ceciliani F, et al. (2000) cDNA cloning of turtle prion protein. FEBS Lett 469: 33–38.
15. Strumbo B, Ronchi S, Bolis LC, Simonic T (2001) Molecular cloning of the cDNA coding for Xenopus laevis prion protein. FEBS Lett 508: 170–174.
16. Suzuki T, Kurokawa T, Hashimoto H, Sugiyama M (2002) cDNA sequence and tissue expression of Fugu rubripes prion protein-like: a candidate for the teleost orthologue of tetrapod PrPs. Biochem Biophys Res Commun 294: 912–917.
17. Premzl M, Gready JE, Jermiin LS, Simonic T, Marshall Graves JA (2004) Evolution of vertebrate genes related to prion and Shadoo proteins–clues from comparative genomic analysis. Mol Biol Evol 21: 2210–2231.
18. Opinion of the Scientific Panel on Biological Hazards on a request from the European Parliament on the assessment of the health risks of feeding of ruminants with fishmeal in relation to the risk of TSE. The EFSA Journal 443: 1–26.
19. Wilesmith JW, Wells GA, Cranwell MP, Ryan JB (1988) Bovine spongiform encephalopathy: epidemiological studies. Vet Rec 123: 638–644.
20. Wells GA, Scott AC, Johnson CT, Gunning RF, Hancock RD, et al. (1987) A novel progressive spongiform encephalopathy in cattle. Vet Rec 121: 419–420.
21. Friedland RP, Petersen RB, Rubenstein R (2009) Bovine Spongiform Encephalopathy and Aquaculture. J Alzheimers Dis.
22. Salas C, Broglio C, Duran E, Gomez A, Ocana FM, et al. (2006) Neuropsychology of learning and memory in teleost fish. Zebrafish 3: 157–171.
23. Panula P, Sallinen V, Sundvik M, Kolehmainen J, Torkko V, et al. (2006) Modulatory neurotransmitter systems and behavior: towards zebrafish models of neurodegenerative diseases. Zebrafish 3: 235–247.
24. Munday BL (2002) Betanodavirus infections of teleost fish: a review. Journal of Fish Diseases 25: 127–142.
25. Castric J, Thiery R, Jeffroy J, de Kinkelin P, Raymond JC (2001) Sea bream Sparus aurata, an asymptomatic contagious fish host for nodavirus. Dis Aquat Organ 47: 33–38.
26. Aranguren R (2002) Experimental transmission of encephalopathy and retinopathy induced by nodavirus to sea bream, Sparus aurata L., using different infection models. Journal of Fish Diseases 25: 317–324.
27. Diaz-San Segundo F, Salguero FJ, de Avila A, Espinosa JC, Torres JM, et al. (2006) Distribution of the cellular prion protein (PrPC) in brains of livestock and domesticated species. Acta Neuropathol 112: 587–595.
28. McLennan NF, Rennison KA, Bell JE, Ironside JW (2001) In situ hybridization analysis of PrP mRNA in human CNS tissues. Neuropathol Appl Neurobiol 27: 373–383.
29. Zanusso G, Liu D, Ferrari S, Hegyi I, Yin X, et al. (1998) Prion protein expression in different species: analysis with a panel of new mAbs. Proc Natl Acad Sci U S A 95: 8812–8816.
30. Race R, Meade-White K, Raines A, Raymond GJ, Caughey B, et al. (2002) Subclinical scrapie infection in a resistant species: persistence, replication, and adaptation of infectivity during four passages. J Infect Dis 186 Suppl 2: S166–170.
31. Collinge J (1999) Variant Creutzfeldt-Jakob disease. Lancet 354: 317–323.
32. Wells GA, Hawkins SA, Austin AR, Ryder SJ, Done SH, et al. (2003) Studies of the transmissibility of the agent of bovine spongiform encephalopathy to pigs. J Gen Virol 84: 1021–1031.
33. Dawson M, Wells GAH, Parker BNJ, Francis ME, Scott AC, Hawkins SAC, Martin TC, Simmons MM, Austin AR (1993) A Consultation on BSE with the Scientific Veterinary Committee of the Commission of the European Community. In: Bradley R, Marchant B, eds. Brussels: European Commission.
34. Westermark P (2005) Aspects on human amyloid forms and their fibril polypeptides. FEBS J 272: 5942–5949.
35. Howie AJ, Brewer DB, Howell D, Jones AP (2008) Physical basis of colors seen in Congo red-stained amyloid in polarized light. Lab Invest 88: 232–242.
36. Budka H (2000) Histopathology and immunohistochemistry of human transmissible spongiform encephalopathies (TSEs). Arch Virol Suppl: 135–142.
37. Unterberger U, Voigtlander T, Budka H (2005) Pathogenesis of prion diseases. Acta Neuropathol 109: 32–48.
38. Butler AaH W (2005) Comparative vertebrate neuroanatomy: Evolution and Adaptation: Wiley-IEEE.
39. Almer G, Hainfellner JA, Brucke T, Jellinger K, Kleinert R, et al. (1999) Fatal familial insomnia: a new Austrian family. Brain 122 (Pt 1): 5–16.
40. Becker CG, Becker T (2008) Adult zebrafish as a model for successful central nervous system regeneration. Restor Neurol Neurosci 26: 71–80.
41. Zupanc GK (2008) Towards brain repair: Insights from teleost fish. Semin Cell Dev Biol.
42. Johansen R (2002) Pathological changes in juvenile Atlantic halibut Hippoglossus hippoglossus persistently infected with nodavirus. Diseases of aquatic organisms 50: 161–169.
43. Barron RM, Campbell SL, King D, Bellon A, Chapman KE, et al. (2007) High titers of transmissible spongiform encephalopathy infectivity associated with extremely low levels of PrPSc in vivo. J Biol Chem 282: 35878–35886.
44. Gambetti P, Dong Z, Yuan J, Xiao X, Zheng M, et al. (2008) A novel human disease with abnormal prion protein sensitive to protease. Ann Neurol 63: 697–708.
45. Lasmezas CI, Deslys JP, Robain O, Jaegly A, Beringue V, et al. (1997) Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein. Science 275: 402–405.
46. Burgos JS, Ripoll-Gomez J, Alfaro JM, Sastre I, Valdivieso F (2008) Zebrafish as a new model for herpes simplex virus type 1 infection. Zebrafish 5: 323–333.
47. Piccardo P, Manson JC, King D, Ghetti B, Barron RM (2007) Accumulation of prion protein in the brain that is not associated with transmissible disease. Proc Natl Acad Sci U S A 104: 4712–4717.
48. Benestad SL, Arsac JN, Goldmann W, Noremark M (2008) Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. Vet Res 39: 19.
49. Beringue V, Vilotte JL, Laude H (2008) Prion agent diversity and species barrier. Vet Res 39: 47.

