JVI Journals.ASM.org

Development of Dose-Response Models of Creutzfeldt-Jakob Disease Infection in Nonhuman Primates for Assessing the Risk of Transfusion-Transmitted Variant Creutzfeldt-Jakob Disease

Yin Huang,[a] Luisa Gregori,[b] Steven A. Anderson,[a] David M. Asher,[b] Hong Yang[a]

Office of Biostatistics and Epidemiology, U.S. Food and Drug Administration, Silver Spring, Maryland, USA[a]; Office of Blood Research and Review, U.S. Food and Drug Administration, Silver Spring, Maryland, USA[b]

ABSTRACT

Estimates for the risk of transmitting variant Creutzfeldt-Jakob disease (vCJD) via blood transfusion have relied largely on data from rodent experiments, but the relationship between dose (amount of infected blood) and response (vCJD infection) has never been well quantified. The goal of this study was to develop a dose-response model based on nonhuman primate data to better estimate the likelihood of transfusion-transmitted vCJD (TTvCJD) in humans. Our model used dose-response data from nonhuman primates inoculated intracerebrally (i.c.) with brain tissues of patients with sporadic and familial CJD. We analyzed the data statistically by using a beta-Poisson dose-response model. We further adjusted model parameters to account for the differences in infectivity between blood and brain tissue and in transmission efficiency between intravenous (i.v.) and i.c. routes to estimate dose-dependent TTvCJD infection. The model estimates a mean infection rate of 76% among recipients who receive one unit of whole blood collected from an infected donor near the end of the incubation period. The nonhuman primate model provides estimates that are more consistent with those derived from a risk analysis of transfused nonleukoreduced red blood cells in the United Kingdom than prior estimates based on rodent models.

IMPORTANCE

TTvCJD was recently identified as one of three emerging infectious diseases posing the greatest immediate threat to the safety of the blood supply. Cases of TTvCJD were reported in recipients of nonleukoreduced red blood cells and coagulation factor VIII manufactured from blood of United Kingdom donors. As the quantity of abnormal prions (the causative agent of TTvCJD) varies significantly in different blood components and products, it is necessary to quantify the dose-response relationship for a wide range of doses for the vCJD agent in transfused blood and plasma derivatives. In this paper, we suggest the first mechanistic dose-response model for TTvCJD infection based on data from experiments with nonhuman primates. This new model may improve estimates of the possible risk to humans.

Variant Creutzfeldt-Jakob disease (vCJD) is a human transmissible spongiform encephalopathy (TSE) first reported in the United Kingdom in 1996 (1). TSEs are slow neurodegenerative diseases affecting humans and animals with long asymptomatic incubation periods (IPs). TSEs are most reliably diagnosed postmortem based on typical histopathological changes and the detection, usually in the brain, of the abnormal, protease-resistant form of the prion protein (PrP^{TSE}) generated during this disease. Experimental and epidemiological evidence indicates that dietary exposure to the bovine spongiform encephalopathy (BSE) agent was responsible for most cases of vCJD in humans. Although foodborne vCJD cases are in decline, secondary transmissions of vCJD through blood transfusions, first reported in 2003, still pose a potential risk to public health. A better understanding of the relationship between the dose of the infectious agent that a person may receive and resulting infection is crucial to estimate the risk of vCJD transmission through blood transfusion.

The greatest challenge for studying the dose-response (DR) relationship for transfusion-transmitted vCJD (TTvCJD) is the scarcity of experimental data. Epidemiological studies typically provide limited information on the infectious doses. The United Kingdom Transfusion Medicine Epidemiology Review (TMER), a survey of human TTvCJD cases (2), reported information on the infection rate in recipients transfused with one unit of infected blood products, but no DR relationship could be derived from such data with a single data point or a narrow range of doses. Ideally, DR studies for intravenous (i.v.) exposure, the route of exposure for TTvCJD, should be based on well-controlled experiments with animals, possibly nonhuman primates, challenged intravenously with a range of doses of infected blood. However, such studies have not been reported. Nonhuman primates are preferred animal models because of their close phylogenetic relationship to humans and similar clinical signs, histopathological lesions, and PrP^{TSE} profiles following infections with either BSE or vCJD agents. Therefore, we considered data from a previously published review of TSE transmission studies conducted at the U.S. National Institutes of Health (NIH) over a number of years with nonhuman primates inoculated intracerebrally (i.c.) with TSE-infected brain tissues (3). We used mechanistic nonlinear DR models to characterize the animal response to different doses of

