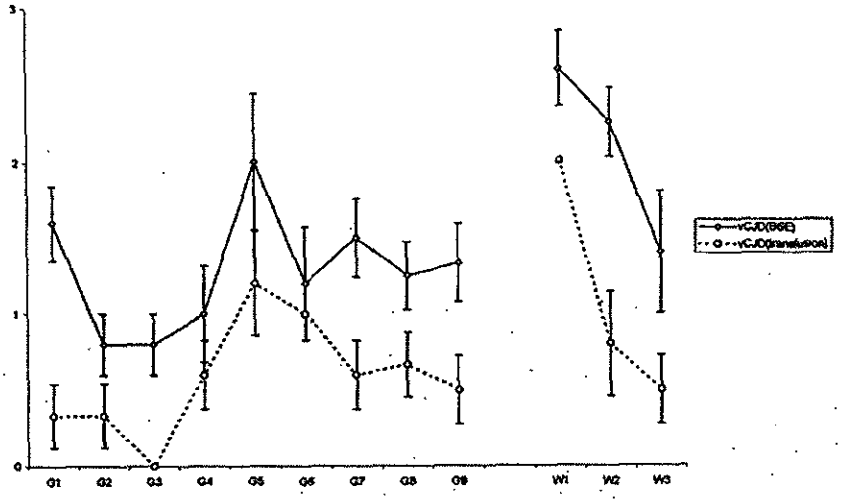
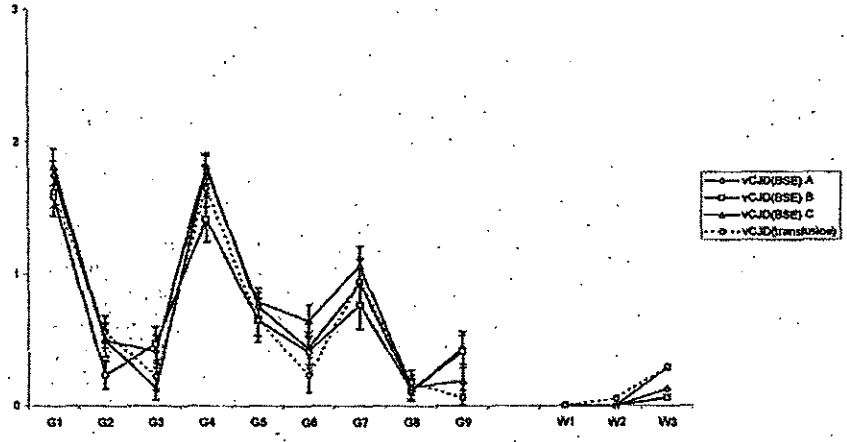
HuMM


vCJD(BSE)
vCJD(transfusion)
G1
G2
G3
G4
G5
G6
G7
G8
G9
W1
W2
W3

RIII


vCJD(BSE) A
vCJD(BSE) B
vCJD(BSE) C
vCJD(transfusion)
G1
G2
G3
G4
G5
G6
G7
G8
G9
W1
W2
W3

VM

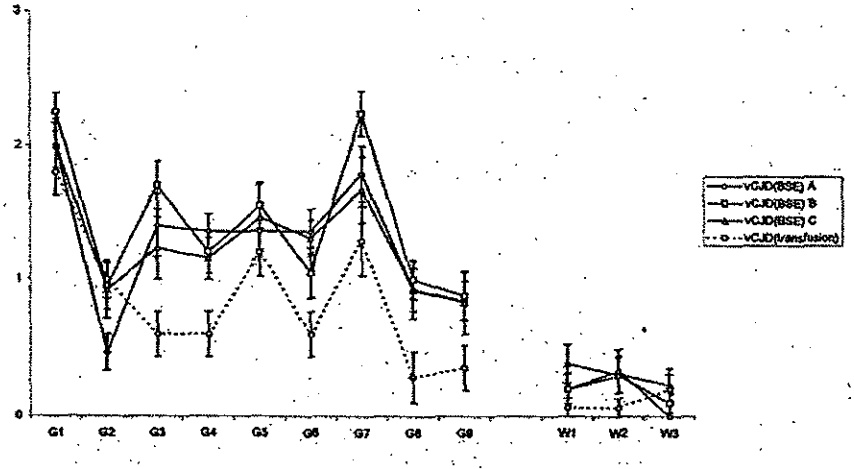

vCJD(BSE) A
vCJD(BSE) B
vCJD(BSE) C
vCJD(transfusion)
G1
G2
G3
G4
G5
G6
G7
G8
G9
W1
W2
W3