Author Contributions

Conceived and designed the experiments: ES CP EK GK TS. Performed the experiments: ES CP KT SP EE FA. Analyzed the data: ES CP NG AN EK GK TS. Contributed reagents/materials/analysis tools: ES CP KT SP EE FA NG AN EK GK TS. Wrote the paper: ES CP TS.

別紙様式第2

別紙(2)-1

医薬品　研究報告　調査報告書

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研究報告の概要

問題点（プリオン遺伝子のコドン 129 遺伝子が MM 型と同一でない特定の人達の中から見つかった）

TTD（Transfusion Transmitted Diseases）Committee が伝達性海綿状脳症（TSE）の状況および輸血の安全性に関する報告書を AABB Board の次回会合で提出予定である。報告の概要は以下のとおりである。

数人の感染者（症状発現なし）が、プリオン遺伝子のコドン 129 遺伝子が MM 型と同一でない特定の人達の中から見つかり、疾患の第二波の可能性や潜在的なキャリアのグループが拡大することに対して懸念されている。供血後に vCJD を発症した供血者 3 人から採取された血液の輸血により vCJD プリオンが合計 4 人に伝播している。

受血者の 3 例は vCJD を発症したが、1 例は vCJD の症状がなく、他の原因で死亡した。その患者の脾臓とリンパ節で異常プリオン蛋白が発見されたが、興味あることにその患者のプリオン遺伝子のコドン 129 が MV 型であることが判明した。非 MM 遺伝子型での感染の潜在的な重要性についての検討を以下に簡潔に示す。最近、供血後に vCJD を発症した供血者の血漿が含まれていたことが判明した血液凝固第 VIII 因子製剤を投与された一人の血友病患者において病原性 vCJD プリオンが発見された。その患者は vCJD の症状が現れなかった。モデリング研究では感染はおそらく血漿分画製剤によると示唆された。アメリカ赤十字社は、Transfusion に掲載された論文で、供血後に古典的 CJD を発症した供血者から伝播するエビデンスが不足していると報告している。その研究では、供血後に CJD を発症した 36 人の供血者の血液を投与された 436 人の受血者のルックバック調査をしたが、CJD の症例はなかった。暴露経験のある受血者を英国の vCJD に暴露した患者のコホートスタディーと比較すると CJD を発症するリスクが有意に低かった（p = 0.012）。この研究は、輸血による古典的 CJD の感染性に関するエビデンスが不足していることをサポートしている。血友病患者及び非 MM 型の特定の人達から vCJD プリオンが発見された結果、米国 FDA は米国由来の血漿分画製剤の受血者に関する vCJD 伝播のリスクモデルを改訂した。新しいモデルは 2009 年 6 月に開催された伝達性海綿状脳症諮問委員会で発表された。一人当たりの年間の最低の推定リスクは 5 倍から 18 倍に上昇したが、まだ 1200 万人に 1 人の低さであり、最大の推定リスクは 12000 人に 1 人のまま変わらず、FDA は、米国患者へのリスクは「極めて低い」と見なしている。

ヒトプリオン遺伝子のコドン 129 には数種のバリエーションがあり、3 つの主要な遺伝子型、MM 型、MV 型と VV 型がある。

現在まで評価された全ての症候性の vCJD 患者のコドン 129 の遺伝子型が MM 型であった。

英国人の約 40%がこの遺伝子型である。しかし病原性プリオンが MV または VV 遺伝子型である無症候性の人達で発見されている。これにより 2 つの疑問が生じてくる。最初の疑問は、非 MM 遺伝子型の人達での vCJD の「第二波」が起こるかどうかということである。これはたぶん潜伏期間がより長期化したためである。二番目は非 MM 遺伝子型を持つ人達が vCJD プリオンの感染キャリアになり得るかどうということである。2 つとも解決が待たれる。

報告企業の意見	今後の対応
当社製品を製造する原料血漿は、ドイツ、米国、オーストリア由来であり、また英国等の滞在期間、通算滞在歴に基づき供血停止基準を設けて収集している。 本剤の添付文書に、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、本剤の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を記載し、注意喚起している。	今後とも新しい感染症に関する情報収集に努める所存である。

使用上の注意記載状況・その他参考事項等

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ABJ2009206

Report to the Board of Directors: Current Status of TSEs and Transfusion Safety

Executive Summary:

There continues to be concern about transmission of variant Creutzfeldt Jakob disease (vCJD) by blood transfusion, with a total of 4 reported cases, plus a potential case of transmission of the prion via a UK-derived plasma derivative, known to have contained potentially infectious donations. Some infections (but no disease) have been found among individuals who are not homozygous for MM at the 129 codon of the PRP gene, raising concern about a possible second wave of disease and an expanded group of potential carriers. There has been no published progress in blood donor testing technologies. One method for prion removal from red cell concentrates has been evaluated by authorities in the UK and Ireland.

Information about transfusion transmitted infectivity:

There have now been a total of four cases of transmission of the vCJD prion by transfusion of blood collected from three donors who subsequently developed vCJD. Three of the cases resulted in the development of vCJD in the recipient, while one was detected in the spleen and one lymph node of a transfused patient who died of other causes. This individual had no symptoms of vCJD (1, 2). Interestingly, he was found to be (MV) heterozygous at codon 129 of the PrP gene. The potential significance of infection among non MM genotypes is discussed briefly below. More recently, evidence of pathologic vCJD prions was found in a hemophilic recipient of F VIII concentrates known to have included plasma from a donor who subsequently developed vCJD (3). Again, the recipient patient was free of vCJD symptoms. Modeling studies imply that the infection was most likely to have come from the plasma products (4).

In a paper published in Transfusion, American Red Cross authors reported on the absence of evidence of transmission of classic CJD from donors who subsequently developed the disease. The study involved lookback on 436 recipients of donations from a total of 36 donors who developed the disease subsequent to their donation, finding no cases of CJD among the recipients. A subset of recipients with exposure histories comparable to the UK cohort of patients exposed to vCJD was shown to be at lower risk of developing CJD: a statistically significant (p = 0.012) observation. This supports the absence of infectivity of classic CJD via transfusion (5).

Risk modeling for recipients of plasma derivatives in the US:

As a result of the finding of the vCJD prion in a hemophilia patient (described above), and the findings of vCJD prions among non MM individuals, the US FDA has revised its model of the vCJD transmission risk for recipients of US-derived plasma derivatives. The new models were presented at a meeting of the Transmissible Spongiform Encephalopathies Advisory Committee in June, 2009. Although the lowest estimated annual per-person risk has risen 5 to 18-fold, it may still be as low as 1 in 12 million and the maximal estimated risk remains unchanged at 1:12,000, the FDA still regards the risk to US patients to be "extremely small".