Received 25 June 2014 **Accepted** 11 September 2014
Published ahead of print 17 September 2014
Editor: B. W. Caughey
Address correspondence to Yin Huang, Yin.Huang@fda.hhs.gov, or Hong Yang, Hong.Yang@ GRO-C

doi:10.1128/JVI.01805-14

TABLE 1 Dose-response data for 83 nonhuman primates exposed via the i.c. route to graded doses of brain tissues from patients with sCJD and fCJD[a]

Dose (g brain tissue)	No. of infected primates	No. of uninfected primates	Total no. of primates
1×10^{-2}	25	0	25
1×10^{-3}	6	0	6
1×10^{-4}	15	5	20
1×10^{-5}	4	8	12
1×10^{-6}	3	5	8
1×10^{-7}	0	6	6
1×10^{-8}	0	3	3
1×10^{-9}	0	3	3

[a] See reference 3.

infectious CJD agents. We further adjusted the model parameters using scaling factors to account for the differences in infectivity between blood and brain tissue and transmission efficiency between the i.v. and i.c. routes. The DR model developed in this study estimates the risk of vCJD infection by blood transfusion as a function of the volume transfused.

MATERIALS AND METHODS

Dose-response data. We searched PubMed and ISI Web of Science for available dose-response data for nonhuman primates exposed to TSE agents (prions) via the i.v. and i.c. routes (English-language original research articles published before March 2014; the search terms were "dose," "prion," "vCJD," "TSE," "primate," "intravenous," "intracerebral," and synonyms). We found a review article that summarized dose-response data from the NIH series of experiments with nonhuman primates infected with prions via the i.c. route (3). This large data set included eight graded doses causing infection rates ranging from 0 to 100% with intermediate responses, which presented a desirable pattern for dose-response modeling.

In these nonhuman primate experiments, diluted brain tissues from 27 patients with CJD and other human TSEs were inoculated into the animals via the i.c. route. Of the 27 patients, 22 were infected with CJD (21 with sporadic CJD [sCJD] and 1 with familial CJD [fCJD]) and 5 other individuals had Gerstmann-Sträussler-Scheinker syndrome (GSS) or kuru. Only data from nonhuman primates (total number = 83) inoculated with CJD-infected brains were used in our analysis to maintain homogeneity of the type of TSE inoculum. Most of the nonhuman primates were squirrel monkeys, with small numbers of chimpanzees, capuchin monkeys, and spider monkeys. The specific numbers of each species of primates were not reported. Linkage between CJD donors and recipient primates was also not described. TSE infection was diagnosed by postmortem neurohistopathological examinations of the brains of the animals. Table 1 shows the extracted data, including doses and numbers of infected/uninfected animals.

Dose-response modeling. Both exponential and beta-Poisson models have been widely used for DR analyses of infectious agents. Both models depend on a mechanistic DR relationship describing an estimated probability of a quantity of infectious agent surviving in the host and successfully initiating infection (4). The statistical programming language "R" (http://www.r-project.org/) was used to program the maximum likelihood estimation (MLE) computations for fitting models to DR data. Binomial MLEs were used to optimize the fit of the likelihood function by selecting the parameters with the best fit that minimized the deviances of the estimated function from the observed data. Confidence intervals (CIs) for best-fit models were determined by using bootstrap analyses with 5,000 bootstrap samples drawn from the DR data sets. To test the goodness-of-fit for a model's ability to predict the data outcomes, we compared the optimal minimum deviance of the model to the critical value of the chi-squared distribution at a 95% confidence level ($\chi^2_{0.95,df}$, where df is the degree of freedom, calculated by the number of dose levels minus the number of model parameters). The data in Table 1 were used to generate the DR model for i.c. inoculations of infected brains into primates. Because the goal of this study was to use the model to generate a DR curve for TTvCJD infection, we adjusted it by using a scaling factor that converted i.c. to i.v. transmissions based on the difference of their transmission efficiencies. In addition, as the infectious dose in the i.c. model was measured as grams of infected brain tissue, the model was adjusted to the infectious dose for the i.v. route and converted to milliliters of blood.