Figure 2. Vacuolation scoring in the mouse brain. Lesion profile comparison of vCJD (transfusion) case versus vCJD (BSE) transmissions to identify similarities in vacuolar pathology levels and regional distribution in mouse brains. (mean score ±SEM; dashed line - vCJD (transfusion) case; solid lines – 3x vCJD (BSE) cases for wild-type mice (diamonds – vCJD(BSE) A; squares – vCJD(BSE) B; triangles – vCJD(BSE) C) and published vCJD (BSE) for HuMM transgenic; G1–G9 grey matter scoring regions; W1–W3 white matter scoring regions)
doi:10.1371/journal.pone.0002878.g002

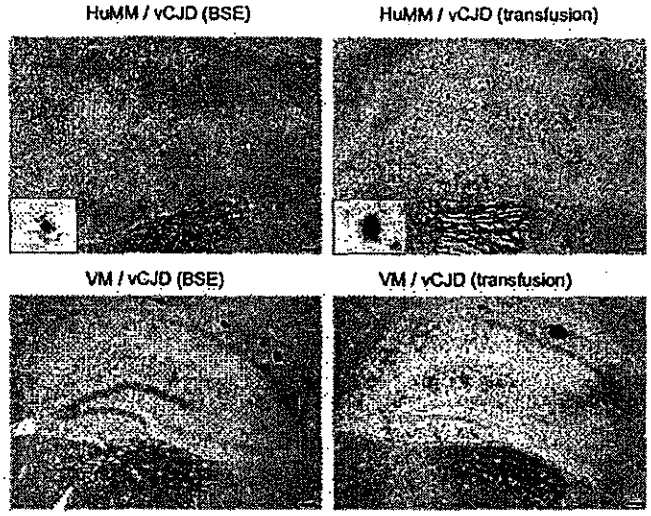


Figure 3. Detection of abnormal PrP in the mouse brain. Immunocytochemical detection of abnormal PrP deposition in hippocampus and thalamus (lateral posterior nucleus) of HuMM transgenic (with additional 40× magnification of florid plaque structure; see box lower left) and VM wild-type mice following inoculation with vCJD (BSE) and vCJD (transfusion) material. (Scale bar 200 μm, anti-PrP antibody 6H4)
doi:10.1371/journal.pone.0002878.g003

sequences, and survive for the same lifespan as non-transgenic mice of the same genetic background (129Ola) with no adverse effects and no features of spontaneous TSE disease. Wild-type mice (lines VM and RIII) are inbred lines used routinely for strain typing of TSEs. RIII is a *Prnp*-a genotype line and VM is a *Prnp*-b genotype line. [33] Use of mice for this work was reviewed and approved by the Neuropathogenesis Division Ethics Committee for Animal Experimentation.

Mice were inoculated as described previously. Groups of 24 wild-type mice received a 0.02 ml dose at 10^{-1} dilution by the intracerebral route, for vCJD (transfusion) and vCJD (BSE). Groups of 18 transgenic mice were injected with inoculum at a higher dilution of 10^{-2} as in previous experiments more concentrated inocula had been found to be toxic to the mice. Inoculum was prepared as a homogenate in sterile saline from frozen frontal cortex (with full consent from the patient's relatives, and approved by the Lothian NHS Board Research Ethics Committee (Reference: 2000/4/157)) to allow accurate comparison with previous data. Cases used for transmission were: the first

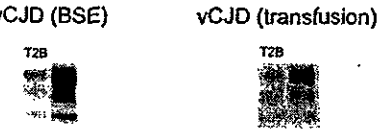


Figure 4. PrP^{Sc} typing by Western blot. Brain homogenates from HuMM mice inoculated with both vCJD (BSE) and vCJD (transfusion) show similar mobility and glycosylation profile (type 2B) as material from vCJD patients. (T2B: control vCJD material; antibody: 6H4)
doi:10.1371/journal.pone.0002878.g004

blood transfusion associated case, designated here as vCJD (transfusion), and three historical vCJD cases designated here as vCJD (BSE) A, B, and C. The historical vCJD cases were not inoculated into the transgenic mice. Data from vCJD (transfusion) inoculation of the transgenic mice was compared with that already published for vCJD (BSE). [12] Data from vCJD (transfusion) inoculation of the wild-type mice was compared with data from the three historical vCJD cases.