Implications of findings of vCJD prions in non MM individuals:

There are several variations at codon 129 of the human PrP gene, resulting in 3 main genotypes, MM, MV and VV, where M signifies methionine and V, valine. To date, all symptomatic cases of vCJD who have been evaluated have been MM homozygous at the 129 codon. Approximately 40% of the UK population has this genotype. However, there have been a number of circumstances (some described above) in which the pathologic prion has been found in asymptomatic individuals with the MV or VV genotype. This has raised two questions. The first is whether there will be a "second wave" of vCJD among individuals with a non MM genotype, perhaps resulting from a much extended incubation period. Second is the question of whether non MM individuals can be infectious carriers of the vCJD prion. This latter concern was included in the newer FDA infectivity model for US-derived plasma derivatives. Both questions await resolution.

Status of interventions against transfusion transmission of TSEs:

No significant progress has been reported in the development of any pre-mortem test that could be used for blood donors or donations, although one of the tests under development has undergone some preliminary clinical evaluation.

Currently, one manufacturer has an available, CE-marked affinity filter intended for use with leukoreduced red-cell concentrates. The procedure has been evaluated in the UK and Ireland, but no decision has been made with respect to

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its implementation. A process has also been developed for use in the manufacture of solvent-detergent treated plasma for transfusion.

References:

1. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG. Creutzfeldt–Jakob disease and blood transfusion: results of the UKTransfusionMedicine Epidemiologic Review study. Vox Sang 2006;91:221-30.

2. Health Protection Agency. CDR weekly, Vol. 16 No 6; 9February 2006. [cited 2009 May]. Available from: http://www.hpa.org.uk/cdr/archives/2006/cdr0606.pdf

3. Health Protection Agency. Asymptomatic vCJD abnormal prion protein found in a haemophilia patient. [cited June 2009]. Available from: http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733818681?e=1225960597236

4. Health Protection Agency. vCJD Risk assessment calculations for a patient with multiple routes of exposure. http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_100357

5. Dorsey K, Zou S, Schonberger LB, Sullivan M, Kessler D, Notari E 4th, Fang CT, Dodd RY. Lack of evidence of transfusion transmission of Creutzfeldt–Jakob disease in a US surveillance study. Transfusion 2009;49:977-84.

Prepared by TTD Committee, October, 2009.

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
				2009. 9. 16	該当なし	
一般的名称	抗HBs人免疫グロブリン		研究報告の公表状況	FDA, CBER. Available from: http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/ucm095070.htm	公表国 米国	
販売名(企業名)	抗HBs人免疫グロブリン「日赤」(日本赤十字社) 抗HBs人免疫グロブリン筋注200単位/1mL「日赤」(日本赤十字社) 抗HBs人免疫グロブリン筋注1000単位/5mL「日赤」(日本赤十字社)					

研究報告の概要

○FDA:米国承認血漿由来第Ⅷ因子製剤によるvCJD感染リスクの可能性:概要
・近年、米国承認血漿由来第Ⅷ因子製剤(pdFVIII, Antihemophilic Factor)の投与を受けた血友病Aおよびフォン・ヴィレブランド(vW)病患者の変異型クロイツフェルト・ヤコブ病(vCJD)感染リスクに関する疑問が提起されている。
・リスク評価に基づき、FDA、CDC、NIHを含む米国の公衆衛生総局(PHS)は、pdFVIII製剤の投与を受けた血友病AとvW病患者のvCJD感染リスクは、はっきりとはわからないものの、非常に小さい可能性が最も考えられる。第Ⅸ因子製剤を含む他の血漿由来製剤からのvCJD感染リスクは同程度か、更に小さいようである。
・新たな情報を得るためには、血友病治療センターの血友病またはvW病の専門家に連絡するのが良い方法である。
(追加情報)
2003年11月～2007年4月に英国で、赤血球輸血によりvCJDに感染したと考えられる患者4名が発生し、血液製剤のvCJD伝播の可能性について懸念が高まった。このためFDAは、vCJDとBSEの発生率が米国と比べて非常に高い国に渡航した人の供血延期を勧告した。米国では、これまでvCJDを発症した人の血漿から作られたpdFVIII製剤はなく、製剤を投与された人がvCJDを発症したこともない。pdFVIII製剤は、他の血漿由来製剤と比べてvCJD感染因子を多く含むと考えられる。また、血漿由来製剤の製造工程における処理でvCJD感染因子は減少すると考えられる。FDA、CDC、NIHの認識している限り、リスクの最も高い英国を含め、血友病、vW病、その他の血液凝固障害患者がvCJDを発症したという報告はない。FDAはvCJD伝播の可能性低減のため、欧州渡航歴のある人の供血延期など様々な対策を実施している。FDAはpdFVIII製剤のvCJD感染リスクを分析したが、有病率について不明な点が多く正確なリスク評価は不可能である。リスクは非常に小さい可能性が最も考えられるが、ゼロではないだろ

使用上の注意記載状況・その他参考事項等

抗HBs人免疫グロブリン「日赤」
抗HBs人免疫グロブリン筋注200単位/1mL「日赤」
抗HBs人免疫グロブリン筋注1000単位/5mL「日赤」

血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

報告企業の意見	今後の対応
米国食品医薬品局が、米国承認血漿由来第Ⅷ因子製剤の投与を受けた血友病AおよびフォンヴィレブランドⅢ病患者のvCJD感染リスクの可能性について、はっきりとはわからないものの、非常に小さい可能性が最も考えられるとの見解を示したとの報告である。生涯反復使用する血漿分画製剤の感染リスクが小さいことは、献血者を米国以上に規制してきた国産製剤はより感染リスクが低いと期待される。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980～96年に1日以上の英国滞在歴のある人の献血を制限している。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

Vaccines, Blood & Biologics

Potential vCJD Risk From US Licensed Plasma-Derived Factor VIII (pdFVIII, Antihemophilic Factor) Products: Summary Information, Key Points

Summary Information

Key Points:

- In recent years, questions have been raised concerning the risk of variant Creutzfeldt-Jakob disease (vCJD) (a rare but fatal brain infection) to hemophilia A and von Willebrand disease patients who receive US licensed plasma-derived Factor Eight (pdFVIII, Antihemophilic Factor) products.
- Based on a risk assessment, the US Public Health Service (PHS), including FDA, CDC, and NIH, believes that the risk of vCJD to hemophilia A and von Willebrand disease patients who receive US licensed pdFVIII products is most likely to be extremely small, although we do not know the risk with certainty. vCJD risk from other plasma derived products, including Factor IX, is likely to be as small or smaller.
- Contacting a specialist in hemophilia or von Willebrand disease at a Hemophilia Treatment Center is a good way to learn about new information as it becomes available.