RESULTS

Dose-response models for i.c. exposure. DR models were fit to the primate data in Table 1. The beta-Poisson model (see the equation below) provided a statistically acceptable fit based on the goodness-of-fit test, while the exponential model did not (data not shown). In the equation below, d represents the infectious dose (grams of infected brain tissue or milliliters of infected blood) and $P(d)$ represents the probability of acquiring TSE infection at dose d. Optimized values for the parameters α and N_{50} were 0.456 and 9.6×10^{-6} (representing a 50% infective dose [ID_{50}] of 9.6 μg brain tissue), respectively, for brain inoculation i.c.; 0.456 and 13.7 (representing an ID_{50} of 13.7 ml blood), respectively, for blood inoculation i.c. (after adjustment with a factor accounting for the difference in infectivity between brain tissue and blood); and 0.456 and 75 (representing an ID_{50} of 75 ml blood), respectively, for blood transfusion (after adjustment with a factor accounting for the difference in efficiencies between the i.c. and i.v. routes of exposure). For brain inoculation, the parameter N_{50} can be interpreted as the amount (in grams) of brain tissue inoculum needed to cause a 50% chance of infection (equal to the ID_{50}). In this case, an N_{50} value equal to 9.6×10^{-6} means that 9.6 μg of infected brain tissue will cause a 50% chance of infection. α is the slope parameter describing the host-pathogen interaction. The experimental data and the beta-Poisson model (mean and 90th percentile values) are presented in Fig. 1.

$$P(d) = 1 - \left[1 + \left(\frac{d}{N_{50}}\right) \cdot \left(2^{1/\alpha} - 1\right)\right]^{-\alpha}$$

Adjustment for infectivity in blood versus brain tissue. We needed to convert the infectivity dose present in a gram of brain tissue to the equivalent infectivity dose in a volume of blood. To estimate this conversion, we analyzed data from studies using different methodologies and approaches and obtained a triangular distribution of scaling factors with the upper bound and most likely value from a transmission study by Douet et al. (5) and the lower bound based on other relevant studies.

Douet et al. (5) inoculated transgenic mice expressing bovine prion protein via the i.c. route using brain tissue and blood fractions (erythrocytes, plasma, and leukocytes) from a patient with confirmed vCJD. By comparing the infection rates, those researchers estimated that the quantity of infectivity present in 1 ml of whole blood was approximately equivalent to that present in 1.4×10^{-6} g of vCJD brain. To the best of our knowledge, this was the only study that quantified the difference in transmission efficiencies between vCJD-infected brain- and blood-derived inocula. In addition to transmission experiments, researchers have also developed laboratory techniques to measure the concentration of PrP^{TSE} in tissue samples directly. Wadsworth et al. (6) estimated

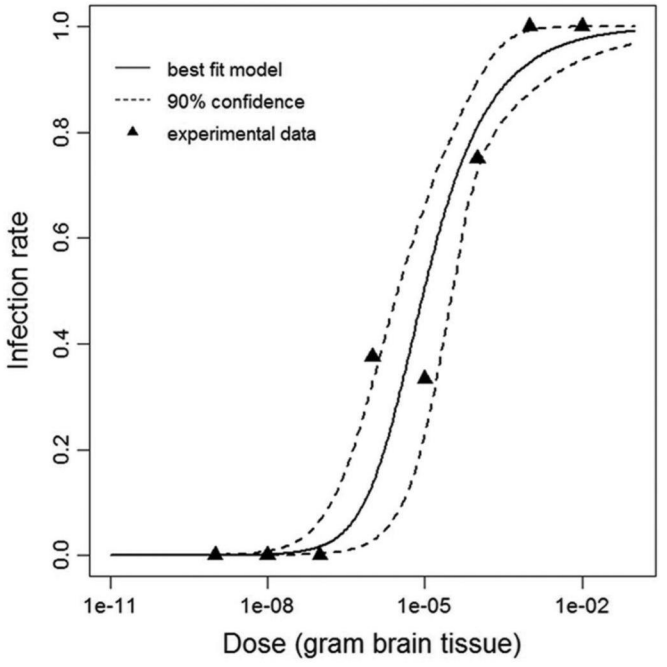

FIG 1 Beta-Poisson model fit to data from Table 1 for i.c. challenge of nonhuman primates (3) with confidence bounds around the optimized model ($\alpha = 0.456$; $N_{50} = 9.6 \times 10^{-6}$ [representing an ID_{50} of 9.6 μg brain tissue]).

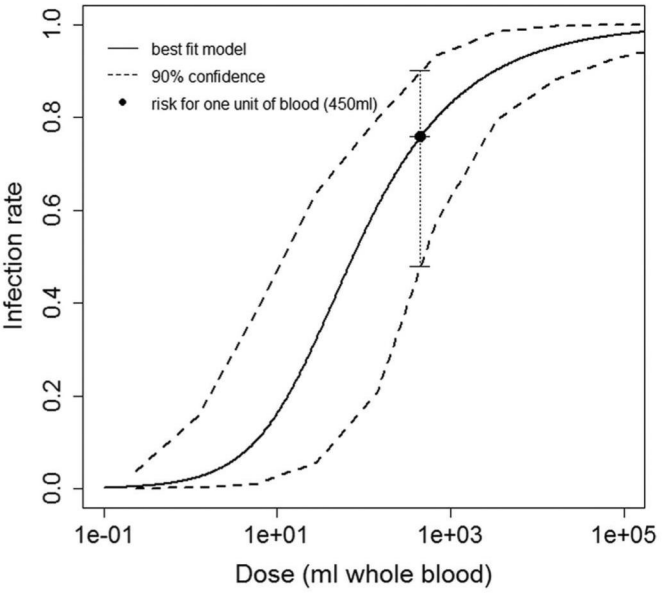

FIG 2 Beta-Poisson dose-response model for risk of transfusion-transmitted vCJD ($\alpha = 0.456$; $N_{50} = 75$ [representing an ID_{50} of 75 ml blood]). The estimated infection rate for one unit of transfused whole blood (450 ml) is 76% (90% CI, 48% to 90%).

PrP^{TSE} concentrations in different human tissues from patients with vCJD in the terminal stages of disease by using high-sensitivity Western blot analysis. Their data suggested that the amount of PrP^{TSE} in 1 ml blood was less than that in 10^{-7} g of human vCJD brain tissue. Both Gregori et al. (7), using a highly sensitive enzyme-linked immunosorbent assay (ELISA)-based assay, and Chen et al. (8), using protein misfolding cyclic amplification, estimated the concentration of PrP^{TSE} in plasma from scrapie-infected hamsters to be approximately 1×10^{-14} to 3×10^{-14} g per ml of plasma, and the PrP^{TSE} concentration in infected brain was estimated to be 2×10^{-5} to 6×10^{-5} g per g of brain tissue, which indicated that for hamsters, the amount of PrP^{TSE} in 1 ml of blood was equivalent to the amount present in $\sim 10^{-9}$ g of brain. These biochemical studies measured PrP^{TSE} present as mixtures with different levels of aggregation, but they did not distinguish between the PrP^{TSE} monomer and multimeric forms that might be more infectious (9).

To account for all these different values from the literature, we assumed that a reasonable scaling factor between the infectivities of 1 ml blood and 1 g brain tissue follows a triangular distribution, and we used 10^{-6} (from the transmission study using human tissues by Douet et al. [5]) as the upper bound as well as the most likely value of the distribution. While other biochemical studies reported lower ratios ($<10^{-7}$ for human by Wadsworth et al. [6] and 10^{-9} for hamster by Gregori et al. [7] and Chen et al. [8]) for the PrP^{TSE} concentrations in blood and brain tissues, the correlation between the reported concentrations and infectivity cannot be verified. Therefore, we used the lowest ratio (10^{-9}) from those studies to define the lower bound of the triangular distribution, which contributed much less to the final estimates than the most likely value (10^{-6}) from the study by Douet et al. The median of the triangular distribution (representing the center of the distribution) was used to convert the N_{50} from 9.6×10^{-6} (g of brain) to 13.7 (ml of blood). The parameter N_{50} for the adjusted model can be interpreted to mean that 13.7 ml of infected blood inoculated through the i.c. route has a 50% chance of transmitting infection.

Adjustment for efficiency of disease transmission by the i.v. versus the i.c. route. The DR model for blood inoculated by the i.c. route was further converted into a DR for the i.v. route by multiplying the parameter N_{50} with a scaling factor, which conceptually accounts for the difference in transmission efficiency between the two routes. Experiments with mice exposed to infectivity by the i.v. and i.c. routes suggested an i.c./i.v. ratio of 5 to 7 (10). Based on those experiments, in a previously reported vCJD risk assessment for blood clotting factors, the FDA suggested a uniform distribution, with 1 and 10 being the lower and upper bounds, respectively, for the ratio between the efficiencies of vCJD transmission via the i.c. and i.v. routes (11), which we adopted as the scaling factor for i.c.-to-i.v. conversion in the current study. Multiplied by the median of this uniform distribution, the parameter N_{50} (ID_{50}) was converted from 13.7 for the i.c. route to 75 for the i.v. route (see above).

Hence, the beta-Poisson DR model for blood transfusion has been derived with ml blood as the dose unit and with the optimized parameters of an N_{50} value of 75 and an α value of 0.456 (see above). The N_{50} value represents an ID_{50} equal to 75 ml blood. This model estimates a 76% infection rate (90% CI, 48% to 90% [CI generated by Monte Carlo simulation with 5,000 iterations]) for individuals receiving one unit (450 ml) of blood from a person with clinical vCJD (Fig. 2).

DISCUSSION

Linear DR models were previously adopted for vCJD and BSE risk assessments, (11–13), assuming a linear relationship between the exposure dose of the TSE agent and the probability of infection for

the entire range of doses. However, this linear relationship is certainly a simplification of the true dose-response interaction and does not account for the interactions between the agent and the host in the infection process, which may affect the final outcome. Nonlinear DR models were previously applied by Gravenor et al. (14) and by Huang (15) to data for hamsters orally exposed to scrapie agent (16), but no adjustments were made for the scenario of human transfusion-transmitted TSEs. In this study, we used a beta-Poisson DR model developed based on data for nonhuman primates to estimate the probability of vCJD infection, or TTvCJD risk, as a function of the volume of blood transfused. We believe this to be the first mechanistic DR model for TTvCJD generated by using data from experimental animal dosing studies.

Gregori et al. (17) analyzed TTvCJD data from the TMER study (2) and estimated that transfusion of a unit of infected nonleukoreduced red blood cells had a 75% infection rate (90% CI, 56% to 96%). The intervals between donation and clinical onset of vCJD in the implicated donors ranged from 1.4 years to 3.5 years. Given that the estimated median IP of food-borne vCJD is about 12 years, it seems that those donors whose blood transmitted the disease donated blood during the last one-third of the IP (17). Thus, the infection rate per unit transfused suggested previously by Gregori et al. represented the risk from blood of clinically normal donors during the later stage of the IP. Our model predicts an infection rate of 76% (90% CI, 48% to 90%) associated with one unit of whole blood transfused. If adjusted for a unit of nonleukoreduced red blood cells (equivalent to 24% to 30% of the infectious dose of whole blood [18, 19]), the rate would be 58% (90% CI, 20% to 78%). The DR model predictions based on nonhuman primate experiments and the results previously reported based on the human epidemiological study appear to be consistent. On the other hand, the ID_{50} (75 ml infected blood or equivalent blood products) given by this primate model is significantly higher than that based on rodent data (0.5 ml infected blood) (10, 11, 18). This implies that although studies of BSE and vCJD in rodents have provided important insights into host susceptibility, efficiency of transmission for different routes, and distribution of tissue infectivity, extrapolation of a dose-response model for humans from rodent data may overestimate the risk. A DR study using species more closely related to humans, e.g., a primate model, may be more relevant.

Nevertheless, due to the limited data available, our analysis necessarily incorporated the following assumptions. (i) The inocula used in the primate experiments were primarily infected brain tissues of sCJD patients, and we assumed that those inocula are a relevant surrogate model for vCJD-infected brains. Rubenstein and Chang (20) compared the tissue distributions and levels of PrP^{TSE} in patients with sCJD and vCJD; they reported no significant differences between the two TSEs in levels of PrP^{TSE} in both brain and other tissues (spleens, lymph nodes, and tonsils). While those findings support our assumption, uncertainties remain about whether infectivity titers in tissues and blood of patients with sCJD and vCJD are also similar. (ii) Different species of primates were used in the experiments. While we assumed all primate species tested followed the same dose-response pattern and could therefore be pooled into one model, such an assumption could not be validated. (iii) Extrapolation of the conversion rate of the i.c. versus the i.v. transmission route was based on imperfect and limited data and may not be precise. (iv) We assumed the ratio between PrP^{TSE} and infectivity in blood to be the same as that in brain. (v) Calculations to convert infectivity from weight of tissue to volume of blood were based on different studies, each with its own uncertainties. (vi) The current model ignores the impact of individual genotypes on susceptibility to vCJD. (vii) Early diagnosis, before symptoms of vCJD appear, is not yet possible because of the difficulty in identifying small quantities of abnormal prion protein in blood. While our model based on infected tissues from persons with overt disease is likely to apply to clinically healthy persons during the late IP of asymptomatic vCJD, the risk of TTvCJD from donors in an earlier stage of the disease will be lower.

Keeping these limitations in mind, this analysis establishes, for the first time, a plausible DR curve needed to quantify the risk of TTvCJD as a function of the volume of blood transfused. Based on these data, future vCJD risk assessment models for blood transfusion need no longer rely on a single-point estimate of infectivity titers. This dose-response curve can be used to better estimate the magnitude of risk, and the associated uncertainty, from exposures to low doses of a vCJD agent in transfused blood and plasma derivatives. This development should assist regulators to make better-informed science-based policy decisions to protect public health.

ACKNOWLEDGMENT

This research was funded by the U.S. Food and Drug Administration through the Oak Ridge Institute for Science and Education.

REFERENCES

1. **Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG.** 1996. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet **347**:921–925. http://dx.doi.org/10.1016/S0140-6736(96)91412-9.
2. **The UK National Creutzfeldt-Jakob Disease Research & Surveillance Unit and the UK Blood Services.** 12 February 2013. Transfusion medicine epidemiology review (TMER). http://www.cjd.ed.ac.uk/TMER/TMER.htm.
3. **Brown P, Gibbs CJ, Jr, Rodgers-Johnson P, Asher DM, Sulima MP, Bacote A, Goldfarb LG, Gajdusek DC.** 1994. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. Ann. Neurol. **35**:513–529. http://dx.doi.org/10.1002/ana.410350504.
4. **Haas CN, Rose JB, Gerba CP.** 1999. Quantitative microbial risk assessment. John Wiley & Sons, New York, NY.
5. **Douet JY, Zafar S, Perret-Liaudet A, Lacroux C, Lugan S, Aron N.** 2014. Detection of infectivity in blood of persons with variant and sporadic Creutzfeldt-Jakob disease. Emerg. Infect. Dis. **20**:114–117. http://dx.doi.org/10.3201/eid2001.130353.
6. **Wadsworth JD, Joiner S, Hill AF, Campbell TA, Desbruslais M, Luthert PJ, Collinge J.** 2001. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. Lancet **358**:171–180. http://dx.doi.org/10.1016/S0140-6736(01)05403-4.
7. **Gregori L, Gray BN, Rose E, Spinner DS, Kascsak RJ, Rohwer RG.** 2008. A sensitive and quantitative assay for normal PrP in plasma. J. Virol. Methods **149**:251–259. http://dx.doi.org/10.1016/j.jviromet.2008.01.028.
8. **Chen B, Morales R, Barria MA, Soto C.** 2010. Estimating prion concentration in fluids and tissues by quantitative PMCA. Nat. Methods **7**:519–520. http://dx.doi.org/10.1038/nmeth.1465.
9. **Silveira JR, Raymond GJ, Hughson AG, Race RE, Sim VL, Hayes SF, Caughey B.** 2005. The most infectious prion protein particles. Nature **437**:257–261. http://dx.doi.org/10.1038/nature03989.
10. **Brown P, Cervenáková L, McShane LM, Barber P, Rubenstein R, Drohan WN.** 1999. 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 **39**:1169–1178. http://dx.doi.org/10.1046/j.1537-2995.1999.39111169.x.
11. **FDA.** 2006. Draft quantitative risk assessment of vCJD risk potentially associated with the use of human plasma-derived factor VIII manufactured under United States (US) license from plasma collected in the US.

US Food and Drug Administration, Washington, DC. http://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/UCM095104.pdf. Accessed 4 January 2013.
12. **United Kingdom Department of Health.** 2002. Risk assessment of exposure to vCJD infectivity in blood and blood products (updated May 2006). United Kingdom Department of Health, London, United Kingdom.
13. **Harvard Center for Risk Analysis, Harvard School of Public Health.** 2003. Evaluation of the potential for bovine spongiform encephalopathy in the United States. Harvard School of Public Health, Boston MA.
14. **Gravenor MB, Stallard N, Curnow R, McLean AR.** 2003. Repeated challenge with prion disease: the risk of infection and impact on incubation period. Proc. Natl. Acad. Sci. U. S. A. **100:**10960–10965. http://dx.doi.org/10.1073/pnas.1833677100.
15. **Huang Y.** 2010. Incorporating time to response into dose-response models used in quantitative microbial risk assessment. Doctoral dissertation. Drexel University, Philadelphia, PA.
16. **Diringer H, Roehmel J, Beekes M.** 1998. Effect of repeated oral infection of hamsters with scrapie. J. Gen. Virol. **79**(Part 3):609–612.
17. **Gregori L, Yang H, Anderson SA.** 2011. Estimation of variant Creutzfeldt-Jakob disease infectivity titers in human blood. Transfusion **51:**2596–2602. http://dx.doi.org/10.1111/j.1537-2995.2011.03199.x.
18. **Brown P, Rohwer RG, Dunstan BC, MacAuley C, Gajdusek DC, Drohan WN.** 1998. The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. Transfusion **38:**810–816. http://dx.doi.org/10.1046/j.1537-2995.1998.38998408999.x.
19. **Bennett P, Daraktchiev M.** 2013. vCJD and transfusion of blood components: an updated risk assessment. United Kingdom Department of Health, London, United Kingdom.
20. **Rubenstein R, Chang B.** 2013. Re-assessment of PrP(Sc) distribution in sporadic and variant CJD. PLoS One **8:**e66352. http://dx.doi.org/10.1371/journal.pone.0066352.