Mice were housed in independently ventilated cages in a Category 3 facility, monitored daily and scored for signs of TSE disease weekly from 100 days post inoculation. Mice were culled, when clinical TSE was evident or for animal welfare reasons, by cervical dislocation and the brain bisected sagittally; one half frozen for biochemical analysis of disease-associated prion protein and the other half fixed in formalin for histology.

Vacuolation scoring was performed according to published protocols and lesion profiles generated. [34,35] Immunocytochemical detection of abnormal PrP deposition was performed as published and Western blotting of disease-associated PrP from the frozen half-brain carried out according to Head *et al.* [12,25]

Acknowledgments

We thank Irene McConnell and the Animal Facility staff in the Neuropathogenesis Division, Anne Suttie and the Pathology staff for sectioning the mouse brains and assessing the levels of TSE vacuolation, and Dot Kisielewski for transgenic mouse genotyping.

This study would not be possible without the continued support of the families of those affected by vCJD, and the neurologists and neuropathologists throughout the UK that assist in CJD surveillance.

Author Contributions

Conceived and designed the experiments: MTB RGW MB JCM. Performed the experiments: MTB DLR VT. Analyzed the data: MTB DLR MWH. Contributed reagents/materials/analysis tools: JWI MWH. Wrote the paper: MTB RGW JWI JCM.

References

1. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, et al. (1996) A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347: 921–925.
2. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, et al. (1997) Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 389: 498–501.
3. Andrews NJ, Farrington CP, Ward HJ, Cousens SN, Smith PG, et al. (2003) Deaths from variant Creutzfeldt-Jakob disease in the UK. Lancet 361: 751–752.
4. Llewelyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, et al. (2004) Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 363: 417–421.
5. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG (2006) Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. Vox Sang 91: 221–230.
6. Zeidler M, Stewart G, Cousens SN, Estibeiro K, Will RG (1997) Codon 129 genotype and new variant CJD. Lancet 350: 668.
7. HPA (2006) New case of blood transfusion-associated variant-CJD. CDR Weekly 16.
8. Wroe SJ, Pal S, Siddique D, Hyare H, Macfarlane R, et al. (2006) Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. Lancet 368: 2061–2067.

9. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 364: 527–529.
10. Ironside JW, Bishop MT, Connolly K, Hegazy D, Lowrie S, et al. (2006) Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study. BMJ.
11. Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, et al. (2004) Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. J Pathol 203: 733–739.
12. Bishop M, Hart P, Aitchison L, Baybutt H, Plinston C, et al. (2006) Predicting susceptibility and incubation time of human-to-human transmission of vCJD. The Lancet Neurology 5: 393–398.
13. Houston F, Foster JD, Chong A, Hunter N, Bostock CJ (2000) Transmission of BSE by blood transfusion in sheep. Lancet 356: 999–1000.
14. Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, et al. (2002) Transmission of prion diseases by blood transfusion. J Gen Virol 83: 2897–2905.
15. McCutcheon S, Hunter N, Foster JD, Macgregor I, Hornsey V, et al. (2007) Transmission of BSE infection in sheep via blood transfusion. Edinburgh.
16. Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, et al. (1997) The same prion strain causes vCJD and BSE. Nature 389: 448–450.
17. Taguchi Y, Mohri S, Ironside JW, Muramoto T, Kitamoto T (2003) Humanized knock-in mice expressing chimeric prion protein showed varied susceptibility to different human prions. Am J Pathol 163: 2585–2593.
18. Capobianco R, Casalone C, Suardi S, Mangieri M, Miccolo C, et al. (2007) Conversion of the BASE Prion Strain into the BSE Strain: The Origin of BSE? PLoS Pathogens 3: e31.
19. Kong Q, Huang S, Zou W, Vanegas D, Wang M, et al. (2005) Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models. J Neurosci 25: 7944–7949.
20. Manson JC, Cancellotti E, Hart P, Bishop MT, Barron RM (2006) The transmissible spongiform encephalopathies: emerging and declining epidemics. Biochem Soc Trans 34: 1155–1158.
21. Korth C, Kaneko K, Groth D, Heye N, Telling G, et al. (2003) Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. Proc Natl Acad Sci U S A 100: 4784–4789.
22. Asante EA, Linehan JM, Gowland I, Joiner S, Fox K, et al. (2006) Dissociation of pathological and molecular phenotype of variant Creutzfeldt-Jakob disease in transgenic human prion protein 129 heterozygous mice. Proc Natl Acad Sci U S A 103: 10759–10764.
23. Bruce ME, McConnell I, Will RG, Ironside JW (2001) Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. Lancet 358: 208–209.
24. Bruce M, Will R, Ironside J, Fraser H (2006) Evidence for a link between variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. In: Hornlimann B, Riesner D, Kretzschmar H, eds. Prions in Humans and Animals Walter de Gruyter. pp 573–578.
25. Head MW, Bunn TJ, Bishop MT, McLoughlin V, Lowrie S, et al. (2004) Prion protein heterogeneity in sporadic but not variant Creutzfeldt-Jakob disease: U.K. cases 1991–2002. Ann Neurol 55: 851–859.
26. Cooper JK, Ladhani K, Minor PD (2007) Reference materials for the evaluation of pre-mortem variant Creutzfeldt-Jakob disease diagnostic assays. Vox Sang 92: 302–310.
27. Herzog C, Sales N, Etchegaray N, Charbonnier A, Freire S, et al. (2004) Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. Lancet 363: 422–428.
28. Baron T (2002) Mouse models of prion disease transmission. Trends Mol Med 8: 495.
29. Bruce M, Chree A, McConnell I, Foster J, Pearson G, et al. (1994) Transmission of bovine spongiform encephalopathy and scrapie to mice - strain variation and the species barrier. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 343: 405–411.
30. Lasmezas CI, Fournier JG, Nouvel V, Boe H, Marce D, et al. (2001) Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt–Jakob disease: implications for human health. Proc Natl Acad Sci U S A 98: 4142–4147.
31. Cervenakova L, Yakovleva O, McKenzie C, Kolchinsky S, McShane L, et al. (2003) Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. Transfusion 43: 1687–1694.
32. Bartz JC, Kincaid AE, Bessen RA (2003) Rapid prion neuroinvasion following tongue infection. J Virol 77: 583–591.
33. Barron RM, Baybutt H, Tuzi NL, McCormack J, King D, et al. (2005) Polymorphisms at codons 108 and 189 in murine PrP play distinct roles in the control of scrapie incubation time. J Gen Virol 86: 859–868.
34. Fraser H, Dickinson AG (1968) The sequential development of the brain lesion of scrapie in three strains of mice. Journal of Comparative Pathology 78: 301–311.
35. Bruce ME, McConnell I, Fraser H, Dickinson AG (1991) The disease characteristics of different strains of scrapie in Sinc congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. J Gen Virol 72 (Pt 3): 595–603.

別紙様式第2-1
番号16

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2008年9月16日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	乾燥濃縮人アンチトロンビンⅢ	研究報告の公表状況	PLoS ONE 2008; 3(8_e3017): 1-8	公表国 フランス
販売名 (企業名)	①ノイアート（ベネシス） ②ノイアート静注用1500単位（ベネシス）			