Additional Information:

- Between December 2003 and April 2007, there have been four reports of people, all in the UK, who probably acquired the vCJD agent through red blood cell transfusions. This has increased concern about the potential transmission of vCJD by blood products.
- Principal concerns are whether persons infected with vCJD could donate plasma in the U.S., and whether clotting factor products made from their plasma donations might transmit the disease.
- To address these concerns FDA recommends the deferral of donors who may have lived in or traveled extensively to countries with a higher prevalence of vCJD and bovine spongiform encephalopathy (BSE) than in the U.S.
- In the United States, pdFVIII products have not been made from the plasma of anyone known to have developed vCJD, and no one who received any of these products is known to have developed vCJD.
- FDA conducted a risk assessment for pdFVIII because the plasma fraction from which it is made is likely to contain more of the vCJD infectious agent, if present, than plasma fractions from which other plasma-derived products are made, such as Factor IX, (used to treat hemophilia B), albumin, and immune globulins. The FVIII-containing fraction is further processed using a variety of methods that are likely to reduce or

Potential vCJD Risk From US Licensed Plasma-Derived Factor VI... http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/B...

potentially eliminate vCJD from the final pdFVIII product. Methods likely to reduce or potentially eliminate vCJD are also used in the manufacture of other plasma-derived products.

- FDA, CDC, and NIH are not aware of any cases of vCJD having been reported worldwide in patients with hemophilia, von Willebrand disease, or other blood clotting disorders. This includes those who have received, over a long period of time, large amounts of blood clotting factor products manufactured from plasma donations from the UK where the risk of vCJD is highest because of a previous higher risk of potential exposure to BSE-infected beef in the UK diet.
- The FDA has taken a number of steps to further reduce the potential vCJD risk from blood components. These steps include donor deferral recommendations, and quarantine and withdrawal of products at increased vCJD risk. Donor deferral guidance, first issued in August 1999 and subsequently updated, includes, among other things, deferral of donors who visited or resided in Europe where BSE prevalence is higher than in the US. Also, blood components and plasma derivatives are to be withdrawn if a donor is later diagnosed with vCJD. The potential spread of vCJD through red blood cell or plasma transfusion is limited by these deferral and quarantine measures that are in place.
- Additional steps FDA is taking to reduce potential vCJD risk from plasma derivatives include gathering, evaluating, and disseminating information about manufacturing processes that potentially could reduce the vCJD infectious agent in blood products. FDA is helping to develop donor screening and diagnostic tests for vCJD, and to inform patients and physicians about the current scientific understanding of vCJD risk from blood products.
- Using a computer model, FDA assessed the potential risk of vCJD infection from the current use of pdFVIII products. However, because so much is unknown about vCJD and its prevalence, the risk assessment performed by FDA has a lot of uncertainty, making it impossible to precisely estimate the risk of vCJD in general, or of the actual risk to individual hemophilia A or von Willebrand disease patients. Meaningful distinctions also could not be made among specific products. There is no test yet available to detect vCJD infection in healthy donors or recipients.
- Although the risk of vCJD exposure from US pdFVIII products is most likely to be extremely small, it may not be zero, and FDA is encouraging physicians and patients to consider this risk, in the context of all remaining real or potential risks and the known benefits of product use, when making treatment decisions.
- At this time, the PHS does not believe there is a need for hemophilia A and von Willebrand disease patients who receive pdFVIII to inform their surgeons or dentists about their potential exposure to vCJD. Also, there is no recommendation for surgeons and dentists to take any special precautions based on such potential exposures. This belief is based on the results of the FDA risk assessment, as well as on the lack of known cases of vCJD transmitted by plasma-derived clotting factor products in the UK or anywhere else in the world. PHS agencies will continue to monitor and reevaluate the situation as new information becomes available.
- vCJD originally came from a disease in cattle called "mad cow disease" or

BSE (bovine spongiform encephalopathy) . Transmission of the BSE agent to humans, leading to vCJD, is believed to occur primarily from eating beef and beef products contaminated with the BSE agent. Both BSE and vCJD are invariably fatal brain diseases with incubation periods typically measured in years.

- From 1995 through April 2007, 202 individuals with vCJD were reported worldwide, with 165 in the United Kingdom (UK), and three in the United States. Two of the individuals in the United States had lived in the UK from 1980-1996 during a key exposure period to the BSE agent. The third US individual with vCJD most likely acquired the disease in Saudi Arabia. The reported incidence of vCJD in the UK based on disease onset peaked in 1999 and has been declining thereafter. In the UK, where most cases of vCJD have occurred, the current risk of acquiring vCJD from eating beef and beef products appears to be negligible.
- More information about vCJD is available on these government websites:
  - FDA: Potential Risk of Variant Creutzfeldt-Jakob Disease (vCJD) From Plasma-Derived Products
  - Centers for Disease Control and Prevention: vCJD (Variant Creutzfeldt-Jakob Disease)
  - US Department of Agriculture
- Information also may be obtained from these non-government sources:
  - Committee of Ten Thousand
  - Hemophilia Federation of America
  - National Hemophilia Foundation and/or HANDI
  - World Federation of Hemophilia

Contact Us

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別紙様式第2-1
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医薬品
医薬部外品
化粧品

研究報告　調査報告書

識別番号・報告回数			報告日		第一報入手日 2009年11月10日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	人ハプトグロビン			研究報告の公表状況	Vox Songuinis 2009; 97: 207-210	公表国 イギリス	
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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
（背景） 輸血や血液製剤の投与を介して変異型クロイツフェルト-ヤコブ病(vCJD)が伝播することの公衆衛生上の危険性は、特に血友病患者の体内で異常プリオンタンパク質が検出されたことが最近報告されたことから、現在でも懸念されている。 （目的） 英国のvCJD臨床症例について過去に血漿分画製剤への暴露があったか説明すること。 （方法） 国立CJDサーベイランスユニット(National CJD Surveillance Unit)に保管されている記録(親族、開業医、および病院からのもの)を調査する。 （結果） 英国の168例のvCJD症例のうち9例が、血漿分画製剤の投与をのべ12回受けたことがあった(その12回のうちの1回は1970年でvCJDの危険性以前であったが、残りの11回は1989～1998年であった)。UK CJD Incident Panelの危険性評価基準によれば、11回は低危険度製品の投与であり、1回は低もしくは中等度危険度製品の投与であった。 （結論） 現在までの英国のvCJD臨床症例のうちのいずれの例についても、血漿分画製剤への暴露を介して感染したものとは考えられない。しかし、将来的にそのような伝播がvCJD感染例をもたらす可能性は排除し得ない。	2. 重要な基本的注意 (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
英国のvCJD臨床症例について過去に血漿分画製剤への曝露があったかについて、国立サーベイランスユニットに保管されている記録を調査した報告である。 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り旨を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。

(18)

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ORIGINAL PAPER

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Variant Creutzfeldt–Jakob disease and exposure to fractionated plasma products

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Background The risk to public health of onward transmission of variant Creutzfeldt–Jakob disease (vCJD) via blood transfusion and plasma product administration is of on-going concern, particularly with the recent reported detection of abnormal prion protein in a person with haemophilia.

Objectives To describe the history of fractionated plasma product exposure in clinical cases of vCJD in the UK.

Methods Through examination of records held at the National CJD Surveillance Unit (from relatives, general practices and hospitals).

Results Nine out of 168 UK vCJD cases had a history of receipt of fractionated plasma products on 12 different occasions (1 pre-vCJD risk in 1970, the remaining between 1989–1998). According to the UK CJD Incident Panel risk assessment criteria, 11 were low-risk products and one was low or medium risk.

Conclusion It is unlikely that any of the UK vCJD clinical cases to date were infected through exposure to fractionated plasma products. However, the possibility that such transmission may result in vCJD cases in the future cannot be excluded.

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Key words: fractionated plasma products, public health, transfusion, vCJD.

Introduction

The risk of onward transmission of variant Creutzfeldt–Jakob disease (vCJD) via blood transfusion and plasma product administration is of on-going concern. This has been highlighted by the recent announcement by the UK's Health Protection Agency of the post-mortem finding of abnormal prion protein in the spleen of a patient with haemophilia who died from a cause unrelated to vCJD [1]. This individual had received UK-sourced fractionated plasma products before 1999, when safety measures were put in place in relation to vCJD, including importation of plasma, mainly from the USA, to manufacture plasma products. There has been no previous documentation suggesting transmission of any type of CJD by fractionated plasma products. On the other hand, variant CJD has been shown to be transmissible via blood component transfusion, with four instances of transfusion-transmitted vCJD infection to date associated with non-leucodepleted red cells [2–5].

Laboratory studies in animal models have shown that infectivity may be present in plasma both during clinical illness and in the incubation period [6]. Although there is experimental evidence that significant infectivity may be cleared during the production process for fractionated plasma products [7], there are doubts about the interpretation of studies that have been largely based on spiking of plasma with brain-derived material rather than endogenous infectivity [8]. In addition, there are varieties of manufacturing processes used in production of plasma products. These findings have drawn attention to the important public health implications of potential secondary transmission of vCJD.

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In addition to recipients of vCJD-implicated labile blood components, more than 4000 UK-sourced plasma product recipients have been classified and notified by the UK CJD Incidents Panel as 'at risk for public health purposes', in part on the basis of a risk assessment [9]. In 2004, the UK CJD Incidents Panel advised that patients who were treated with UK-sourced fractionated plasma products between 1980 and 2001 and who were exposed to a 1% risk of infection in addition to the background risk of the UK population through diet, should be contacted and advised to take public health precautions. Fractionated plasma products were categorized into three groups according to the number of treatments that were likely to result in a patient reaching this risk threshold: high risk (one treatment with factor VIII, factor IX or antithrombin), medium risk (several infusions of intravenous immunoglobulin or 4·5% albumin) and low risk (intramuscular immunoglobulins or 20% albumin) [1, personal communication]. An exercise was undertaken to trace recipients, estimate individual risk and inform all those who reached this threshold that they were 'at risk of vCJD for public health purposes'. The amount of potential infectivity in the low-risk category was estimated to be so small that the likelihood of surpassing the threshold was extremely unlikely and so individual recipients did not need to be traced or notified.

Data from actual cases of vCJD are important in attempting to determine the potential risk from fractionated plasma products. This paper describes a number of UK vCJD cases reported to have received such products before the onset of illness. The characteristics of the specific plasma products involved suggest that these exposures are most unlikely to have been the source of vCJD in these cases.

Methods

The UK National CJD Surveillance Unit (NCJDSU) routinely collects information on potential risk factors for all cases of vCJD referred to the unit [10], including data on blood transfusion, plasma product administration, vaccination and 'injection' histories. The information is obtained from interviews with relatives of cases and, when available, primary care and hospital records. Where possible, batch numbers of fractionated plasma products were obtained for vCJD cases found to have received such products and compared with the list of product batches derived from plasma donated by individuals who later went on to develop vCJD. Eleven of the 168 cases of vCJD referred to the NCJDSU up to end of March 2009 are known to have made 25 plasma donations which had been used to manufacture 191 batches of fractionated products, prior to the UK importing plasma from abroad in 1999.

Results

To examine whether any of the 168 vCJD cases had received fractionated plasma products, we examined records held at NCJDSU. One hundred and fifty-eight had data available from relatives and general practice/hospital records, seven from relatives only, two from general practice records only and in one case there was minimal information available (this patient was investigated in another country).

Nine cases of vCJD, with onset of symptoms between December 1994 and April 2006, had recorded receipt of fractionated plasma products on 12 occasions (Table 1). Five

Table 1 Variant CJD clinical cases reported to have received plasma products

vCJD case	Plasma product	Year given	Year clinical onset of vCJD	Batch number known (country of plasma origin, if known)
1	Human normal immunoglobulin[a] (gammaglobulin for travel)	1990	1994	✓ (non-UK)
2	Rh(D) immunoglobulin[a]	1992	1995	✗
	Human normal immunoglobulin[a] (gammaglobulin for travel)	1993		✓ (non-UK)
3	Rh(D) immunoglobulin[a]	1991	1996	✓ (UK)
4	Albumin	1993	1998	✗
5	Rh(D) immunoglobulin[a]	1989	1998	✗
	Rh(D) immunoglobulin[a]	1993		✓ (UK)
	Rh(D) immunoglobulin[a]	1998		✗
6	Human normal immunoglobulin[a] (gammaglobulin for travel)	1993	1999	✓ (non-UK)
7	Human normal immunoglobulin[a] (for travel)	1991	2000	✓ (UK)
8	Rh(D) immunoglobulin[a]	1970[b]	2001	✗
9	Rh(D) immunoglobulin[a]	1997	2006	✗

[a]Administered intramuscularly; [b]before the considered start of the vCJD at risk period in 1980.

cases had received Rh(D) immunoglobulin to protect against Rh isoimmunization, four in childbirth (on six occasions) and one (case 9, Table 1) with receipt of fresh frozen plasma. Before travel, four cases had received normal human immunoglobulin for intramuscular use (three gammaglobulin, one human normal immunoglobulin), including one case (case 2, Table 1) who had received Rh(D) immunoglobulin previously. The remaining case was given albumin (unknown concentration) for 'cover' during a paracentesis procedure. One of the nine cases received Rh(D) immunoglobulin in 1970 before the considered start of the vCJD at risk period (1980) and the other eight received products between 1989 and 1998.