研究報告の概要

＜背景＞
ヒトのvCJDは、古典的BSEに罹った畜殺牛のプリオンを食料として摂取することで感染する。非定型BSEは、高齢牛では殆どが無症候であるが、最近ヨーロッパと北米の畜殺場で確認され、これらの新しいプリオン株に対するヒトの感受性についての問題が提起されている。
＜方法/主な所見＞
古典的BSEと非定型BSEに感染した牛の脳のホモジネートを、以前に古典的BSEのオリジナル株に感受性が高いことを示したヒト以外の霊長動物モデルであるカニクイザルに脳内接種した。こうして発現させた疾患を、臨床兆候、組織学、異常プリオンたん白の生化学の点から比較した。非定型BSEに感染した1頭のサルは生存期間が短く、古典的BSEまたはvCJD接種動物のいずれとも異なる臨床的展開、組織変化、プリオン蛋白(PrPres)パターンを示した。加えて、非定型BSEの接種動物におけるPrPresの生化学的特徴として、octa-repeat領域のプロテイナーゼKへの高い感受性を有していることが判明した。我々は、感染牛と同じ郡に住んでいた孤発性CJDおよびMM type 2 PrP遺伝型の4人の患者のうちの3人に、同じ生化学的特徴があるのを見出した。
＜結論＞
我々の結果は、霊長動物において、古典的BSEよりも非定型BSEの方が病原性が高い可能性があることを示し、加えて外見上孤発性CJDに見えるまれな症例群と結びついている可能性についての問題を提起している。これより、古典的BSEの流行が衰えているにも関わらず、非定型株の発生によって、BSE汚染製品による偶発的汚染から公衆衛生を保護するために現在実施されている措置を緩和することを推進することは抑えるべきである。

報告企業の意見	今後の対応
霊長動物では非定型BSEの方が古典的BSEよりも病原性が高く、孤発性CJDに見える症例と結びついている可能性があるとの報告である。 これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	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。

使用上の注意記載状況・その他参考事項等

代表としてノイアート（献血）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。

OPEN ACCESS Freely available online

PLoS one

Atypical BSE (BASE) Transmitted from Asymptomatic Aging Cattle to a Primate

Emmanuel E. Comoy[1]*, Cristina Casalone[2], Nathalie Lescoutra-Etchegaray[1], Gianluigi Zanusso[3], Sophie Freire[1], Dominique Marcé[1], Frédéric Auvré[1], Marie-Magdeleine Ruchoux[1], Sergio Ferrari[3], Salvatore Monaco[3], Nicole Salès[4], Maria Caramelli[2], Philippe Leboulch[1,5], Paul Brown[1], Corinne I. Lasmézas[4], Jean-Philippe Deslys[1]

1 Institute of Emerging Diseases and Innovative Therapies, CEA, Fontenay-aux-Roses, France, 2 Istituto Zooprofilattico Sperimentale del Piemonte, Turin, Italy, 3 Policlinico G.B. Rossi, Verona, Italy, 4 Scripps Florida, Jupiter, Florida, United States of America, 5 Genetics Division, Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States of America.

Abstract

Background: Human variant Creutzfeldt-Jakob Disease (vCJD) results from foodborne transmission of prions from slaughtered cattle with classical Bovine Spongiform Encephalopathy (cBSE). Atypical forms of BSE, which remain mostly asymptomatic in aging cattle, were recently identified at slaughterhouses throughout Europe and North America, raising a question about human susceptibility to these new prion strains.

Methodology/Principal Findings: Brain homogenates from cattle with classical BSE and atypical (BASE) infections were inoculated intracerebrally into cynomolgus monkeys (*Macaca fascicularis*), a non-human primate model previously demonstrated to be susceptible to the original strain of cBSE. The resulting diseases were compared in terms of clinical signs, histology and biochemistry of the abnormal prion protein (PrPres). The single monkey infected with BASE had a shorter survival, and a different clinical evolution, histopathology, and prion protein (PrPres) pattern than was observed for either classical BSE or vCJD-inoculated animals. Also, the biochemical signature of PrPres in the BASE-inoculated animal was found to have a higher proteinase K sensitivity of the octa-repeat region. We found the same biochemical signature in three of four human patients with sporadic CJD and an MM type 2 PrP genotype who lived in the same country as the infected bovine.

Conclusion/Significance: Our results point to a possibly higher degree of pathogenicity of BASE than classical BSE in primates and also raise a question about a possible link to one uncommon subset of cases of apparently sporadic CJD. Thus, despite the waning epidemic of classical BSE, the occurrence of atypical strains should temper the urge to relax measures currently in place to protect public health from accidental contamination by BSE-contaminated products.