Batch numbers were available for only two of the seven Rh(D) immunoglobulin products, which indicated the UK as origin of the plasma in these two cases. However, batch numbers were available for all four intramuscular human normal immunoglobulin/gammaglobulin products and one of these was of UK origin. The albumin batch number was not recorded. No batch number matched any others, nor did the batch numbers match any of those from products known to have included plasma donated from individuals who subsequently went onto develop vCJD.

Discussion

Of the nine vCJD patients who had received fractionated plasma products, the batch numbers of the plasma products, where known, did not correlate with any of the batches derived from pools containing a donation from a person who went on to develop vCJD. Eight had received products considered by the UK CJD Incidents Panel as low risk and one person had received a low-/medium-risk product (albumin of unknown concentration). It is, therefore, unlikely that administration of plasma product was the source of vCJD infection in these cases.

Thirty-two of the 74 female vCJD cases had children and, of these, four (13%) were reported to have received Rh(D) immunoglobulin. In the UK, 17% of all women are RhD negative. Approximately 10% of all UK pregnancies are in RhD negative mothers bearing RhD positive babies, and these women should all receive routine Rh(D) immunoglobulin after delivery [11]. Although less likely now, in the past RhD negative women may have been given Rh(D) immunoglobulin without the blood group of the baby being known, resulting in more than 10% receiving Rh(D) immunoglobulin. However, the median age at death in vCJD is only 28 years and the proportion of women with vCJD who received Rh(D) immunoglobulin is comparable to the likely exposure rate in the general population.

The lack of evidence of transmission of vCJD through fractionated plasma products assumes that accurate and thorough information has been obtained on relevant exposure [10]. Information was available from relatives, hospital notes at the time of admission for the terminal illness and from primary care records in 158 of the 168 cases included in this analysis. In this group, it is unlikely that any plasma product exposure was missed and, in particular, it is unlikely that higher-risk exposures involving long-term treatment with plasma products, such as treatment of haemophilia, were undetected. This is probably also true for the seven cases in which information from relatives was the only source of data on past exposures. However, it is possible that prior treatment, for example with albumin or intravenous immunoglobulin, could have been missed and it was only possible to identify batch numbers in half of plasma products identified as having been used in vCJD cases. There is also the possibility that infection via plasma products might result in a protracted incubation period because of the relatively low dose exposure and that cases of vCJD infected through this mechanism have yet to occur.

In conclusion, it is unlikely that any of the UK vCJD clinical cases to date were infected through exposure to fractionated plasma products. However, the possibility that such transmission may result in vCJD cases in the future cannot be excluded.

References

1 Variant CJD and plasma products: Health Protection Agency. Available at http://www.hpa.org.uk/webw/HPAwebRHPAweb Standard/HPAweb_C/1195733818681?p=1225960597236 [Accessed 31 March 2009]

2 Llewelyn CA, Hewitt PE, Knight RSG, Amar K, Cousens S, Mackenzie J, Will RG: Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 2004; 363:417–421

3 Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW: Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 2004; 364:527–529

4 Health Protection Agency: Fourth case of transfusion-associated variant-CJD. *Health Protection Report* 2007, 1. Available at http://www.hpa.org.uk/hpr/archives/2007/hpr0307.pdf [Accessed 8 June 2009]

5 Wroe SJ, Pal S, Siddique D, Hyare H, Macfarlane R, Joiner S, Linehan JM, Brandner S, Wadsworth JD, Hewitt P, Collinge J: Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. *Lancet* 2006; 368:2061–2067

6 Brown P, Cervenakova L, McShane LM, Barber P, Rubenstein R, Drohan WN: Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. *Transfusion* 1999; 39:1169–1178

7 Foster PR: Removal of TSE agents from blood products. *Vox Sang* 2004; 87 (Suppl. 2):S7–S10

8 Gregori L, Gurgel PV, Luthrop JT, Edwardson P, Lambert BC, Carbonell RG, Burton SJ, Hammond DJ, Rohwer RG: Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins. *Lancet* 2006; 368:2226–2230

9 Risk of Infection from variant CJD in Blood: Det Norske Veritas Consulting, April 2004. Available at http://www.dnv.com/news_events/news/2004/riskofinfectionfromvariantcjdinblood.asp [Accessed 18 November 2008]

10 Ward HJT, Everington D, Cousens SN, Smith-Bathgate B, Leitch M, Cooper S, Heath C, Knight RSG, Smith PG, Will RG: Risk factors for variant Creutzfeldt-Jakob disease: a case-control study. *Ann Neurol* 2006; 59:111–120

11 Haemolytic disease of the fetus and newborn: in Klein HG, Anstee DJ (eds): *Mollison's Blood Transfusion in Clinical Medicine*, 11th edn. Oxford, Blackwell Science, 2005:504

B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

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

血対ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用
100071	2009/12/17	90805	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	無	有	無
100072	2009/12/17	90806	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	無	有	無

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第13回	13-1	臨床検査	Ｃ型肝炎陽性	米国	男性	65歳	2009/09	未回復	症例報告	当該製品	識別番号：09000017（完了報告） 報告日：2009年11月5日 MedDRA: Version(12.1)
	13-2	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	32歳	2009/07/12	未回復	症例報告	当該製品	識別番号：09000013（完了報告） 報告日：2009年9月24日 MedDRA: Version(12.1)
	13-3	感染症および寄生虫症	Ｂ型肝炎	米国	女性	40歳	2009/05	回復	症例報告	当該製品	識別番号：09000012（完了報告） 報告日：2009年8月19日 MedDRA: Version(12.1)
	13-4	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	37歳	2009/04/23	未回復	症例報告	当該製品	識別番号：09000014（完了報告） 報告日：2009年10月8日 MedDRA: Version(12.1)
	13-5	臨床検査	Ｂ型肝炎抗体陽性	米国	不明	新生児	2009/04/23	未回復	症例報告	当該製品	識別番号：09000015（完了報告） 報告日：2009年10月8日 MedDRA: Version(12.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第12回	12-1	感染症および寄生虫症	肝炎ウイルスキャリアー	米国	不明	不明	1993	不明	症例報告	当該製品	識別番号：08000002（完了報告） 報告日：2008年12月22日 MedDRA: Version(11.1)
	12-2	感染症および寄生虫症	Ｃ型肝炎	米国	女性	48	2008/12/09	未回復	症例報告	当該製品	識別番号：08000034（完了報告） 報告日：2008年1月19日 MedDRA: Version(11.1)
	12-3	感染症および寄生虫症	Ｃ型肝炎	米国	女性	不明	不明	不明	症例報告	当該製品	識別番号：09000004（完了報告） 報告日：2008年5月18日 MedDRA: Version(12.0)
第11回	11-1	臨床検査	Ｂ型肝炎抗体陽性	米国	男性	17	2008/05	不明	症例報告	当該製品	識別番号：08000007（完了報告） 報告日：2008年6月5日 MedDRA: Version(11.0)
	11-2	感染症および寄生虫症	Ｃ型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号：08000018（追加報告） 報告日：2008年11月12日 第11回症例番号11-2において10月17日に報告したものの追加報告 MedDRA: Version(11.1)
	11-2	感染症および寄生虫症	Ｃ型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号：08000018（完了報告） 報告日：2008年10月17日 MedDRA: Version(11.0)
	11-3	感染症および寄生虫症	Ｂ型肝炎	スペイン	女性	不明	2008/6/3	未回復	症例報告	外国製品	識別番号：08000026（完了報告） 報告日：2008年10月31日 MedDRA: Version(11.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回		0*	0	0	0	0	0	0	0	0	* 当該調査期間に対象となる感染症報告はなかった
第9回		0	0	0	0	0	0	0	0	0	
第8回		0	0	0	0	0	0	0	0	0	
第7回	7-1	臨床検査	ＨＩＶ抗体陽性	米国	不明	小児	不明	不明	症例報告	外国製品	識別番号： 06000022 (完了報告) 報告日：2006年8月24日 MedDRA: Version (9.0)
第6回	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号： 05000456 (追加報告) 報告日：2006年2月15日 第6回症例番号5-1は前回報告における第5回症例番号5-1において報告したものの追加報告 MedDRA: Version (8.1)

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別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第5回	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号： 05000456 (追加報告) 報告日：2005年11月11日 MedDRA: Version (8.1)
	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号： 05000456 (完了報告) 報告日：2005年10月27日 MedDRA: Version (8.1)
	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男性	26歳	2002/11/19	不明	症例報告	当該製品	識別番号：03000006 (追加報告) 報告日：2005年7月4日 第2回症例番号1-3において報告したものの追加報告 MedDRA: Version (8.0)
	1-3	感染症および寄生虫症	Ｂ型肝炎	米国	男性	26歳	2002/10/4	不明	症例報告	当該製品	識別番号：03000006 (追加報告) 報告日：2005年7月4日 第2回症例番号1-3において報告したものの追加報告 MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-1 血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号： 05000001 (追加報告) 報告日：2005年6月27日 第4回症例番号4-1において報告したものの追加報告 MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-2 血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号： 05000001 (追加報告) 報告日：2005年6月27日 第4回症例番号4-1において報告したものの追加報告 MedDRA: Version (8.0)

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別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第４回	4-1	臨床検査	HTLV-1 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001（追加報告） 報告日：2005 年 4 月 25 日 MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-1 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001（完了報告） 報告日：2005 年 4 月 7 日 MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-2 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001（追加報告） 報告日：2005 年 4 月 25 日） MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-2 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001（完了報告） 報告日：2005 年 4 月 7 日 MedDRA: Version (8.0)
	4-2	感染症および寄生虫症	C型肝炎	フランス	男性	不明	不明	不明	症例報告	外国製品	識別番号： 04000129 報告日：2005 年 3 月 31 日） MedDRA: Version (8.0)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第３回	3-1	感染症および寄生虫症	C型肝炎	米国	女性	37 歳	2004/5/21	不明	症例報告	当該製品	識別番号：04000023 報告日：2004 年 6 月 30 日 MedDRA: Version (7.0)
	3-2	臨床検査	B型肝炎抗体陽性	米国	女性	63 歳	2004/7/27	不明	症例報告	当該製品	識別番号：04000059 報告日：2004 年 9 月 7 日 MedDRA: Version (7.0)
	3-2	臨床検査	A型肝炎抗体陽性	米国	女性	63 歳	2004/8/16	不明	症例報告	当該製品	識別番号：04000059 報告日：2004 年 9 月 7 日 MedDRA: Version (7.0)
	3-3	臨床検査	B型肝炎抗体陽性	米国	女性	50 歳代	2004/9 月	不明	症例報告	当該製品	識別番号：04000082 報告日：2004 年 10 月 20 日 MedDRA: Version (7.1)
	3-3	臨床検査	A型肝炎抗体陽性	米国	女性	50 歳代	2004/9 月	不明	症例報告	当該製品	識別番号：04000082 報告日：2004 年 10 月 20 日 MedDRA: Version (7.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第２回	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男性	26 歳	2003/8/30	軽快	症例報告	当該製品	識別番号：03000006 報告日：2004 年 1 月 7 日 第 1 回症例番号 1-3 において報告したもの（FAX 報告）の完了報告 MedDRA: Version (6. 1)
	2-2	感染症および寄生虫症	Ｃ型肝炎	ドイツ	女性	6 歳	1994/6/21	未回復	症例報告	外国製品	識別番号：04000013 報告日：2004 年 5 月 27 日 MedDRA: Version (7. 0)
第１回	1-1	臨床検査	Ｃ型肝炎ウイルス	米国	男性	不明	不明	未回復	症例報告	外国製品	識別番号：D03-31 報告日：2003 年 8 月 6 日 MedDRA: Version (6. 1)
	1-2	臨床検査	Ｃ型肝炎ウイルス	米国	男性	不明	不明	未回復	症例報告	外国製品	識別番号：A03-32 報告日：2003 年 8 月 6 日 MedDRA: Version (6. 1)
	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男	26 歳	2003/8/30	軽快	症例報告	当該製品	FAX 報告 報告日：2003 年 11 月 19 日 （識別番号：03000006 2003 年 11 月 28 日） MedDRA: Version (6. 1)

100071	2009/12/17	90805	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG

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別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第13回	13-1	臨床検査	Ｃ型肝炎陽性	米国	男性	65 歳	2009/09	未回復	症例報告	当該製品	識別番号： 09000017（完了報告） 報告日：2009 年 11 月 5 日 MedDRA: Version (12. 1)
	13-2	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	32 歳	2009/07/12	未回復	症例報告	当該製品	識別番号： 09000013（完了報告） 報告日：2009 年 9 月 24 日 MedDRA: Version (12. 1)
	13-3	感染症および寄生虫症	Ｂ型肝炎	米国	女性	40 歳	2009/05	回復	症例報告	当該製品	識別番号： 09000012（完了報告） 報告日：2009 年 8 月 19 日 MedDRA: Version (12. 1)
	13-4	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	37 歳	2009/04/23	未回復	症例報告	当該製品	識別番号： 09000014（完了報告） 報告日：2009 年 10 月 8 日 MedDRA: Version (12. 1)
	13-5	臨床検査	Ｂ型肝炎抗体陽性	米国	不明	新生児	2009/04/23	未回復	症例報告	当該製品	識別番号： 09000015（完了報告） 報告日：2009 年 10 月 8 日 MedDRA: Version (12. 1)