Citation: Comoy EE, Casalone C, Lescoutra-Etchegaray N, Zanusso G, Freire S, et al. (2008) Atypical BSE (BASE) Transmitted from Asymptomatic Aging Cattle to a Primate. PLoS ONE 3(8): e3017. doi:10.1371/journal.pone.0003017

Editor: Neil Mabbott, University of Edinburgh, United Kingdom

Received April 24, 2008; **Accepted** August 1, 2008; **Published** August 20, 2008

Funding: This work has been supported by the Network of Excellence NeuroPrion.

Competing Interests: CEA owns a patent covering the BSE diagnostic tests commercialized by the company Bio-Rad.

* E-mail: emmanuel.comoy@cea.fr

Introduction

Classical Bovine Spongiform Encephalopathy (cBSE), the first prion disease identified in cattle, was initially reported in 1986 in the UK. Food-borne transmission of cBSE to humans was observed ten years later as a variant form of Creutzfeldt-Jakob Disease (vCJD) [1], leading to a major public health crisis.

This strain of cBSE is now rapidly disappearing as a result of appropriate containment measures. However, atypical forms of BSE have recently been identified in Europe and North America as a consequence of cBSE testing performed in these countries [2-4]. Because these cases are only found sporadically in older animals (≥8 years) coming to slaughter with few or no signs of disease, it would be plausible to suppose that atypical forms of BSE may have a lower virulence than cBSE and be innocuous to humans. However, recent studies suggest that one of the two main forms of atypical BSE, initially discovered in Italy and referred to as the bovine amyloidotic spongiform encephalopathy (BASE), might be at the origin of the cBSE epidemic: inoculation of the BASE strain into transgenic and inbred mice showed an apparent natural evolution towards the typical BSE strain [5,6]. Moreover, a possible link has been suggested between BASE and one subtype (MV2) of human sporadic CJD (sCJD) on the basis of biochemical similarities [2,7]. In contrast to vCJD, sCJD is believed to occur de novo without food-borne transmission. However, specific contaminating events by ingestion are difficult to rule out because human prion diseases can have silent incubation periods exceeding 50 years, as demonstrated for kuru [8].

One strategy to evaluate the risk of BASE for humans consists in assessing the susceptibility to disease transmission and the degree of pathogenicity in a non-human primate model that has already been shown to have characteristic clinical signs, histopathological lesions and PrPres profiles following infections with either BSE or vCJD [9,10]. We therefore inoculated cynomolgus macaque monkeys (*Macaca fascicularis*) intracerebrally with BASE, cBSE and vCJD prion strains. The BASE strain, prepared from brain extract of a 15-

Table 1. Survival times of macaques inoculated intracerebrally with brain homogenates from cattle with BASE or BSE, and from humans with vCJD.

Strain	Source	Dose*	Survival time (months)
BASE	cattle	100 mg	40
BSE	cattle	100 mg	40
vCJD	human	40 mg	25
vCJD	human	40 mg	32
vCJD	human	40 mg	32

*Amount of crude brain in 10% brain suspension inoculated intracerebrally. BSE brain had a 10-fold greater concentration of PrP^{Res} than the BASE brain). Animals inoculated with vCJD also received the equivalent of 8 mg of brain by intra-tonsillar injection.

doi:10.1371/journal.pone.0003017.t001

year-old asymptomatic cow induced a distinctive and more rapidly fatal disease than cBSE, and showed a biochemical signature similar to that of the MM2 cortical subtype of human sCJD.

Methods

Cattle and human samples

The BASE inoculum (mix of brainstem and thalamus) from an asymptomatic 15 year-old Italian Piemontese cow [2]: 250 µl of a 10% brain homogenate in 5% glucose were inoculated intracerebrally (i.c.) to a single macaque monkey. As controls, we used two macaques inoculated i.c. with cBSE (brainstem from infected UK cattle) and 4 macaques inoculated i.c. with human vCJD [9,11]. Twenty-one subjects with a diagnosis of definite sCJD were referred to the Medical Center in Verona, Italy during the period 2000–2004. Tissues were processed 4–18 hours post-mortem according to established guidelines regarding safety and ethics. Brains were cut longitudinally into two halves. Hemi-brains were frozen and stored at −80°C until biochemical studies were performed. The patient group encompassed all of the different

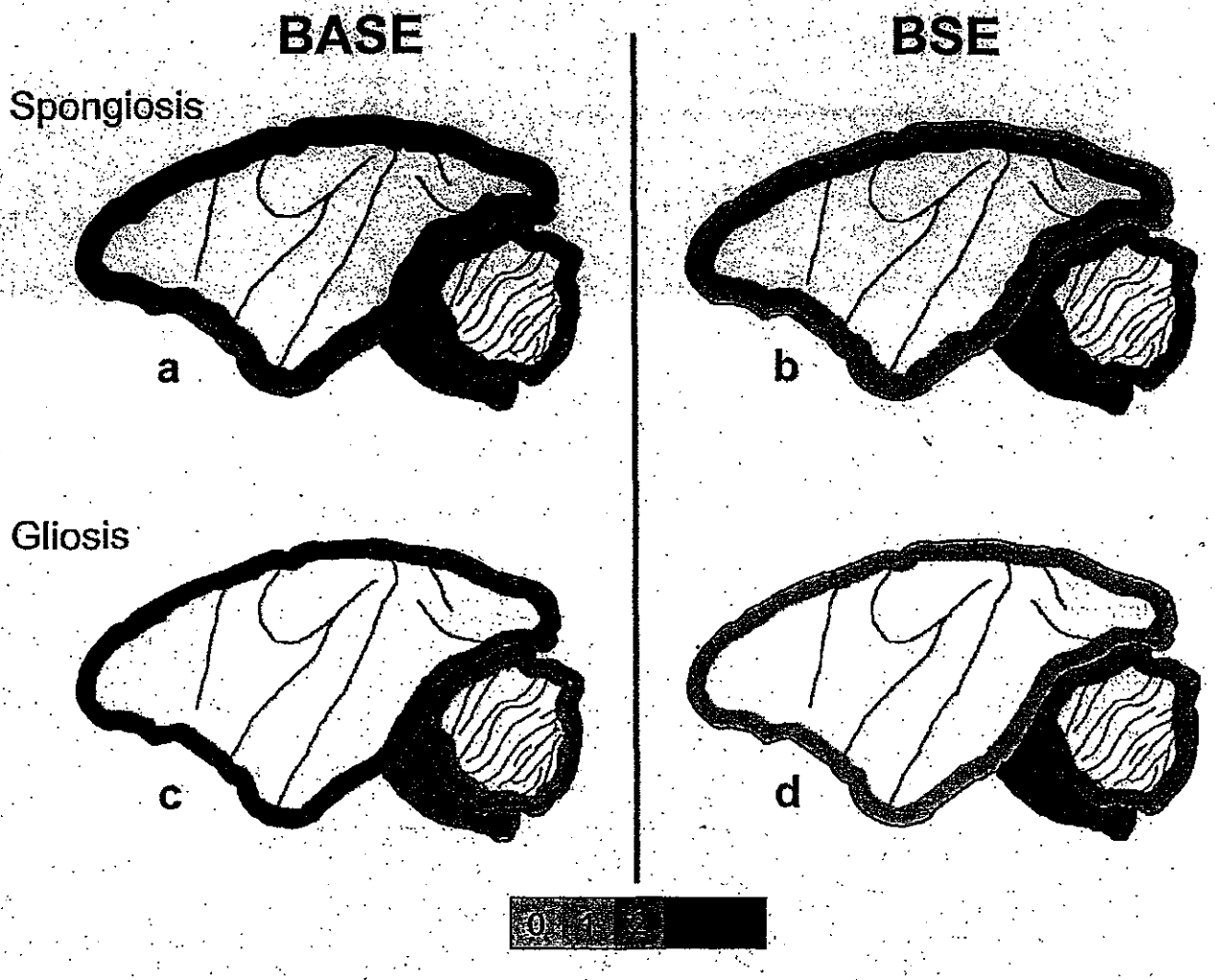


Figure 1. Diagrammatic representation of histologic lesions. Topographic distribution of spongiosis (a and b) or gliosis (c and d) in BASE and cBSE-infected primates. The lesions were scored from 0 to 4 (negative, light, mild, moderate, and severe).

doi:10.1371/journal.pone.0003017.g001