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別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第12回	12-1	感染症および寄生虫症	肝炎ウイルスキャリアー	米国	不明	不明	1993	不明	症例報告	当該製品	識別番号：08000002（完了報告） 報告日：2008年12月22日 MedDRA: Version(11.1)
	12-2	感染症および寄生虫症	Ｃ型肝炎	米国	女性	48	2008/12/09	未回復	症例報告	当該製品	識別番号：08000034（完了報告） 報告日：2008年1月19日 MedDRA: Version(11.1)
	12-3	感染症および寄生虫症	Ｃ型肝炎	米国	女性	不明	不明	不明	症例報告	当該製品	識別番号：09000004（完了報告） 報告日：2008年5月18日 MedDRA: Version(12.0)
第11回	11-1	臨床検査	Ｂ型肝炎抗体陽性	米国	男性	17	2008/05	不明	症例報告	当該製品	識別番号：08000007（完了報告） 報告日：2008年6月5日 MedDRA: Version(11.0)
	11-2	感染症および寄生虫症	Ｃ型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号：08000018（追加報告） 報告日：2008年11月12日 第11回症例番号11-2において10月17日に報告したものの追加報告 MedDRA: Version(11.1)
	11-2	感染症および寄生虫症	Ｃ型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号：08000018（完了報告） 報告日：2008年10月17日 MedDRA: Version(11.0)
	11-3	感染症および寄生虫症	Ｂ型肝炎	スペイン	女性	不明	2008/6/3	未回復	症例報告	外国製品	識別番号：08000026（完了報告） 報告日：2008年10月31日 MedDRA: Version(11.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回		0*	0	0	0	0	0	0	0	0	* 当該調査期間に対象となる感染症報告はなかった
第9回		0	0	0	0	0	0	0	0	0	
第8回		0	0	0	0	0	0	0	0	0	
第7回	7-1	臨床検査	ＨＩＶ抗体陽性	米国	不明	小児	不明	不明	症例報告	外国製品	識別番号：06000022（完了報告） 報告日：2006年8月24日 MedDRA: Version(9.0)
第6回	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号：05000456(追加報告) 報告日：2006年2月15日 第6回症例番号5-1は前回報告における第5回症例番号5-1において報告したものの追加報告 MedDRA: Version(8.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第５回	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年９月	未回復	症例報告	当該製品	識別番号：05000456(追加報告) 報告日：2005年11月11日 MedDRA: Version(8.1)
	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年９月	未回復	症例報告	当該製品	識別番号：05000456(完了報告) 報告日：2005年10月27日 MedDRA: Version(8.1)
	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男性	26歳	2002/11/19	不明	症例報告	当該製品	識別番号：03000006(追加報告) 報告日：2005年７月４日 第２回症例番号1-3において報告したものの追加報告 MedDRA: Version(8.0)
	1-3	感染症および寄生虫症	Ｂ型肝炎	米国	男性	26歳	2002/10/4	不明	症例報告	当該製品	識別番号：03000006(追加報告) 報告日：2005年７月４日 第２回症例番号1-3において報告したものの追加報告 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-1血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号：05000001(追加報告) 報告日：2005年６月27日 第４回症例番号4-1において報告したものの追加報告 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号：05000001(追加報告) 報告日：2005年６月27日 第４回症例番号4-1において報告したものの追加報告 MedDRA: Version(8.0)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第４回	4-1	臨床検査	HTLV-1血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号：05000001(追加報告) 報告日：2005年４月25日 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-1血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号：05000001(完了報告) 報告日：2005年４月７日 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号：05000001(追加報告) 報告日：2005年４月25日) MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号：05000001(完了報告) 報告日：2005年４月７日 MedDRA: Version(8.0)
	4-2	感染症および寄生虫症	Ｃ型肝炎	フランス	男性	不明	不明	不明	症例報告	外国製品	識別番号：04000129 報告日：2005年３月31日) MedDRA: Version(8.0)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第３回	3-1	感染症および寄生虫症	Ｃ型肝炎	米国	女性	37歳	2004/5/21	不明	症例報告	当該製品	識別番号：04000023 報告日：2004年6月30日 MedDRA: Version(7.0)
	3-2	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	63歳	2004/7/27	不明	症例報告	当該製品	識別番号：04000059 報告日：2004年9月7日 MedDRA: Version(7.0)
	3-2	臨床検査	Ａ型肝炎抗体陽性	米国	女性	63歳	2004/8/16	不明	症例報告	当該製品	識別番号：04000059 報告日：2004年9月7日 MedDRA: Version(7.0)
	3-3	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	50歳代	2004/9月	不明	症例報告	当該製品	識別番号：04000082 報告日：2004年10月20日 MedDRA: Version(7.1)
	3-3	臨床検査	Ａ型肝炎抗体陽性	米国	女性	50歳代	2004/9月	不明	症例報告	当該製品	識別番号：04000082 報告日：2004年10月20日 MedDRA: Version(7.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第２回	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男性	26歳	2003/8/30	軽快	症例報告	当該製品	識別番号：03000006 報告日：2004年1月7日 第1回症例番号1-3において報告したもの（FAX報告）の完了報告 MedDRA: Version(6.1)
	2-2	感染症および寄生虫症	Ｃ型肝炎	ドイツ	女性	6歳	1994/6/21	未回復	症例報告	外国製品	識別番号：04000013 報告日：2004年5月27日 MedDRA: Version(7.0)
第１回	1-1	臨床検査	Ｃ型肝炎ウイルス	米国	男性	不明	不明	未回復	症例報告	外国製品	識別番号：D03-31 報告日：2003年8月6日 MedDRA: Version(6.1)
	1-2	臨床検査	Ｃ型肝炎ウイルス	米国	男性	不明	不明	未回復	症例報告	外国製品	識別番号：A03-32 報告日：2003年8月6日 MedDRA: Version(6.1)
	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男	26歳	2003/8/30	軽快	症例報告	当該製品	FAX報告 報告日：2003年11月19日 （識別番号：03000006 2003年11月28日） MedDRA: Version(6.1)

100072	2009/12/17	90806	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン