vCJD AND TRANSFUSION OF BLOOD COMPONENTS: AN UPDATED RISK ASSESSMENT

Peter Bennett and Maren Daraktchiev

Health Protection Analytical Team
Department of Health
Wellington House
133-155 Waterloo Road, London SE1 8UG

February 14th 2013

CONTENTS

ANNEXES

SUMMARY

This paper considers the transmission of variant Creutzfeldt-Jakob Disease (vCJD) from person to person though receipt of donated blood components. It presents a mathematical model, primarily designed to examine how many future clinical cases might be caused in this way. Key inputs to the model include the number of donors that might be carrying vCJD infection without showing any symptoms, the infective dose in blood components sourced from such a donor, and the likelihood of recipients surviving long enough to develop symptoms of vCJD if infected. In addition, the results of the model need to be consistent with the number of cases seen to date. Much of the analysis concerns the potential transmission of vCJD though red cell transfusion: this is the component most commonly transfused, and the small number of known transmissions have all been associated with red cells. However, the analysis is also extended to consider the possibility of transmission via Fresh Frozen Plasma (FFP).

Any attempt to quantify the potential impact of transmission has to take account of many scientific uncertainties about vCJD. We therefore use a scenario-based approach. After reviewing some of the key evidence, the analysis uses a range of values for each of the key inputs, endorsed by the relevant expert scientific group. The model then generates a range of possible scenarios for the number of transfusion-related cases that might appear in future - and how many of these future cases would be caused by transfusions yet to happen. The analysis presented here is intended to be precautionary, and may be subject to substantial change as understanding of disease develops.

Despite the small number of clinical cases seen to date that might plausibly be associated with transfusion, current knowledge leaves open a substantial range of scenarios as regards future cases. Nevertheless, this range has narrowed with the passage of time, as compared with previous analyses. For red cell transmission, plausible numbers of future vCJD cases associated with red cell transfusion range from almost zero up to about 1,000 spread over the next 60 years. About half these cases would be caused by transfusions that have already taken place. The cental estimate for the number of clinical cases that might be caused by *future* red cell transfusions is roughly 160, with an upper limit of 460. For Fresh Frozen Plasma, the central estimate is roughly 45, and the upper limit 120. However, the number of “silent” vCJD infections associated with transfusion would be much higher than the number of clinical cases. It is therefore important to maintain, and if possible enhance, measures to prevent onward transmission of infection, notably the exclusion of recipients from donating blood.

1. INTRODUCTION

Variant Creutzfeldt-Jakob Disease (vCJD) is one of a small number of neurological diseases associated with an abnormal form of prion protein. Despite efforts to develop effective treatments, it has proven to be fatal in all known instances where symptoms have developed. First identified in the late 1980s, it almost certainly first spread to humans via cattle infected with Bovine Spongiform Encephalopathy (BSE), or "Mad Cow" disease. Earlier fears of large numbers of vCJD deaths have fortunately not been realised, and the number of new cases each year has been in decline for several years. But because it can take many years for symptoms to develop, the disease remains something of an enigma (Holmes, 2012), and there is evidence of a much larger number of people having been infected. A survey of stored tissue samples published in 2004, suggested that about 1 in 4,000 people might be carrying the abnormal prion protein indicative of vCJD, whilst larger survey completed in 2012 suggests a figure of the order of 1 in 2,000. It is therefore essential to consider the possible risks of secondary transmission – i.e. of infection being passed on from person to person. One way in which this can occur is if someone infected with vCJD, but without showing any symptoms, donates blood. There is strong evidence of this having happened in a few instances, whilst the delay on onset of symptoms again makes it difficult to assess the possible extent of "silent" infection.

From the first identification of vCJD, UK policy has been based on the presumption that infection *might* be transmissible via transfusion, and steps have been taken to reduce the risks. From late 1999 onward, all donations have undergone removal of white cells (leucodepletion) in order to reduce any vCJD infectivity present. Although this is considered unlikely to eliminate the risk completely, all the blood-borne transmissions identified so far occurred prior to this step. Also from 1999, plasma derivatives have been fractionated from imported plasma (or, more recently, manufactured using recombinant methods), rather than being sourced from UK donors. Fresh Frozen Plasma (FFP) used for children and certain groups of adults needing frequent transfusions is also imported. From 2004 onward, recipients of blood components have been excluded from donating blood, in order to prevent vCJD - or potentially other infections - being "recycled" within the population (Bennett and Dobra, 2006).

Assessing the benefit of steps to reduce the risks of vCJD transmission depends critically on establishing a plausible range of scenarios for how many future vCJD infections might be caused by donated blood, and how many of these infections might result in patients suffering clinical symptoms of the disease.

Despite a good deal of research on both the basic science and on the epidemiology of vCJD, great uncertainties about the risks of blood-borne transmission remain (e.g. Knight, 2010; Watkins *et al*, 2012). Decisions thus need to have a strongly precautionary element, while still having regard to the available evidence. Risk assessment to inform such decisions relies on mathematical modelling of how infection might spread, and how many future clinical (symptomatic) cases of vCJD might result. Earlier models used by the Department were based on separate inputs - for example on the level of infectivity in blood, the prevalence of infective donors and the susceptibility of recipients to clinical disease – using ranges for each that were consistent with the available evidence.[1] This

[1] http://webarchive.nationalarchives.gov.uk/+/www.dh.gov.uk/en/Publichealth/Communicablediseases/CJD/CJDgeneralinformation/DH_4136944

produced a very wide range of scenarios for the number of vCJD cases that might eventually result from blood-borne transmission of infection. However, the passage of time has also made it more feasible to "calibrate" such models against the observed numbers of clinical vCJD cases. In earlier models, combinations of plausible inputs led to some scenarios inconsistent with the case data seen since. Although one cannot necessarily expect the future to resemble the past, mathematical models need to be consistent with what has been seen so far. In particular, it is important to consider whether blood-borne transmission could still lead to large numbers of clinical vCJD cases in future, and in what circumstances. This is a key question in assessing the benefit of new risk reduction measures, or indeed those already in place (see e.g. Dodd, 2010; Will, 2010).

The updated risk assessment presented here aims to provide a range of scenarios consistent with observation, whilst remaining precautionary. It has been produced through a number of stages, each considered and endorsed by TSE Risk Assessment Subgroup of the Advisory Committee on Dangerous Pathogens (ACDP). This is an expert scientific advisory committee, referred to as "the ACDP Subgroup" in what follows. To summarise the process:

i. DH analysts prepared a paper (Bennett and Daraktchiev, 2011) setting out what is known (and unknown) about vCJD transmission via blood components, the results of previous modelling exercises, and the challenge of "calibrating" transmission models against observed clinical case numbers. It also set out a partial methodology for producing calibrated scenarios, developed with the assistance of the Clinical Operational Research Unit (CORU) at University College London.

ii. Using that paper as a starting point, the ACDP Subgroup reviewed the evidence in July 2011[2], and agreed some principles to guide development of a revised risk assessment. In particular, it endorsed the proposal to calibrate transmission models against observed clinical case numbers, subject to taking a precautionary approach in estimating how many vCJD infections would have shown up as clinical cases, as well as how many known cases might have been due to blood-borne infection. As detailed below, the Subgroup also endorsed specific modelling inputs and assumptions – e.g. on the levels of infectivity that might be present in blood from an infected donor.

iii. DH analysts developed a more complete modelling method in accordance with the Subgroup's advice, and produced a preliminary risk assessment using these revised methods and assumptions.

iv. In parallel with this, researchers were completing a large-scale study led by the Health Protection Agency (HPA), in which samples of appendix tissue taken during surgery were tested for presence of abnormal prion protein. This provided important new evidence on the prevalence of vCJD infection within the population.[3] Having earlier received interim results of the study, the ACDP Subgroup considered a

[2] Minutes of the meeting (14th July 2011) can be found at: http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@ab/documents/digitalasset/dh_129920.pdf

[3] http://www.hpa.org.uk/hpr/archives/2012/news3212.htm#bnrmlprn

summary of the final results in July 2012 and produced a statement setting out their interpretation.[4]

The DH analytical team produced successive updates of the risk assessment model in response to this new evidence, and to other suggestions made by the Subgroup. These revisions have informed the work of expert committees advising on CJD-related risk management decisions, notably the CJD Incidents Panel, and the Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO), both in offering new advice and in reviewing past recommendations where necessary. This paper sets out the revised model, incorporating changes agreed up to the end of 2012. Both the Risk Assessment itself, and recommendations based on it, will be kept under review and revised as and when new evidence becomes available.

2. CASE EVIDENCE, PREVIOUS SCENARIOS AND MODEL CALIBRATION

2.1 Background

Usage of blood components

There are two fundamental uses of donated blood: transfusion of components, and use of fractionated plasma products. For blood *components* (most commonly red cells, but also including Fresh Frozen Plasma (FFP), Platelets and cryoprecipitate[5]), each unit transfused exposes the recipient to substantial volume of material from one (or a few) donors. By contrast, plasma products – e.g. clotting agents such as Factor-VIII used to treat haemophilia - are produced by "fractionating" plasma in pools of many thousand donations. Recipients are thus exposed to tiny amounts of material sourced from very many donors. Since 1999, plasma for fractionation has been imported, though a substantial number of recipients are regarded as at increased risk of vCJD due to earlier exposure to UK-sourced products.

This paper concentrates on the risks of vCJD transmission *via blood components*, rather than fractionated products, though the analysis has some indirect implications for the latter. Clearly, the scale of any such risk will depend on the number of units transfused. If the order of 3,000,000 units of blood components are transfused each year, including roughly 2,000,000 units of red blood cells: a small risk per unit could therefore result in a large number of infections within the population. More detail on year-by-year usage of components, distribution by age of recipient and post-transfusion survival is given in **Annex A1-3**.

It is also important to note that components are not transfused in pure form. For example, a unit of "red cells" will actually contain a significant volume of plasma, other cells, and so on, in quantities depending on the processing method used. This is a key point in

[4] Advisory Committee on Dangerous Pathogens (ACDP) TSE Risk Assessment Subgroup. Position Statement on occurrence of vCJD and prevalence of infection in the UK population. July 2012. Available at: http://www.dh.gov.uk/ab/ACDP/TSEguidance/DH_125868.

[5] Terminologically, it is not clear whether cryoprecipitate should count as a component or a product. For present purposes, however, it is considered as a component.

considering how much of any infectivity in a donation would end up in a transfused unit. Similar comments apply to FFP, while for some platelets and all cryoprecipitate there are additional complications due to units being produced by pooling donations from several donors.

From late1999 onward, all blood donations have undergone a process known as leucodepletion (or leucoreduction), involving removal of almost all white cells, or leucocytes. As discussed below, this should have reduced the risk of vCJD being transmitted via an infected donation: however, there are uncertainties as to how great a reduction this step achieves.

Clinical cases of vCJD

As of December 2012, 176 patients in the UK have been found to have definite or probable[6] clinical vCJD, and over 220 symptomatic cases have been reported world-wide. The UK vCJD patients comprise a single wave of clinical cases: numbers of known onsets peaked in 1999, with diagnoses and deaths both peaking in 2000. The most recent onset of symptoms in a UK patient was in October 2010, and there are currently no living vCJD patients in the UK[7].

As with other prion diseases, development of disease is influenced by genetic factors, in particular at position ("codon") 129 of the prion protein gene. About 40% of the UK population have the *methionine* amino-acid at this position: they are described as methionine homozygous, or MM. 10% have the *valine* amino-acid (valine homozygous, or VV), while the remaining 50% have both amino-acids (methionine-valine heterozygous, or MV). Most patients with probable or definite diagnoses of vCJD have been genetically tested, and all have been MM homozygous at codon 129.

As discussed below, there is evidence of subclinical (asymptomatic) infection amongst the other codon 129 genotypes, making it plausible that further clinical cases may appear in the remaining 60% of the population. One MV heterozygote patient has been classified as a *possible* clinical case (Kaksi, Mead, Hyare *et al*, 2009) though the diagnosis of vCJD could not be designated as "probable" or "definite" due to insufficient evidence.

Cases attributable to transmission via blood components

Clearly, it is important to establish how many clinical cases of vCJD might already have been caused by transfusion. Evidence on this comes primarily from the Transfusion Medicine Epidemiology Review (TMER) study, undertaken jointly by the National CJD Research and Surveillance Unit (NCJDSU) and the UK Blood Services and (Hewitt *at al*, 2006), which investigates both donors and recipients found to have vCJD.

As detailed in **Annex A4**, three of the 176 patients with clinical vCJD appear to have been infected by transfusion of blood components. All received red cells from donors who later went on to develop the disease (Llewelyn, Hewitt, Knights *et al*, 2004; Wroe, Pal, Siddique *et al*, 2006; Head *et al*, 2009), and they developed symptoms of vCJD 6.5, 7.8 and 8.3 years after transfusion. Two were linked to the *same* pre-symptomatic donor, which

[6] In this context, these terms refer to specific criteria developed for epidemiological studies of prion disease agreed by the World Health Organisation. Details can be found on the National CJD Research & Surveillance Unit website at http://www.cjd.ed.ac.uk/criteria.htm

[7] Two remain alive in France: see http://www.cjd.ed.ac.uk/vcjdworld.htm

establishes blood-borne transmission beyond all reasonable doubt.[8] Whilst all these recipients were MM-homozygotes, one MV-heterozygote recipient of red cells from a vCJD-infected donor showed signs of sub-clinical infection, after dying of unrelated causes (Peden *et al*, 2004). All the "implicated" transfusions were from MM-homozygotes, and took place between 1996 and 1999, prior to the implementation of leucodepletion. Other recipients of blood components from donors who developed vCJD have been followed up (Gillies, Chohan, Llewelyn *et al*, 2009). Some survived (or are surviving) for significant periods with no symptoms of vCJD, and 17 are still alive as of January 2013. In addition to the one "sub-clinical transmission" already noted, autopsy tissue has been obtained from four who died of other causes. Although investigations have to be completed, none is thought to show evidence of prion infection.

2.2 Modelling transmission: some fundamentals

Key factors

The risk of vCJD infection resulting from transfusion of a *single unit* of (say) red cells will depend on:

- the prevalence of infected donors within the population
- the chance of a unit from an infected donor infecting the recipient. This "transmission probability" depends in turn on the level of infectivity in blood, its distribution between different components, and the timing of its appearance within the incubation period between the donor's initial infection and the onset of clinical symptoms. For donations that have been leucodepleted, the possible effects of this step have also to be allowed for. All these factors are highly uncertain.

The number of new infections *within the population* will then depend on the number of units transfused.

The number of *clinical cases* of vCJD that eventually result will depend on the susceptibility of recipients to clinical disease, and the speed with which symptoms develop following blood-borne infection, as compared with the patients' survival post-transfusion. Allowing for survival is particularly important for an infection that may have a long incubation period. A good deal of blood is transfused into patients with relatively short life-expectancy: it is likely that only a minority of patients infected with vCJD would survive long enough to develop clinical symptoms of the disease.

Many of these factors are highly-uncertain: modelling allows for this by using ranges of inputs compatible with the best evidence available. This is discussed in more detail below. However, it is also essential to check model *outputs* for plausibility: this brings us to the issue of model calibration.

Model calibration

[8] One transmission of sub-clinical vCJD is thought to have occurred via Factor VIII. (Peden, McCardle, Head *et al*, 2010; Bennett and Ball, 2009). Given the numbers of patients exposed to UK-sourced plasma products up until 1999, the lack of clinical cases amongst recipients of these fractionated products is interesting. As compared with components, however, risk assessment is complicated by major additional uncertainties regarding the distribution of infectivity, and the effects of manufacturing processes in removing it.

A credible transmission model needs to produce scenarios for the number of *clinical (symptomatic) cases* of vCJD caused by blood-borne transmission that are consistent with what has been seen so far. Early in the vCJD outbreak, risk assessment had necessarily to be based on precautionary inputs. With the passage of time, however, it has become increasingly meaningful to compare model projections with actual numbers of clinical cases. This provides both "positive" and "negative" evidence. As in other areas, positive evidence consists of quite prominent and fully-investigated events - primarily the appearance of new clinical cases. Negative evidence, by contrast, comprises non-appearance of cases that one might have expected to occur. Whilst it can be easy to overlook a gradual accumulation of non-events, models require calibration against both types of evidence. Consistency with negative evidence provides a necessary reality-check, especially where models multiply precautionary assumptions and inputs.

Considering the basic model structure just set out, it is easy to show how "reasonable" inputs for each separate factor can produce scenarios in which impossibly large numbers of blood-borne vCJD cases would already have occurred. This can be illustrated by a simple calculation.

Suppose that exposure to BSE led to a 1 in 2,000 prevalence of vCJD infection (a figure of the order suggested by the recent appendix survey), spread throughout the donor population and typically occurring circa 1990-1. Since then, roughly 2m units of red cells have been transfused each year. If the dose in an infective unit were sufficient to transmit infection, there would therefore have been roughly 1,000 transmissions of infection *every year*. Allowing for a modest delay in blood becoming infective, this would have been happening from about 1992 onward.

Clearly, recent transmissions would not yet have shown up as clinical cases, whilst some infected recipients would have died of unrelated causes. However, this may not suffice to explain the lack of observed cases. For illustration, suppose that only MM recipients (40% of the population) would be liable to develop clinical disease within about 10 years - as has happened in all three "known" instances. Data on post-transfusion survival suggest that 25-30% of units are transfused into patients surviving at least this long.

- Using the lower survival figure, the net effect would be that one in every ten transmissions of infection would result in a clinical vCJD case within 10 years. In the 10 years from 1992 to 2002, there would have been 10,000 secondary infections due to red cell transmission alone, leading to 1,000 clinical cases by 2012.
- Alternatively, suppose that only 10% of infected MM recipients developed symptoms within ten years. Even then, one might have expected to see around 100 transfusion-related cases. This is still strongly at variance with what has been seen.

This simplistic illustration ignores statistical distributions (e.g. around the time at which donors were infected, and around incubation periods amongst those infected). Up to a point, more elaborate modelling can provide some greater realism and further insights. But it does not remove the basic problem of over-prediction. Combinations of plausible inputs produce scenarios in which "predicted" numbers of blood-borne clinical cases seen to date can range from a handful up to several hundred. The upper end of this range is clearly unrealistic when compared with what has been seen in the last few years.

2.3 Calibration criteria: model outputs and clinical cases

To calibrate the transmission model in a realistic way, we need to consider how many vCJD cases might actually have been caused by blood-borne infection. As already noted, three of the 176 UK patients with clinical vCJD are presumed to have been infected through transfusion. In comparing model outputs with case numbers, however, we cannot assume that *only* these three have occurred. Some other vCJD cases *might* be attributable to blood-borne transmission, even though no symptomatic donor has been identified. Transfusion histories of all identified vCJD patients in the UK are routinely investigated, and 14 of the other 173 patients had histories of transfusion. For some of these, the timing of the transfusions, or of their disease, preclude transfusion as the source of infection. However, 4-5 had transfusions at relevant times.[9] In theory, any or all of these cases might have been due to blood-borne infection, making a maximum of 7-8 clinical cases in total. All these transfusions were of non-leucodepleted components, except for that involving exposure to two donors. The recipient with three exposures received Fresh Frozen Plasma, and this *might* therefore represent a transmission via FFP. No other vCJD patients have recorded transfusions that could provide a plausible route of transmission.

Transfusion records may not be complete, despite the best practicable follow-up of individual cases. In addition, 100% case ascertainment of vCJD cannot be taken for granted: there *might* be under-reporting, especially in age groups in which dementia is relatively common and post mortem investigations rare. However, checks currently in place, and the level of research interest in prion disease, make gross under-reporting less likely. In addition, we can "calibrate" transmission models by considering predicted numbers of cases *amongst MM homozygotes alone*. Such cases are less likely to have been missed, whereas any presentation of vCJD in other genotypes may be more uncertain.[10,11]

Our 2011 paper therefore suggested that plausible scenarios should "predict" between 3 and 10 clinical vCJD cases to have occurred by the start of that year, due to transmission via blood components. This would represent about one such case per year during the period 2000-2010. The upper limit of 10 cases was designed to allow for any or all the cases just discussed to have been blood-borne, plus some "margin of error" for undetected transfusions or linkages. As will be seen, this proposition was endorsed by the ACDP Subgroup, later updated and refined to consider separate limits for transmission via red cells and FFP.

In considering the plausibility of transmission scenarios, two further questions are relevant.

---

9 http://www.cjd.ed.ac.uk/TMER/reverse.htm. Four patients were transfused between 1993 and 2002, being exposed to 2, 3, 4 and 103 donors. If these cases were indeed blood-borne, the incubation periods from transfusion to onset of symptoms would range from 4.6 to 6.25 years. A "reverse risk assessment" (Dept of Health, 2005; 2010; Bennett, Dobra and Gronlund, 2006) was used to determine the implied risk of the donors being infected. One further recipient was exposed to 4 donors in 1989: a blood-borne route might be considered less likely in this instance, but cannot be ruled out. The incubation period in this case would be nearly 17 years, following transfusion as an infant. All identifiable and contactable (107 out of 112) donors have been notified that they are at increased risk of carrying vCJD infection.

10 For MM homozygotes, there is no evidence of the strain of the agent or the characteristics of disease changing significantly following blood-borne infection (Head *et al*, 2009).

11 In addition, the distribution of units transfused is highly skewed, with a few recipients receiving many units. This does not greatly affect the total number of blood-borne vCJD cases to be expected. However, the chance of exposure to an infective donor rises with the number of units received, and some patients have received hundreds of units (Department of Health, 2010). From an epidemiological point of view, highly-transfused patients are a key "sentinel" group.

- *Why did blood-borne cases not appear earlier?* The three "known" patients all had incubation periods of under 10 years. If a large number of infected units were transfused from the early 1990s onwards, why were no clinical cases found prior to 2003?
- *Why have there not been more subsequent cases?* One might have expected the three "known" cases to be the start of a more substantial wave, resulting from more frequent transmissions as more donors became infective. Instead, there has been a gap of almost seven years since onset of symptoms in the last such case in February 2006. Although we cannot conclude that there have been no clinical cases of transfusion-transmitted vCJD, the small *overall* incidence of cases in recent years means that there could only have been a few.

Although these questions do not have a definitive answer, it is helpful to take account of the *timing* of cases seen so far (as well as their number) when considering whether model outputs have "face plausibility".

3. INPUTS AND ASSUMPTIONS REVISITED

Given the challenge of calibrating models against case data, each of the key inputs was reconsidered in the light of the most recent evidence available. We consider these in turn, and summarise the advice given by the ACDP Subgroup.

3.1 Infectivity in donated blood

Prior to 2011, scenarios used by DH were based on evidence from rodent models, applying infective titres found *per ml* to human blood. This approach leads to very large estimated doses *per unit* of any infected component. However, further evidence is now available from the results of ongoing experimental work on transmission of prion disease through sheep-to-sheep transfusion, while the passage of time has provided more evidence – both positive and negative - on human transmission.

Whilst rodent models continue to provide a quick and convenient means of investigating infectivity, ovine transmission (Houston *et al*, 2000; 2008; McCutcheon, Alejo Blanco, Houston *et al,* 2011) arguably provides the best available experimental analogue to the human situation. Not only are sheep more similar to humans in many ways (as compared with rodents), but blood and its components can be transfused in similar volumes. This in turn makes it possible to prepare components for experimental purposes using the same procedures as for human transfusions. Results from these studies confirm that prion infection can be efficiently transmitted (in sheep) by any of the components commonly transfused in humans. While this suggests that infectious doses *per unit* are substantial, these results do not necessarily support the very high values used previously.

If a transfusion contains infective material, the probability of transmitting the infection will depend on the dose-response relationship. Two dose-response models are in common use: linear and Poisson. The former is a continuous model, in which an ID_{50} is defined as the dose needed to infect 50% of recipients. For any individual, probability of infection is then proportional to the dose received, up to a limit of 2 ID_{50}, at which point infection is – at least in the simple version of the model - regarded as certain. The Poisson model assumes that some minimal Infectious Dose (ID) is needed to transmit infection: the chance of

transmission thus depends on the probability of at least one ID being present. Whilst the linear model has merit as a simple and precautionary working assumption, the Poisson model is arguably more realistic, so the following discussion is couched in those terms. A dose of 0.7 ID in the Poisson model can be regarded as similar to: 1 ID_{50} in the linear model: both essentially imply a 50% chance of the infection being transmitted.

A paper by Gregori, Yang and Anderson (2011) estimated the levels of infectivity likely to be present in human blood, based on evidence both from human transmissions and the sheep experiments already referred to. They find that both calculations support similar conclusions. The human data suggest an infectivity in the range 0.3-0.75 intravenous ID per unit of non-leucodepleted red cells, depending on the method of calculation (the method supporting the higher figure appears the more realistic). This in turn suggests that an infective donation of whole blood would contain "a few" IDs. Their analysis of the published ovine data suggests a dose of the order of 0.8 ID per unit, equivalent to roughly 0.002 ID per ml.

The ACDP Subgroup considered this research to provide the best available evidence on the likely levels of infectivity present in human blood, recommending that modelling be based on doses *of the order of* 1 ID per unit of infected red cells, prior to leucodepletion. Although this is much lower than the range used before, the change has a less dramatic effect on the "predicted" number of infections (simply because the previous values were so high). Nevertheless, doses of this order make it easier to explain why large numbers of blood-borne cases have not appeared with short incubation periods - as might have been expected following receipt of very large doses via an efficient person-to-person route.

Specifically, the Subgroup endorsed a range of 0.7 ID upward, producing a probability of transmission of 50-100%.

A further question is that of how rapidly infectivity appears in blood, following infection - specifically, via the oral route. Clearly, any substantial delay in onset of infectivity would help explain a lack of observed secondary cases, while leaving open the possibility of substantially more appearing in future (Dept of Health, 2003). One would not expect blood to become infectious instantaneously. The known human transmissions involved donations taken fairly close to onset of symptoms in the donor - the largest interval being just under 3.4 years - leaving infectivity earlier in the incubation period as an open question. However, the sheep transfusion experiments already noted show that blood taken no more than 25% through the donor animal's incubation period can infect. There might plausibly have been a few years' delay before significant infectivity appeared in the blood of human donors, with those infected at the peak of the primary outbreak typically becoming infective in the early-to-mid 1990s – e.g. circa 1994. This would go *some* way toward explaining the absence of earlier secondary cases. For modelling purposes, we consider a wide range of scenarios, including the possibility that this delay may vary by genotype (matching the more rapid onset of symptoms in MM homozygotes).

3.2 Distribution of infectivity and the effect of leucodepletion

A further key question is the distribution of infectivity within a donation of whole blood, which affects both the risks associated with the different components "as transfused", and the effects of leucodepletion. Both red cells and platelets "as transfused" contain significant quantities of plasma. Previous models have taken infectivity to be associated both with white cells and plasma, in roughly equal quantities. The former would be almost

completely removed through leucodepletion, whereas the latter would be unaffected. Current evidence on the likely effectiveness of leucodepletion remains somewhat mixed.

- Significant association of infectivity with plasma is supported both by rodent studies and by the published and ongoing research on sheep-to-sheep transmission of BSE already noted (McCutcheon, Alejo Blanco, Houston *et al, op cit*). In this model, leucodepletion does not eliminate the risks of transmission: transmissions have been observed following transfusions of all components, whether leucodepleted or not.
- As against this, the pattern of human cases associated with red cell transfusion is difficult to explain unless leucodepletion had *some significant* effect. We now know that in the four years leading up to implementation of this measure (1996-99 inclusive) there were three transmissions of vCJD, in which both donor and recipient developed clinical disease by the end of 2006. Had transmissions continued at a similar rate, and with similar doses leading to similar incubation periods, one would have expected about three more such pairs to be detected by the end of 2010. Two years later, none has appeared. Though the small numbers prevent this argument from being conclusive, it supports the effectiveness of leucodepletion. As will be seen, it becomes much easier to produce scenarios consistent with what has been seen so far by way of cases associated with red cell transfusion if we allow a significant step change in transmission risks to have occurred in 1999.
- If vCJD infectivity in human blood is largely associated with white cells rather than plasma, this would also help explain the lack of cases associated with FFP transfusion, and the lack of clinical cases amongst recipients of plasma derivatives, including those *known* to have been exposed to vCJD-infected donors (Zaman *et al*, 2011).

It is also important to distinguish between effects of leucodepletion on the infective dose present in whole blood, on that present in a unit of red cells. For example, Gregori *et al* (2004) suggest that leucodepletion removes roughly 40% of the infectivity present in a whole blood donation, based on a scrapie/hamster model. However, this is *not* equivalent to reducing the infectivity of the red cell transfusions by 40%. The latter will contain almost all the white cells in the original donation, but less than 10% of the plasma. Further studies referred to by Gregori, Yang and Anderson (2011) suggest that about 20% of the infectivity present in whole blood remains in the red cell component "as transfused". Removal of White Cells will thus have a much greater proportionate effect on the infectivity of red cell units.

Whilst it is difficult to reconcile all these pieces of evidence, it is possible to construct scenarios *roughly* consistent with most. To illustrate:

- Suppose that a donation of infective whole blood contains roughly 4 Infectious Doses (IDs) of which 1 ID is associated with White Blood Cells (WBC) and 3 with plasma.
- Empirically, a unit of red blood cells as transfused contains 8% of the original plasma (for Top/Top processing), or 4% for the now more common Bottom-and Top (BAT) method.
- *Without* leucodepletion, the red blood cell unit would therefore contain:

  1 ID associated with WBC + 0.2 in plasma = **1.2 ID** for Top/Top processing

1 ID associated with WBC + 0.1 in plasma = **1.1 ID** for BAT

- *Leucodepletion* reduces the WBC count by a factor at least 10,000, making cell-associated infectivity negligible compared to that in plasma. The residual infectivity per unit would thus be **0.2 ID** for leucodepleted T/T units, or **0.1 ID** for leucodepleted BAT units.

In such a scenario, whole blood would contain the "few IDs" suggested by Gregori, Yang and Anderson, 25% of it (rather than 40%) associated with WBC. A unit of non-leucodepleted red cells would contain about 25% of the infectivity in the whole donation, close to their suggested 20% – with the absolute levels somewhat higher than their estimated range. Though not eliminating the risk, leucodepletion would have a substantial effect. It is also noteworthy that in scenarios such as this, the progressive replacement of Top/Top processing by BAT would have led to a significant further decrease in transmission risk.

Clearly, this illustrative scenario does not provide a perfect fit with all the evidence: it could probably be improved. In particular, one might attach different weights to the evidence on human and animal transmission: these may be impossible to reconcile precisely.

For the present analysis, the ACDP Subgroup endorsed a range of inputs of 0.1 – 0.7 ID per unit for red cells following leucodepletion, producing probabilities of transmission ranging from 10% to 50%.

3.3 Prevalence of subclinical infection

The key evidence for subclinical infection in the population comes from testing tissue samples for prevalence of abnormal prion protein.

- Until recently, estimation was reliant on the retrospective Hilton *et al* (2004) study, which found three "positive" appendices in approximately 12,000 tested, using Immunohistochemisty (IHC). This suggested a prevalence of abnormal prion of the order of 1 in 4,000, with 95% confidence intervals ranging from approximately 1 in 20,000 to 1 in 1,200. All three samples were from patients born between 1961 and 1985, and it was suggested that this might represent a "high risk" cohort, with infection being rarer in both older and younger groups. This would be broadly consistent with the age distribution of clinical cases.
- A large-scale prospective survey of tonsils was then instigated, the NATA (National Anonymous Tonsil Archive) study (Clewley, Kelly, Andrews *et al*., 2009). This tested large numbers of samples from different cohorts. All tested negative using the methods originally specified (dual ELISA and Western Blotting). However, 10,000 samples in the 1961-85 cohort were then re-tested using IHC. One sample was found to be positive (de Marco *et al*, 2010), albeit with limited presence of the abnormal protein. This re-test result was consistent with the Hilton study, while also casting doubt on the negative results obtained using the original methods in the other age cohorts.
- A further, large-scale appendix survey was then commissioned, testing tissue samples from over 30,000 patients (roughly 20,000 in the 1961-85 cohort, and 10,000 in the 1941-1960 cohort) using IHC. Positives have been found in both

cohorts[12], with prevalence in the older group appearing to be at least as great as in those born 1961-85. ***In round figures, the study shows a prevalence of roughly 1 in 2,000 across both cohorts, with a 95% Confidence Interval running from approximately 1 in 3,500 to 1 in 1,250.***

- From 1996 onward, our working assumption remains that measures in place to prevent BSE entering the human food chain would have led to a negligible incidence of new primary vCJD infections.[13] However, there is at present no IHC evidence relating to this cohort. This reflects the point that none of the tissue surveys has been subject to a *negative control* – i.e. testing of samples from a population expected to have had no appreciable exposure to BSE or vCJD. It is now proposed to start a further study of two further sets of appendices (a) taken from patients born after 1st January 1996 and (b) extracted during surgery carried out prior to the BSE outbreak

Attempts have also been made to set up a survey of spleen (and brain) tissues to be collected post mortem, and different ways of setting this up have been piloted. Unfortunately, the practical difficulties have proven to be insuperable (McGowan and Viens, 2011).

The most fundamental question, however, is whether presence of abnormal prion protein - whether in appendix, tonsil, or some other tissue - indicates that a donor's blood would necessarily be infective. The precautionary approach to risk assessment has been to assume that those *infected* with abnormal prion protein would indeed be – or at least, would become - *infective*, with consequent transmission risks not only via donation of blood, tissues and organs but also from re-use of surgical and dental instruments.[14] A single notion of "prevalence" has been assumed to characterise all these risks. Any discussion of prevalence requires careful specification as to what is meant, whether prevalence of people with abnormal prion protein in a specific site, or "somewhere in the body", or prevalence of specific forms of infectivity

This question received considerable attention on the part of the ACDP Subgroup. Although the contrary assumption would have made it much easier to explain the small number of clinical vCJD cases attributable to secondary transmission, ***the Subgroup strongly advised that the results of the appendix survey should be used as an indication of the prevalence of infective donors.*** The modelling presented below is based on this assumption.[15]

---

[12] Pending publication of the detailed findings (Gill, Spencer, Boyes *et al*, in preparation, 2013)**,** a summary of results is available at http://www.hpa.org.uk/hpr/archives/2012/news3212.htm#bnrmlprn

[13] There is also a case for assuming a lower prevalence for the relatively narrow cohort born between 1985 and 1st 1996, given that exposure to BSE in the food chain should have started to decline.

[14] For potential transmission of vCJD via re-use of surgical instruments, the relevant DH risk assessment was last revised in 2005: see http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4113541. Since then, evidence on the efficacy of instrument decontamination may have lent more weight to scenarios toward the pessimistic end of the ranges considered. Yet there have still been no detected cases of vCJD transmitted via surgery. Although any association with surgery may be more difficult to establish than a link to transfusion, and there are obvious issues of survival – especially for neurosurgical patients - this absence requires explanation.

[15] The model assumes that the "positive" appendix samples reflect prevalence of *primary* infection, present since the period of BSE exposure. If some of the positive findings resulted from secondary infection, prevalence would have increased more gradually before reaching the level suggested by the survey. This alternative cannot be ruled out: however, the appendix samples would have come predominantly from

In future, a more direct measure of blood-borne transmission risks may be offered by adapting an assay developed for screening purposes to investigate prevalence. This will require a test with sensitivity sufficient to detect "true positives" reliably, though specificity requirements could be less stringent than for a test to screen donations prior to transfusion. A number of tests are under development (e.g. Edgeworth , Farmer, Sicilia *et al*, 2011). It is hoped such a prevalence survey can be taken forward, ideally with some method of establishing whether samples are infective, rather than simply indicating presence of abnormal prion.[16]

3.4 Response to infection: Susceptibility and Incubation Periods

The final set of factors affecting the appearance of clinical cases is the response of individual recipients to the receipt of infected donations. This can be broken down into a series of questions: are all individuals susceptible to vCJD infection, or are some resistant? If individuals are infected, are they all susceptible to the development of clinical vCJD, and over what timescale? How do these factors vary – and why? For example, are variations caused by differences between the individuals infected, by differences in strain for the infective agent, or some combination of both? These are major scientific unknowns that affect our whole understanding of the vCJD phenomenon, and the challenge for modelling is to allow for these uncertainties in a practical way. The following approach has been endorsed by the ACDP Subgroup, at least as a provisional way forward pending further evidence.

Firstly, we assume that all individual recipients are susceptible to vCJD *infection* via blood transfusion. This is clearly a precautionary approach, but reflects the fact that signs of sub-clinical infection appear to be quite widespread and have been found in all genotypes[17], though clinical disease has been rare and (with one possible exception) been confined to MM homozygotes. It allows the possibility of most of those infected – perhaps including many MM homozygotes as well as other genotypes - remaining in a long-term, asymptomatic but *possibly* infectious, "carrier state".

Given this initial assumption, there is clear evidence for Codon-129 genotype as one determinant for the development of clinical vCJD (e.g. Bishop *et al*, 2006), and this has been factored-into existing models for exploring future case numbers (e.g. Clarke and Ghani, 2005). However, the disparity between prevalence of infection and clinical case numbers requires some additional factor: put crudely, a small proportion of those exposed - much smaller than 40% - have developed symptoms quite rapidly (by the standards of prion disease) while the majority appear clinically unaffected. This appears to be the case both for primary and secondary infection. A number of explanations can be given, singly or in combination.

---

fairly young patients less likely to have undergone prior transfusion (or neurosurgery). Further analysis suggests that model results are not greatly affected if this assumption is varied.

[16] At present, the effects of tissue fixation mean that no conclusions can be drawn from the failure of the positive Hilton samples themselves to transmit infection to transgenic mice (Wadsworth, Dalmau-Mena I Joiner et al, 2011).

[17] The assumption other genotypes are susceptible to vCJD *infection* is supported by the discoveries of abnormal prion protein in other genotypes in both the Hilton *et al* survey (Ironside, Bishop, Connolly *et al*, 2006) and the recently-completed HPA survey, as well as in patients in receipt of red cells (Peden *et al*, 2004) and plasma products (Peden, McCardle, Head *et al*, 2010). Susceptibility to clinical vCJD may be illustrated by one possible MV case (Kaski, Mead , Hyare *et al*, 2009).

The first is that not all infected individuals – even MM homozygotes – are susceptible to the development of clinical disease. Perhaps this requires some other physiological condition, or some genetic characteristic yet to be discovered. In principle, this provides a simple way of explaining the apparent disparity between clinical case numbers and prevalence of infection. For example, Clarke and Ghani (2005) suggested that an assumption of 10% susceptibility to disease within the population could reconcile the then-current case numbers with the results of the Hilton *et al* prevalence survey. Previous DH scenarios for blood-borne transmission accordingly used a range of values for susceptibility to disease of 10-100%. At the low end of this range, development of clinical disease within (say) 10 years of transfusion might plausibly be confined to just 10% of MM homozygotes (i.e. 4% of recipients). However, any such explanation remains rather ad hoc unless and until some specific biological basis can be given for the differential in susceptibility. So far no definitive factor other than codon-129 genotype has been found (Mackay, Knight and Ironside, 2011), though other candidate loci have been identified and mapped using a mouse cellular model (Mead , Poulter, Uphill *et al*, 2009).

A further possibility is that susceptibility to clinical vCJD following transfusion might be markedly *age-dependent*, for example through some maximum age "cut-off". This has some support from animal models (Brown *et al*, 2009), and might plausibly be invoked to help explain the pattern of cases due to primary infection (Cooper and Bird, 2003; Boelle, Cesbron and Valleron, 2004). In the context of secondary transmission, however, further examination (Bennett and Daraktchiev, *op cit*) suggests that such a cut-off would have a relatively modest effect on expected case numbers, after allowing for age-related differentials in post-transfusion survival. In addition, two of the three vCJD patients presumed to have been infected via transfusion were aged over 60 at transfusion, whilst the other was under 20. This indicates that neither youth nor comparative old age precludes development of clinical vCJD through this infection route.

A further possibility is that there are different strains of the infective agent for vCJD. Recent studies using transgenic mice expressing ovine or human PrP (Béringue *et al,* 2012) suggest that cross-species transmission may result in the emergence of multiple strains of infection. Furthermore, these strains may be predominantly "lymph-seeking" (lymphotropic), typically causing long-term asymptomatic carrier-state infection, or "brain-seeking" (neuroinvasive), causing relatively rapid onset of clinical symptoms. The experiments also suggest that the former may be much more common.[18] This work builds on previous models of prion infection (e.g. Collinge and Clarke, 2007; Li *et al*, 2010), which view prion strains as "clouds" of different prion protein entities. These compete and evolve: different tissues can allow one strain to become dominant over another, with other strains still present at much reduced concentrations.

Such a hypothesis could provide a major advance in our understanding of how BSE/vCJD has affected the human population (Collinge, 2012), and could readily explain the contrast between clinical vCJD case numbers and tissue-based prevalence estimates. Nevertheless, some caution is warranted. To date, studies carried out on vCJD-infected human tissues provide no evidence of different strains in tonsil/spleen and brain. However, all but one of these investigations have been of tissues from individuals who went on to develop clinical disease - and who (if different strains do exist) would all presumably have been infected with a brain-seeking strain. One patient investigated after death from other causes (Bishop,

[18] Béringue *et al*(op cit): of the humanised mice inoculated with BSE, 7% showed signs of PsR^{res} in brain, as compared with 65% in spleen.

Diack, Ritchie *et al*, 2013) was of MV genotype, and found to have evidence of vCJD infection in the spleen, but not the central nervous system. Whilst the strain of infection appears similar on first passage to those found in symptomatic cases, this provides evidence only for a single patient. There is no way of knowing whether he or she would have developed symptoms of vCJD or remained asymptomatic indefinitely, an outcome likely in any case to depend on the titre of infection. Unfortunately, the stored tissues collected for prevalence studies do not lend themselves to biological investigations, as they are formalin-fixed. The current absence of direct evidence for multiple strains of human vCJD infection thus leaves the question open, rather than excluding such a possibility.

In addition to the known influence of Codon-129 genotype, there are thus two lines of explanation as to why some individuals develop clinical vCJD quite rapidly, while others may never do so. Biologically, one focuses initially on differences between infectious strains, the other on differences between infected individuals. However, they have the same mathematical effect, decreasing the proportion of infections expected to show up as clinical cases.[19] Nor are these foci mutually exclusive, as the course of infection in any individual may depend on a complex interaction between infective strain, route of transmission and individual characteristics (some of which may change over time).

For present purposes, there is therefore no need to choose between these explanations. Under either or both hypotheses, we would expect a small proportion of those infected with vCJD via transfusion to develop symptoms comparatively rapidly (say within 10 years): *the secondary clinical cases seen so far would have come from within this sub-group of recipients*.

The key remaining question is whether other vCJD infections would eventually lead to clinical cases. This remains uncertain, whether they are distinguished by differences in infectious agent, in recipient characteristics or both.

- It may be that clinical disease is destined never to develop – because these are infections with agents that remain lymphotropic rather than neuroinvasive, and/or because these individuals are non-susceptible.
- The alternative is that clinical disease *will* develop, but with significantly longer incubation periods than those seen so far. For example, a part-adapted lymphotropic strain of vCJD might more readily adapt to the CNS (Collinge, *op cit*). If so, we would expect at least some of those infected with such a strain to develop clinical vCJD, though not as rapidly as those initially infected with the neuroinvasive strain.

In considering how many infections might eventually appear as clinical cases, the first possibility corresponds to scenarios in which "absolute" susceptibility to clinical disease is

[19] To simplify for illustration, suppose that the cattle-to-human species barrier resulted in 10% of the vCJD infections caused by exposure to BSE being exclusively of a "brain-seeking" strain, the other 90% being exclusively "lymph-seeking". This would be equivalent to assuming that only 10% of those infected are susceptible to clinical disease. In either case, we assume that typical incubation periods for those who *do* develop clinical vCJD will be dependent on Codon-129 genotype. We would then expect relatively rapid development of clinical vCJD symptoms to be confined to those MM homozygotes infected with the "brain seeking" strain – in this scenario about 4% of the infected population. Other genotypes infected with the same strain could be expected to follow (albeit with greater reductions in numbers due to intercurrent deaths), while the other 90% of infections would remain "silent".

allowed to vary, the second to those in which susceptibility is fixed (in the limiting case, at 100%) but incubation periods are bimodal for a *given* Codon-129 genotype.

The ACDP subgroup advised inclusion of both types of scenario. Specifically, we need to allow for the possibility that ***the clinical cases seen so far come from a small minority of MM homozygotes in whom – for whatever reason - clinical vCJD developed atypically fast following infection***. However, all other infected recipients ***might also develop clinical vCJD*** if they survive long enough. In varying the hypothetical incubation periods for these other patients, it is interesting to explore the effect on the maximum number of future cases. This is illustrated in the scenario analysis below. Unless incubation periods are significantly longer than for the first group[20], some of these recipients would have developed clinical vCJD already. Conversely, if the incubation periods are typically very long, few will survive long enough to do so. In the limit, incubation periods longer than a human life-span become equivalent to non-susceptibility.

One could also separate the assumptions made for MV and VV genotypes, but little is currently known about the likely relativities between these groups. Their respective proportions in the population – 50% MV compared with 10% VV – also suggests that numerical projections will be strongly dominated by the former.

4. MODELLING METHODS

4.1 Basic principles

As already stressed, plausible scenarios may be bounded by *combinations* of inputs, rather than by separate ranges for each. For example, if we assume that an infected unit is highly-likely to transmit vCJD and that prevalence of infective donors has been relatively high, then consistency with case data requires us to assume limited susceptibility to blood-borne disease (and/or very long incubation periods). Conversely, 100% susceptibility would imply a very low prevalence of infective donors. We need to characterise the "feasible space" created by these trade-offs.

This principle can be illustrated by considering some alternative scenarios that would each lead to about one clinical case per year during the10 years up to the end of 2010 - roughly the maximum credible rate, as discussed previously. The following tables set out the *maximum possible proportion of infective donations* during the 1990s compatible with this rate, given different assumptions about the proportion of infected recipients liable to develop symptoms within 10 years. Column (a) assumes that transmission via an infective donation would be virtually certain, Column (b) a probability of 50%. All figures are "rounded" in ways that widen the feasible range of scenarios – e.g. taking conservative estimates of 3m units of components transfused annually, with 25% going to recipients surviving at least 10 years.

These simple calculations demonstrate the trade-offs between key parameters. For example, the top row assumes that all infected (and surviving) MM homozygotes would develop symptoms of vCJD within 10 years of transfusion. If an infective component is

[20] As previously noted, onset of clinical vCJD in the three "known" cases occurred 6.5, 7.8 and 8.3 years after transfusion.

virtually certain to transmit, as in column (a), over-prediction of cases could only be avoided by assuming that historically, no more than 1 in 300,000 donations were *infective at the time of donation*. By contrast, if the transmission probability is taken to be 50%, and development of symptomatic vCJD within 10 years is confined to just 1% of MMs, then as many as 1 in 1,500 donations might have been infective (as in the lower-right cell).

Table 1: Proportion of infective donations leading to 1 clinical case per year after 10 years: alternative scenarios.

% infected recipients symptomatic within 10 years	**Feasible proportion of infective donations**	
	(a) With certain transmission	(b) With 50% chance of transmission
All MM homozygotes (40% population)	**1 in 300,000**	**1 in 150,000**
10% of MMs	**1 in 30,000**	**1 in 15,000**
1% of MMs	**1 in 3,000**	**1 in 1,500**

Though this is a separate question from ascertaining the possible number of future clinical cases, estimating the risks of recipients having been infected though historical exposure to blood components is of some importance in its own right. Because the risk of infection clearly accumulates with the number of units received, it may be appropriate to apply additional precautions to "highly-transfused" patients, in order to mitigate risks of onward transmission – even if none of the donors to these patients has shown any sign of vCJD infection. The question of how such a threshold should be established is a challenging one[21], and the ACDP Subgroup considered that it could best be done using this approach of working backward from the numbers of observed cases, provided this was done on a sufficiently precautionary basis. The calculation endorsed by the Subgroup in 2011 is set out in **Annex B**, and is now being used to inform risk management decisions.

Turning again to the overall transmission model, we also need to consider how the prevalence of infective donors may have varied by birth cohort. As noted earlier, evidence from the latest appendix survey implies that prevalence is at least as high in the 1941-60 cohort, rather than being concentrated in the 1961-85 "Hilton cohort". Nevertheless, the rate of blood-borne transmissions would have varied over time, as more individuals born between 1941 and 1985 became eligible to donate blood, then eventually too old to do so. It is generally accepted that precautions put in place to remove BSE from the food chain should have greatly reduced the risk of primary infection amongst those born from 1996 onward. These individuals will soon become eligible to donate blood, and as more donors are drawn from this group, future blood-borne transmission risks should gradually decrease and may eventually disappear.[22] It may also be feasible to speed the decrease in risk by

[21] The more general issues around the use of "risk thresholds" in the context of vCJD infection, are discussed in CJD Incidents Panel (2005); Pryer and Hewitt (2010).

[22] This depends on there being no potential for the outbreak becoming "self-sustaining" through onward transmission of infection. This now appears to be a reasonable working assumption, especially given the prohibition on recipients of blood components from donating. However, we cannot assume the

deliberately recruiting younger donors in the coming years: this is subject to further analysis outside the scope of this paper.

Throughout, we distinguish between clinical vCJD cases *that have already appeared* (critical for model calibration), future cases caused by infections that *have already occurred*, and those caused by infections that *have yet to occur*. Only the last could still be prevented. Changes in scenario inputs and assumptions will affect each of these in different ways.

In Sections 4.3 and 4.4 below, these principles are applied through two distinct stages of modelling:

- using a spreadsheet model to generate and explore individual scenarios consistent with the known evidence,
- establishing an overall range of such scenarios, by running this model a large number of times.

4.2 Working Assumptions

First, we summarise the inputs and assumptions endorsed by the ACDP Subgroup, used to determine an acceptable range of inputs and assumptions.[23]

a) Model outputs (secondary clinical cases, after allowance for post-transfusion survival, incubation periods and susceptibility to clinical vCJD) are calibrated against observed clinical case numbers.

   - As discussed above, it was originally proposed that scenarios be constrained to those in which transmission via blood components would lead to 3 - 10 clinical cases appearing by the start of 2011.
   - The upper limit of 10 included the three cases linked to vCJD-infected donors, four others with known transfusion histories and a further "margin for error". Six of the seven transfused vCJD cases, including all three linked to known-infected donors, received Red Cell transfusions, whereas one was exposed only to FFP. Given the need to model transmission via Red Cells and FFP separately, the Subgroup therefore endorsed a slight modification, constraining the **Red Cell model** to produce **3-9** clinical vCJD cases "to date", and the **FFP model** to produce **0-3** such cases.
   - No further clinical cases linked to blood transfusion have been detected in 2011 or 2012. In July 2012, the Subgroup agreed that the "cutoff date" for model calibration be moved to the start of 2012, and the analysis set out here updates this further to provide a cutoff at the **end of 2012.**

b) Model calibration uses a precautionary approach in estimating **how many infections would have shown up as clinical cases**, as well as how many cases might actually have been blood-borne.

---

prevalence of vCJD in the post-1996 cohort to be zero, due to the theoretical possibility of infection by other routes – e.g. hospital or dental surgery, or maternal transmission.

[23] These working assumptions – especially as regards infectivity and prevalence – also have implications for the assessment of vCJD transmission risks via fractionated blood products. These are not discussed in the present paper, but are being explored separately.

- Specifically, appearance of clinical symptoms within 10 years of infection might be confined to a small minority of the population, at minimum comprising 10% of MM homozygotes.
- Other recipients may not all be susceptible to development of clinical vCJD, though all are taken as susceptible to vCJD *infection*. We allow for population susceptibility to vary between 10% and 100% (across all genotypes).
- Alternatively, all recipients may be susceptible to clinical vCJD, but many would have very long incubation periods.

c) On **prevalence of infected donors**, the default assumption remains that detectable prion protein in lymphoid tissue indicates that the individual would have - or develop - infectivity in his or her blood. We therefore use values consistent with the HPA-led appendix survey already noted.

d) We consider the following ranges for the mean infectious dose per unit transfused:
   - 0.7 ID or greater for non-leucodepleted red cells, and for FFP.
   - 0.1 – 0.7 ID for red cells following leucodepletion

4.3 Model for scenario generation

Rationale

This model is designed to help explore the consequences of alternative beliefs about the factors affecting blood-borne vCJD transmission. Developed in collaboration with staff at the Clinical Operational Research Unit at University College London (Crowe *et al*, 2012). It does not attempt to capture every possible aspect of vCJD transmission, but provides a reasonably transparent way of exploring what happens as inputs and assumptions are varied. The model itself consists of an Excel spreadsheet, structured as in Figure 1 below. There are four main steps, following the underlying "logic" of transmission.

Step 1: Prevalence of infectivity in the donor population

The model splits the donor population into *5-year birth cohorts* (and by gender), allowing primary vCJD prevalence to vary between these. Primary infections are assumed to have occurred around 1990: the timing and distribution of these infections can be varied, but results are not highly-sensitive to this. Key user inputs characterise the *delay between primary infection and onset of infectivity in blood*, by choosing the mean and standard deviation of an assumed normal distribution. Choices can be varied between MM-homozygous and other donors. These inputs are important in calibrating the model, as delayed onset of infectivity will mainly remove earlier secondary infections and cases.

Step 2: Percentage of units infective

The model contains default assumptions on the provenance of donated units in terms of the age and sex profile of the donor population. The assumed distribution of donated units by age of donor is based on studies by NHS Blood and Transplant: for simplicity, this is taken to be static over time, and the same for male and female donors. Combining this with the inputs from Step 1, the model calculates the percentage of units transfused each year that would be infective.

Step 3: Number of recipients infected

Given the number of units transfused each year – again taken as constant for simplicity – the model next calculates the number of infective units transfused, and hence the number of secondary vCJD infections, by calendar year. The latter depends on the probability of transmission via an infected unit. Initially, transmission was taken to be certain, reflecting the previous assumption that each unit would contain a large infectious dose. However, the current version allows the user to enter any Infectious Dose per unit, before and after leucodepletion: it then calculates the resulting transmission probabilities using a Poisson Dose-response curve.

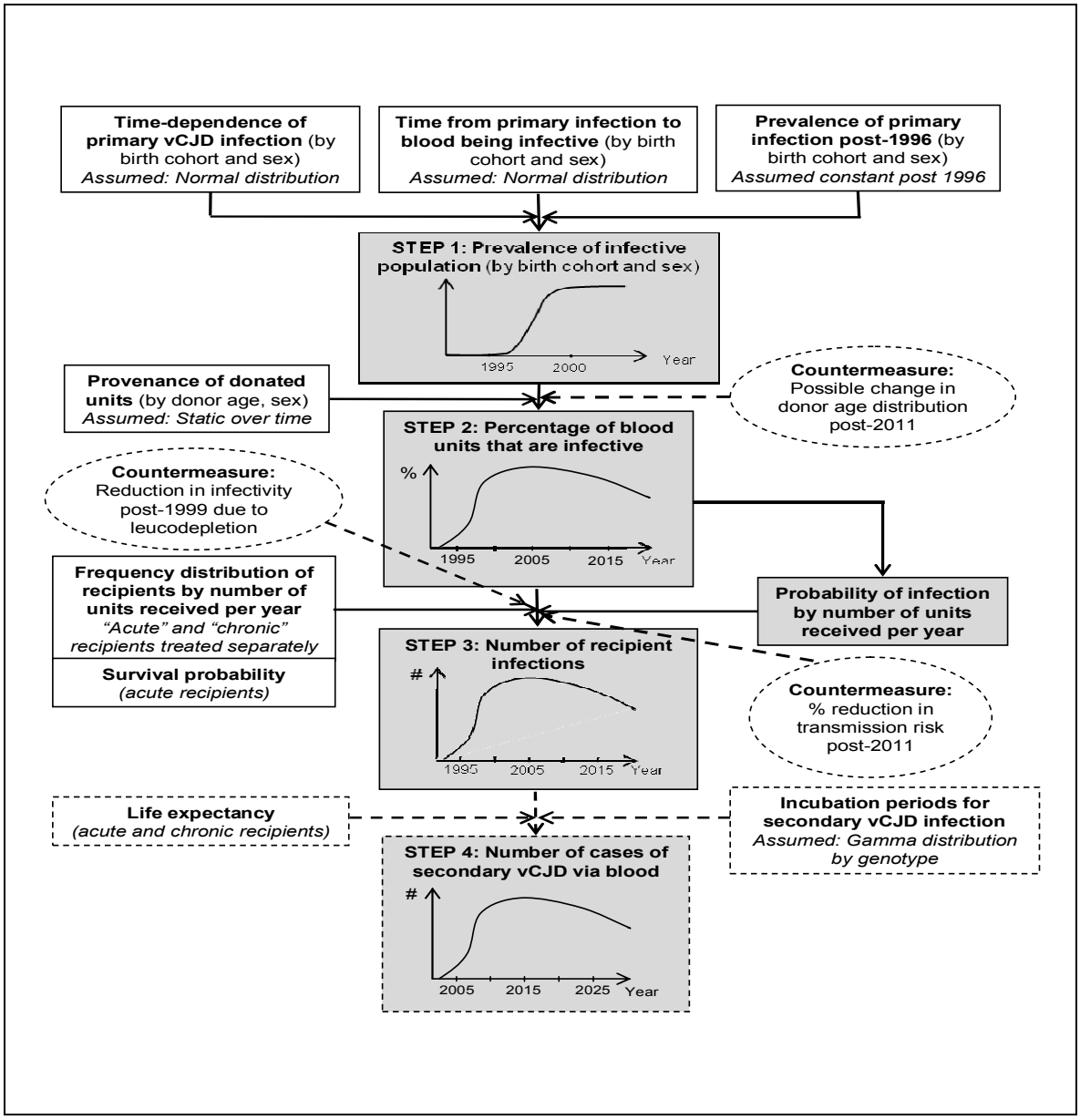
Transfusion recipients are classified as "acute" or "chronic", the former typically undergoing one-off transfusion - e.g. during surgery - the latter undergoing repeated transfusions for ongoing medical conditions. These are treated separately, due to the different patterns of transfusion and survival. A significant proportion of "acute" recipients do not survive the immediate episode necessitating the transfusion, and the model shows both the total number of infections and infections of immediate survivors. This output is displayed in graphical form, showing numbers of infections per year.

Step 4: Estimation of secondary clinical cases

The key additional inputs needed at this stage concern the *longer-term survival* of recipients and the *incubation periods* between secondary infection and appearance of clinical vCJD. The former can be checked against empirical data (e.g. Llewelyn, Wells, Amin *et al*, 2009; Wells, Llewelyn, Casbard *et al*, 2009), and the model has default survival curves built-in for both acute and chronic recipients. Incubation periods are much more uncertain. The model allows the user to choose both mean and (gamma) distribution for secondary incubation periods, taking MMs and other genotypes separately. The *susceptibility* of infected recipients to developing clinical vCJD at all can also be varied, for MM and other genotypes. A final refinement to the model has been to split infections of MM recipients into two, with separately-defined incubation periods. This allows us to define a subgroup of infected recipients who would develop clinical symptoms significantly faster than the rest. (Making the same distinction amongst donors also allows us to model the possibility of this group also developing infectivity in blood faster, following primary infection.)

Figure 1: Overview of model for scenario generation

White and grey rectangles denote model inputs and outputs: ellipses show further inputs relating specifically to counter-measures against vCJD transmission. Note that "cutoff" dates between past and future events have been successively updated.



Specific inputs and assumptions are shown in more detail in Table 2 below. Those shown deal with red cell transfusion, but analogous considerations apply to transfusion of other blood components, notably FFP.

Table 2: Overview of inputs to spreadsheet model (Red Cell transmission)

Model stage	Input	Type, unit	Values	Comments
Prevalence of infective donors, by calendar year	Timing of primary infections	Mean (calendar year), normal distribution	Default is sharp peak around 1990	Can be varied, but not critical
	Time from infection to onset of infectivity in blood	Mean, std deviation (yrs).	Separate inputs for MM and other genotypes	Important for calibration of case numbers
	Population prevalence of vCJD infection	By 5-year birth cohort, from 1931 onward	*Variable input*	
Percentage of units infective	Distribution of units by age of donor	Static, for historic donations	From Blood Services studies. Can be varied for future donations	Can also be varied by gender, but not currently done.
vCJD transmissions by calendar year	Number of Red Cell units transfused	Constant (simplifying assumption)	2.15m units per year	Usage has slowly declined, so model will slightly over-estimate future cases
	Infectious dose per unit, no leucodepletion	ID per unit	*Variable input*	Model calculates transmission risk, using Poisson dose-response
	Ditto, with leucodepletion	ID per unit	*Variable input*	
	Immediate survival of "acute" recipients	Percentage	Based on survival studies	Distinguishes infection of surviving patients
Incidence of secondary vCJD cases by calendar year	Long-term survival of "acute" and "chronic" recipients	Survival curves as defined in model.	Overall survival rate checked against empirical studies.	Some uncertainties, but small compared with other inputs.
	Secondary incubation periods for MM and non-MM recipients	Gamma distribution for each group	*Variable input* Default distribution has α = mean IP; $\beta = 1$	MM group can also be split
	Susceptibility	% recipients susceptible to clinical vCJD	*Variable input:* can be varied by genotype.	All recipients assumed to be susceptible to vCJD infection.
	Reduction in transmission risk from end of 2012.	% reduction	*Variable input:* default is no reduction	For exploration of further risk reduction steps

Outputs: scenarios for blood-borne clinical vCJD cases

For any set of inputs, the model provides graphs showing the yearly incidence of blood-borne vCJD transmissions, and the subsequent appearance of clinical vCJD cases. All results can be broken down by genotype. Clinical cases are shown both before and after allowing for post-transfusion survival - highlighting the point that in most scenarios, most infected recipients die of other causes without developing symptoms of vCJD. For each scenario, the model total the number of clinical cases *that would have appeared by the end of 2012* - providing the necessary comparison with observation - and those projected to occur after then. The latter are broken down into those cases caused by past transfusions, and those caused by transfusions yet to take place – only the latter being open to change by any possible intervention.

Exploring the effects of interventions

The model allows any assumption to be made about the effect of leucodepletion on red cell transmission risks after 1999. To explore the potential impact of further interventions, the user can choose to apply any percentage reduction to the rate of new transmissions and/or to vary the donor age profile for future years, e.g. to explore the effect of attracting more donors born after 1996 once they become eligible.

Initial use and development

Initial versions of the model were prototyped, and various illustrative scenarios checked for face plausibility and consistency with independent calculations. For red cell transmission, for example:

- Simple model runs with prevalence of infective donors consistent with the HPA appendix survey and high probabilities of transmission produce over-predictions similar to the simple calculations provided earlier in this paper.
- Using input values similar to those featuring in the published Imperial College model (Ghani and Garske, 2010) allowed us to reproduce key features of that model's central scenario: that of a long wave of secondary cases, with relatively small numbers appearing each year but persisting for several decades. As discussed elsewhere (Bennett and Daraktchiev, *op cit*; Crowe *et al*, *op cit*), this degree of consistency was encouraging, though specific inputs on population prevalence of infection have since been overtaken by more recent evidence.
- If leucodepletion is assumed to have had a substantial effect from 1999 onward, one can produce scenarios matching the timing of cases seen so far - though their number is still exaggerated unless susceptibility to disease is significantly below 100%, or almost all recipients have very long incubation periods.

Numerical scenarios were presented to the ACDP Subgroup meetings in January and July 2012, and some features in the current model followed suggestions made by members.

Illustrative scenarios

To investigate whether and how scenarios consistent with all requirements set out in the previous section could be generated, various combinations of inputs have been considered. The results suggest that the conditions can be met, though only if some inputs are constrained toward the "low risk" limits of their defined ranges - particularly if all recipients are assumed susceptible to clinical vCJD.

For example, Figure 3 below shows a scenario with the following inputs:

- *Prevalence of infected donors*: 1 in 3,300 for all 1941-85 birth cohorts (and 1 in 10,000 for those born 1986-95, and negligible thereafter).
- *Mean Incubation periods* following blood-borne vCJD infection: 9 years for 10% of MM infections, (designated "MM(1)"); 25 years for other MM infections; 30 years for other genotypes.
- *Mean delay in onset of infectivity in donors:* 7 years for MMs; 12 years for other genotypes.
- *Per unit infectivity:* 0.7 ID pre-leucodepletion, 0.1 ID after.

The first graph (Figure 3a) shows the number of secondary *infections* caused by red cell transfusion in this illustrative scenario, by calendar year. Figure 3b then shows how these would translate into the appearance of *clinical cases*, typically after considerable delay and in much smaller numbers due to deaths from other causes. Finally, Figure 3c shows a breakdown of these by genotype.

This scenario has 9 clinical cases appearing prior to 2011, and 182 thereafter. This is in accordance with the number of actual and possible secondary cases seen so far – all amongst MM patients - and, to a fair approximation, with their timing. The assumed prevalence of infected donors is consistent with the appendix survey data, albeit close to the lower end of the feasible range.

To achieve this, however, some other inputs have been set at values that reduce projected case numbers. In both sets of scenarios,

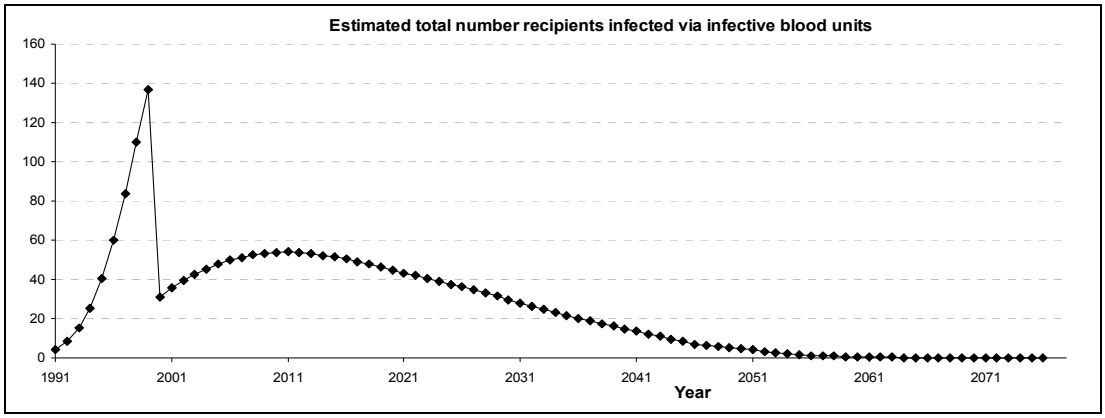
- The proportion of infected recipients developing clinical vCJD relatively quickly is very small, despite this being a highly-efficient transmission route. In addition, onset of infectivity in donors (following primary infection) is substantially delayed.
- Leucodepletion is taken to be highly effective. It should be noted that obtaining any dip in case numbers during the last few years - as appears to have happened in reality - requires post-leucodepletion infectivity to be low *and* almost all incubation periods to be long *regardless* of other inputs.[24]

In such scenarios, the long incubation periods for most recipients mean that only a small proportion of infections would lead to patients developing clinical symptoms of vCJD, even though all are assumed to be susceptible.

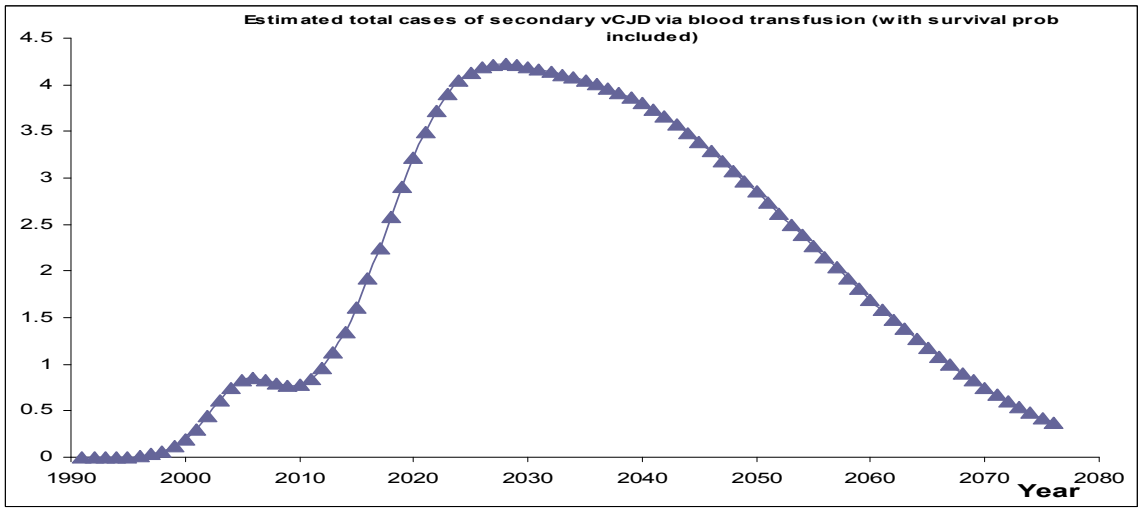
[24] For comparison, and leaving all else unchanged, inputs of 0.6 and 0.2 ID before and after leucodepletion give pre-and post-2012 case numbers of 10 and 313. Values of 0.5 and 0.2 ID give 9 and 298. Both scenarios have less realistic timing of early cases.

Figure 3: Illustrative scenario for Red Cell transmission

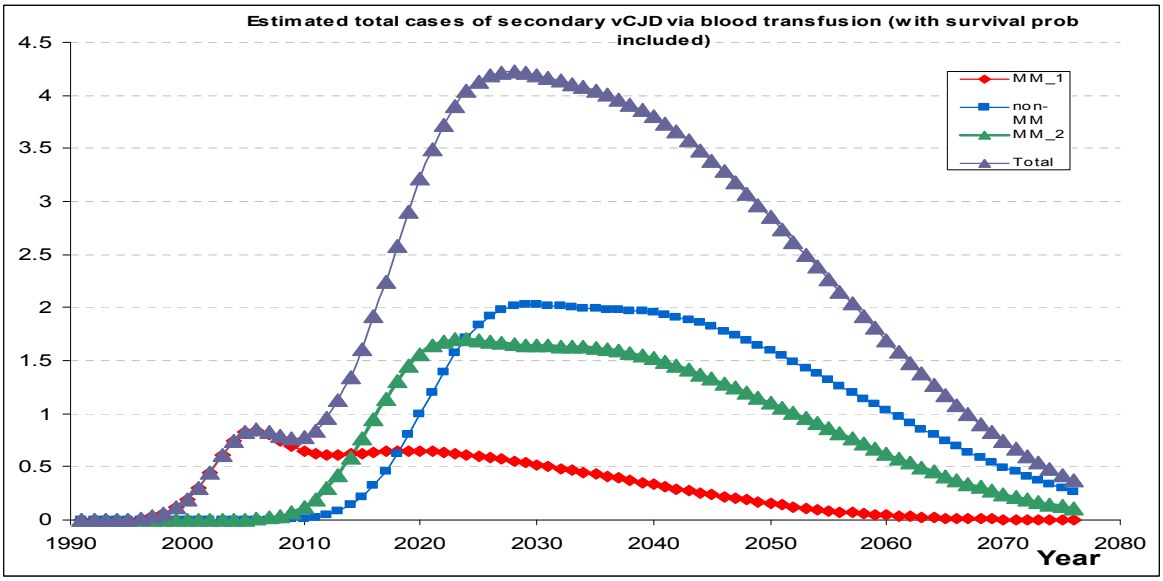
Occurrence of secondary infections, by calendar year



(b) Appearance of clinical cases amongst transfused patients (NB change in scale)



(c) Breakdown of clinical vCJD cases by patient genotype



Finally, the maximum potential effect of any further intervention in this scenario can be seen from the finding that 86 of these cases would be caused by transfusions occurring after the end of 2012, in contrast to the 81 caused by earlier transmissions.

The proportion of future cases that remain "in principle preventable" is around 50% in many of the scenarios considered here (regardless of the absolute numbers involved). This contrasts with existing models that largely confine prevalence of infectivity to the 1961-85 "Hilton" cohort (e.g. Ghani and Garske, 2010), in which a higher proportion of future cases come from future infections. This difference is to be expected. With greater prevalence of infectivity in the earlier cohort of donors, more infections would have occurred earlier in earlier years. In addition, longer incubation periods are required if scenarios are not to "over-predict" the number of cases occurring to date. Consequently, a higher proportion of future cases would be caused by transmissions that have already occurred.

Of course, this is merely one scenario. Other ways of reconciling outputs with existing case numbers can be chosen, demonstrating the trade-off between different parameters. These can produce quite wide variations in the expected number of future secondary cases: further examples are provided in **Annex C.**

4.4 Generating a feasible range of scenarios

Methodology

Choosing and manually inputting different combinations of assumptions can serve to illustrate alternative scenarios, but is somewhat haphazard – some significant set of combinations may be missed, while attempting to cover all possibilities would be inordinately time-consuming. We have therefore developed an additional program that, in effect, automates this process. Key inputs can be fixed, or constrained to fall within specified ranges. The additional program will then repeatedly sample from these ranges (without replacement) and input the values into the spreadsheet already described. This "Monte Carlo" approach of choosing input values at random (see e.g. Stevenson, 1992) makes it possible to run the model some thousands of times, under the chosen constraints. The program then identifies all the runs with outputs meeting specified "calibration" requirements. In this case, we simply require scenarios to have *numbers of clinical cases appearing to date* that fall within the ranges discussed earlier. That is, scenarios for red cell transmission must produce between 3 and 9 secondary vCJD cases by the end of 2012, and those for FFP transmission no more than 3. Scenarios not meeting these criteria are discarded, and those that do so are explored further.

Extended in this way, the model has some conceptual similarities with the Imperial College simulation (Ghani and Garske, *op cit*): nevertheless, it remains simpler and significantly narrower in scope. It models only blood-borne transmission, rather than the whole vCJD outbreak. The effects of the primary outbreak thus appear as inputs - the prevalence of infected donors. These prevalence inputs are set to follow the probability distributions generated by the HPA appendix survey (on the assumption of binomial distributions). For other inputs, there may be no basis on which to assign prior probabilities: all values are therefore treated as equally likely within the ranges set. No attempt is made to attach posterior probabilities to the model outputs. Rather, each scenario simply represents a possible outcome *consistent with the constraints given*. Nevertheless, examining the spread of such outputs provides a way of arriving at a range of credible scenarios for future case numbers. Maintaining a relatively simple model also allows changes to be made, and the model re-run comparatively quickly.

Red Cell transmission model

In line with the discussion and advice set out above, we have carried out this modelling exercise using the following constraints.

- Prevalence of infected donors is set to follow the results of the HPA appendix survey, which found 16 verified "positive" samples from just over 32,000 tested, 6 from 8,181 in the 1961-85 cohort and 10 from 24,260 in the 1941-60 cohort. [25]
- Two different sets of scenarios are then generated, in each case by running the model 30,000 times. "Set 1"uses the assumptions detailed in Table 3 below: all infected recipients are taken to be susceptible to clinical vCJD, but infections with relatively short incubation periods are confined to a sub-group of recipients comprising at least 10% of MM homozygotes.[26] . "Set 2" differs by assuming that incubation periods following infection of all MMs follow a single distribution, but allows susceptibility to clinical disease amongst all recipients to vary between 100% and 10%.

Table 3: Input ranges for Red Cell model runs

Input	Detail	Value / range	Comments
Prevalence of infected donors	1941-60	733 (269-1596) per mn	Means & 95% CI from appendix data; binomial distribution.
	1961-85	412 (198 – 758) per mn	
Mean onset of infectivity in donors, after infection	MM(1)	1-5 yrs (variance 0-3) in Set 1 1 -13 yrs (variance 0-7) in Set 2	
	MM(2)	1 - 13 yrs (variance 0-7 yrs)	
	Others	1 – 19 yrs (variance 0-10 yrs)	
Mean prob. of transmission from infected RC unit	Pre-leucodepletion	50 – 100 %	$\geq$ 0.7 ID per unit (Poisson model)
	Post-leucodepletion	10 – 50%	Roughly 0.1 – 0.7 ID
Secondary Incubation Periods (mean)	MM(1)	3 – 11 yrs in Set 1 3 – 35 yrs in Set 2	
	MM(2)	12 – 35 yrs in Set 1 As MM(1) in Set 2	
	Others	20 – 40 yrs	
	Shape of γ-distributions	$\beta = 1$	Fixed in current model runs.
MM(1) infections	% of infected recipients	$\geq$ 4%	> 10% of MM infections
Susceptibility	To clinical vCJD	100% in Set 1 10-100% in set 2	All recipients susceptible to infection

[25] As noted, the prevalence estimates for the two cohorts show some difference, though this is not statistically significant. In these model runs, prevalence values for the two cohorts are sampled from the two separate distributions. However, results remain similar if the overall finding of 16 in 32,441 is used to generate a single distribution covering both cohorts.

[26] As explained previously, this differential might be caused by differences infective agent, characteristics of individual recipients, or both.

Within each set of 30,000 model runs, the "calibrated" scenarios in which 3 - 9 secondary clinical cases appear up to the end of 2012 are identified. These are then characterised, primarily in terms of the number of future clinical cases, and the number of those caused by transmissions yet to occur.

Scenarios for FFP transmission

The need to calibrate the transmission model applies equally to FFP transfusions. Broadly similar knowns and unknowns exist as for red cells. Units of FFP transfused per year have averaged about 1/7th the number of red cell units. Long-term survival rates are slightly lower than for Red Cell recipients. There have been no known vCJD transmissions via FFP: of the vCJD cases with transfusion histories but no known infected donor, one was exposed solely to FFP and one to FFP in combination with other components and products. As before, however, some allowance should be made for possible under-ascertainment of blood-borne cases.

The limited evidence available suggests that per-unit infectivity of FFP is of the same order as for red cells, though with no significant reduction from leucodepletion.

We therefore apply the same modelling approach as for Red Cell transmission, subject to the following changes:

- Reducing numbers of units transfused annually by a factor of 7.25, and adjusting post-transfusion survival
- Considering only scenarios with no effect from leucodepletion
- Calibrating the model to produce no more than 3 clinical cases associated with FFP transfusion appearing by the end of 2012.

5. RESULTS AND DISCUSSION

5.1 Cases due to Red Cell Transmission

Only a minority of the model runs produce the required 3-9 cases prior to the end of 2012 These comprise:

- Just 5 from 30,000 from "Set 1" (with susceptibility set at 100% and a sub-set of MM infections characterised by much shorter IPs)
- 3,491 from 30,000 in "Set 2", in which susceptibility to clinical disease is allowed to vary between 10% and 100%.

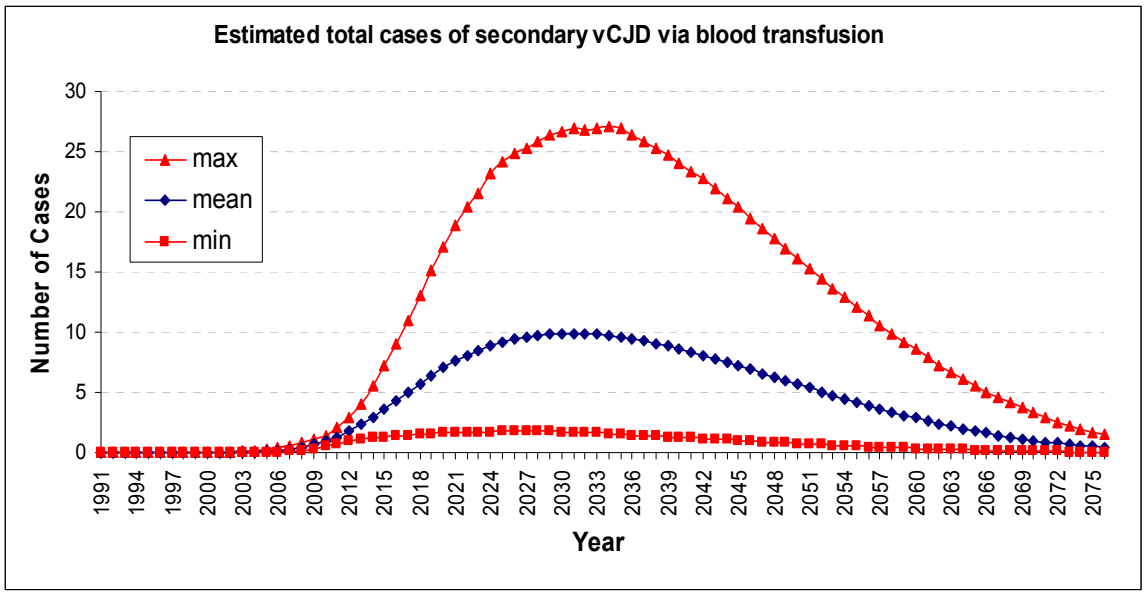
Not surprisingly, it is easier to produce calibrated outputs by allowing the susceptibility to clinical vCJD to vary across all infections of all recipients (indeed the small number in "set 1" indicates that the constraints imposed are almost mutually incompatible).

More details of these model runs (and those for FFP transmission, discussed below) are given in **Annex D**. Figure 4 illustrates the resulting ranges of scenarios in Set 2 for secondary clinical cases. Figure 4(a) shows cases to be expected in the absence of any further interventions to reduce transmission risks. However, a substantial proportion of the future cases would be due to transfusions that have already taken place: Figure 4(b) shows the range of cases to be expected *even if all transmissions were stopped from the beginning*

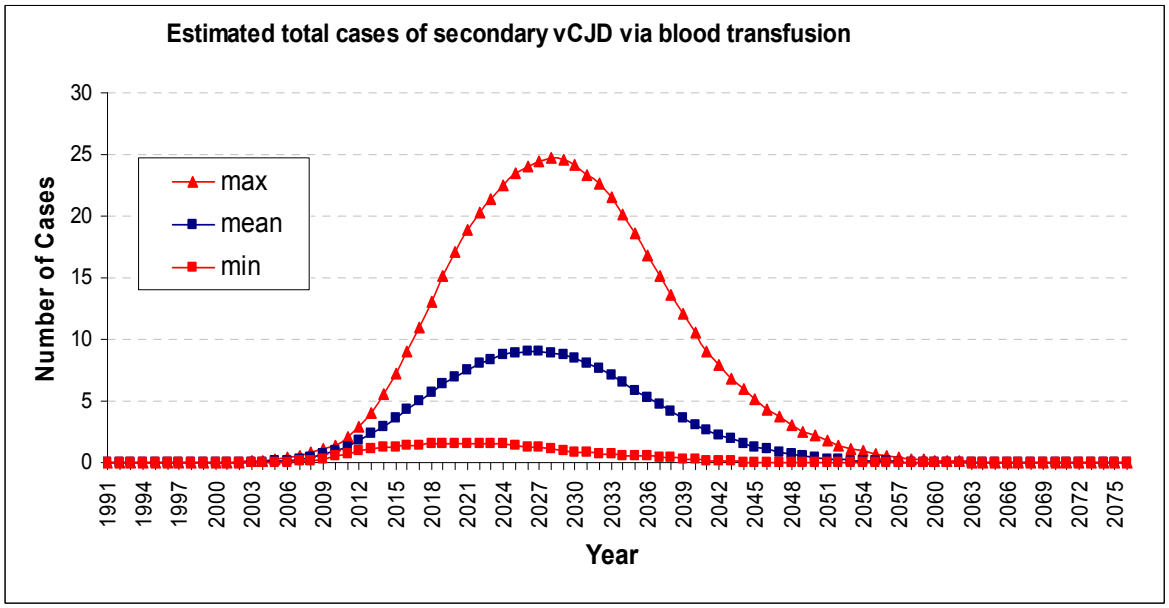
of 2013. In any given scenario, the maximum benefit of any future intervention is represented by the difference between the two figures: Figure 4(c) gives the distribution of these "in-principle preventable" future cases.

Figure 4: Range of scenarios for clinical cases due to Red Cell transfusion

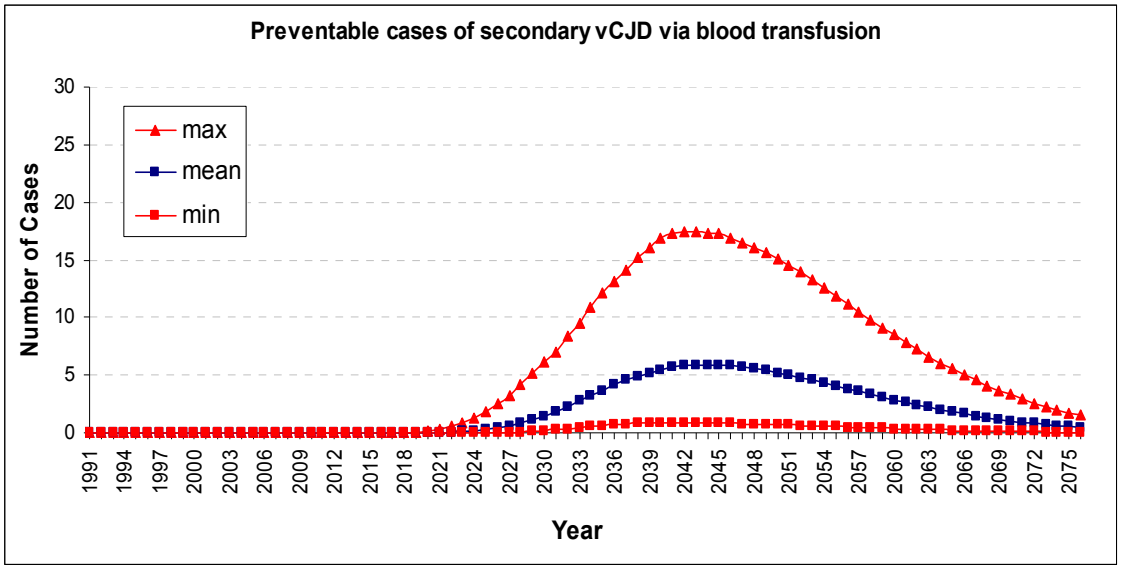
(a) With no further interventions



(b) Cases if all future infections were prevented



(c) Cases caused by transmissions yet to happen



These "preventable" clinical cases would be spread over several decades, with incidence peaking at about 5-6 (for the central estimate) in the 2040s.

Summary results are set out in Table 4. Given the much larger number in Set 2, these suggest a central scenario in which roughly 350 future clinical cases would be caused by red cell transfusions, 160 due to transfusions yet to happen. Wide ranges of uncertainty remain, as indicated by the 95% intervals shown - the range being given by excluding the 2.5% of scenarios lying at each extreme.

Table 4: "Calibrated" scenarios for transmission via Red Cells: summary of properties

	Set 1	**Set 2**
Calibrated model runs, from 30,000	5*	3,491
Susceptibility	100% (fixed)	52% (mean)
Secondary clinical cases, onset from end of 2012 onward	220 (mean)	350 (70 – 930)
Clinical cases caused by *infections* occurring from end 2012	130 (mean)	160 (30 – 460)

* The small number of scenarios in this set make statistical characterisation of the range problematic. The mean result is therefore given as an indicative figure.

Overall, then, there appear to be many scenarios compatible with current evidence in which 300-400 clinical vCJD cases caused by Red Cell transfusion might appear over the coming 65 years in the absence of further interventions. However, possible totals range from a few dozen up to nearly a thousand. The central estimate for the number of clinical cases *caused by future transmissions* is roughly 160, with a range running from about 30 to 460. These are the only cases that could be affected by any further intervention – even one that hypothetically eliminated all risk of transmission via Red Cells.

5.2 Cases due to FFP transmission

As with the Red Cell model, we again generate a range of scenarios by sampling input parameters. The constraints used are as before, except that mean probability of infection from an infected unit of FFP is constrained to lie between 0.5 and 1.0, with no change due to leucodepletion. Results are summarised in Table 5:

Table 5: "Calibrated" scenarios for transmission via FFP Transfusion

	Set 1	**Set 2**
Calibrated model runs, from 30,000	22*	14,487
Susceptibility	100% (fixed)	51% (mean)
Secondary clinical cases, onset from end of 2012	110 (mean)	90 (20 – 220)
Clinical cases caused by infections occurring from end 2012	70 (mean)	45 (10 – 120)

* The small number of scenarios in this set make statistical characterisation of the range problematic. The mean result is therefore given as an indicative figure.

Again taking summary figures from the much more numerous scenarios in Set 2, these suggest a central estimate for the number of clinical cases caused by *future* transmissions via FFP of roughly 40-50 with a range running up to about 120.

These figures might appear rather high when compared with the outputs of the Red Cell transmission model. A number of factors are at work here, one being the lack of any effect from leucodepletion. It might also be argued that allowing up to three existing cases to have been caused by FFP transmission is "over-precautionary" given that no such transmissions have yet been detected and the general lack of exposure to FFP amongst known clinical cases. In general, all working assumptions about FFP transmission remain more speculative than for Red Cells.

6. CONCLUDING COMMENTS AND CAVEATS

We again stress that the model set out here does not attempt to capture every aspect of vCJD transmission, but concentrates on those factors most affecting the broad pattern of infections, and especially of clinical cases. The scenarios provided should not be regarded as predictions, but rather as reasonable (and in general precautionary) working estimates that can be used to assess the potential benefits of any further interventions.

Key inputs to the analysis remain provisional. This includes not only those related to vCJD, but also those on survival of transfusion recipients. This is of particular significance given a model in which incubation periods for many recipients would be of the order of 30 years. Data on survival for this length of time post transfusion are lacking, and estimation of projected vCJD case numbers in such scenarios is dependent on extrapolation from shorter-

term survival data. Some further checking will become possible with completion of an update to the EASTR study already referred to. This tracks patients up to ten years from transfusion, and results will be available later in 2013. Even so, substantial uncertainties about longer-term survival will remain. If more patients survive for long periods than assumed in the existing model – or if survival prospects improve substantially in future – then the proportion of vCJD infections that lead to clinical disease would clearly increase. This will be a topic for continuing analysis.

One might also consider how much weight to give to the recent gap in the appearance of clinical cases. Of the known patients with vCJD that might plausibly be attributed to blood-borne transmission, the last suffered onsets of disease in February 2006. If – as we must hope – this gap continues, it will become steadily more significant in terms of epidemiology. We might then require scenarios to show a significant "dip" in expected secondary cases from about 2006: some of those appearing in the existing red cell model do so (for example that in Figure 3), but this is not currently a general feature. As Figure 3 also illustrates, such a dip may be compatible with a *relatively* large number of future cases, so the model may also serve to guard against premature optimism. Nevertheless, a continuing absence of clinical cases attributable either to Leucodepleted Red Cells or FFP (or cryoprecipitate or platelets), all of which contain significant volumes of plasma, *might* suggest that infectivity in human blood is less strongly associated with plasma than appears to be the case with rodents or sheep.

For the present, the model presented here is intended to remain precautionary – at least on balance - for example in calibrating secondary cases appearing so far to a maximum of 9 from Red Cell transmission plus 3 from FFP, rather than the "3 + zero" actually detected. However, this would not be sufficient to allow for large-scale under-detection of blood-borne clinical cases.

The model also assumes both that presence of abnormal prion protein in lymphoid tissue is indicative of infectivity in blood, and that all recipients of blood are susceptible to vCJD infection. Though compatible with available evidence, these assumptions are in tension with the small number of secondary clinical cases detected so far. To resolve this, the model has to allow the relatively short incubation periods seen for cases attributable (or possibly attributable) to blood-borne transfusion to be highly atypical, even amongst MM-homozygotes. This allows the various pieces of evidence to be reconciled, but the explanation remains largely ad hoc unless or until there is some definitive evidence to explain this variation – whether in terms of differences in infective agent, individual susceptibility or both.

For the present, this approach leads to scenarios with large numbers of secondary infections, very few of which ever present as clinical cases. Individuals who remain asymptomatic suffer no apparent harm. Nevertheless, they might represent sources of potential onward infection, if not through blood donation - given the ban on transfused donors, and the non-eligibility of many recipients with "chronic" conditions - then through donation of tissues and organs or re-use of surgical instruments. It may be possible to provide some quantification of these risks "per infected recipient"; however, this lies beyond the scope of this paper. It should also be stressed that while we have presented scenarios with sub-clinical secondary infections that could range into the thousands, these same scenarios already have large numbers of silent *primary* vCJD infections. As shown in several previous analyses, there is no potential for vCJD infection by this route to become

self-sustaining. We have therefore concentrated on the "first order" effects of transmission, i.e. the number of cases that might *directly* be caused by transfusion.

The continuing uncertainties in the basic science of TSE disease remain of critical importance in attempting to understand vCJD epidemiology. Notably, model calibration could be made much more definite given a better understanding of how clinical disease might or might not develop following blood-borne exposure to infection. This risk assessment remains provisional, and may be updated as and when new information becomes available. Meanwhile, thorough follow-up of "at risk" individuals remains essential if both "positive" and "negative" evidence on transmission risks is to accumulate. This includes the "highly transfused" as a key sentinel group.

References

Bennett PG and Daraktchiev M (2011): Blood-borne transmission of vCJD: re-examination of scenarios. Health Protection Analytical Team, Dept of Health, available at http://www.dh.gov.uk/health/2011/09/vcjd-scenarios/

Bennett PG and Dobra SA (2006): Risk Assessments for Variant CJD and Blood Transfusion: a Perspective from the UK. In: Turner ML, Ed, *Creutzfeldt-Jakob Disease: Managing the Risk of Transmission by Blood, Plasma and Tissues*, Bethesda, MD: AABB Press, 2006 pp 245-285

Bennett PG, Dobra SA and Gronlund J (2006): The implications for blood donors if a recipient develops vCJD" *OR Insight* 19(4)**:** 3-13

Béringue V *et al*. (2012): Facilitated cross-species transmission of prions in extraneural tissue. *Science*, 27 January 2012, 335:472-5

Bishop MT *et al*. (2006): Predicting susceptibility and incubation time of human-to-human transmission of vCJD. *Lancet Neurology* 5: 393-398

Bishop MT, Diack AB, Ritchie DL, Ironside JW, Will RG and Manson JC (2013): Prion infectivity in the spleen of a *PRNP* heterozygous individual with subclinical variant Creutzfeldt-Jakob disease. Brain, *in press*, 2013

Boelle PY, Cesbron JY and Valleron AJ (2004): Epidemiological evidence of higher susceptibility to vCJD in the young. *BMC Infect Dis* 2004; 4:26 [Available at http://www.biomedcentral.com/1471-2334/4/26 (accessed March 2, 2006).]

Brown KL, Wathne GJ, Sales J, Bruce ME and Mabbott NA (2009): The effects of host age on follicular dendritic cell status dramatically impair scrapie agent neuroinvasion in aged mice. *J. Immunology*, 28th Sept 2009

CJD Incidents Panel (2005): *Management of possible exposure to CJD through medical procedures: framework document*. Health Protection Agency, London

Clarke P and Ghani AC (2005): Projections of the future course of the primary vCJD epidemic in the UK: Inclusion of subclinical infection and the possibility of wider genetic susceptibility. *J R Soc Med* 2:19-31.

Clewley JP, Kelly CM, Andrews N et al (2009): Prevalence of disease-related prion protein in anonymous tonsil specimens in Britain: cross sectional opportunistic survey. BMJ 338: b 1442 doi: 10.1136/bmj.b1442

Cooper JD and Bird SM (2003): Predicting incidence of vCJD from UK dietary exposure to BSE for the 1940 to 1969 and post-1969 birth cohorts. *Int J Epidemiol* 32: 684-791

Collinge J, and Clarke AR (2007): A general model of prion Strains and their pathogenicity. *Science* 318: 930-936 DOI: 10.1126/science.1138718

Collinge J (2012): The risk of prion zoonoses. *Science*, 27th January 2012, 335:411-413

Crowe S, Bennett PG, Daraktchiev M and Utley M (2013): Use of modelling to inform public health policy: a case study on the blood-borne transmission of variant CJD. Clinical OR Unit, UCL. *J.Opl Res.Soc*, in press 2013

Department of Health (EOR) (2003): On vCJD Transmission through Blood Components: Reconciling Modelled Risks with Case Evidence. May 2003, available at http://www.dh.gov.uk/en/Publichealth/Communicablediseases/CJD/CJDgeneralinformation/DH_4136944

Department of Health (2005): Assessing the Implications for Blood Donors if Recipients are infected with vCJD. Available at http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4115311

Department of Health (2009): The risk of secondary vCJD infection of patients receiving a high number of blood transfusions. Available at: http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_103331

Dodd, RY (2010): Prions and precautions: be careful for what you ask. Editorial, *Transfusion* 50: 956-958

Edgeworth JA, Farmer M, Sicilia A et al (2011): Detection of prion infection in variant Creutzfeldt-Jakob disease: a blood-based assay. *The Lancet*, 377, 9764: 487- 493

Ghani AC and Garske T (2010): Uncertainty in the tail of the Variant Creutzfeldt-Jakob Disease epidemic in the UK. *PLoS ONE* 5(12) e 15626

Gill ON, Spencer Y, Boyes L *et al* (2013): Presence of subclinical abnormal prion protein in all *PRNP* genotypes: Results of a second United Kingdom survey of archived appendix specimens. Health Protection Agency (in preparation)

Gillies M, Chohan G, Llewelyn CA, MacKenzie J, Ward HJT, Hewitt PE and Will RG (2009): A retrospective case note review of deceased recipients of vCJD-implicated blood transfusions *Vox Sanguinis* 97: 211-218

Gregori L *et al* (2004): Effectiveness of leucodepletion for removal of infectivity of TSEs from Blood. *Lancet* 364: 529-531

Gregori L Yang H and Anderson S (2011): Estimation of variant Creutzfeldt-Jakob disease infectivity titers in human blood. *Transfusion* 2011 Jan 3, doi: 10.1111/j.1537-2995.2011.03199.x.

Head MW, Yull HM, Ritchie DL, Bishop MT and Ironside JW (2009): Pathological investigation of the first blood donor and recipient pair linked by transfusion-associated variant CJD transmission. *Neuropathology and Applied Neuropathology* 35: 433-436

Hewitt PE, Llewelyn CA and Will RG (2002): Follow-up of donations from patients with vCJD. *Transfusion Med* 12(S1):3

Hewitt PE, Llewelyn CA MacKenzie J and Will RG (2006): CJD and blood transfusion: results of the UK transfusion epidemiological review study. *Vox Sanguinis* 91: 221-230

Hilton DA, Ghani AC, Conyers L *et al* (2004): Prevalence of lymphoreticular prion protein accumulation in UK tissue samples *J Pathol* 203: 733-9

Holmes D (2012): vCJD remains an enigma as UK deaths fall to zero. *The Lancet Infectious Diseases* 12(12): 914-915, December 2012

Houston F, Foster JD, Chong A, Hunter N and Bostock CJ. (2000): Transmission of BSE by blood transfusion in sheep. *Lancet* 356: 999-1000

Houston F, McCutcheon S, Goldmann W, Chong A, Foster J, Sisó S, González L, Jeffrey M, Hunter N. (2008): Prion diseases are efficiently transmitted by blood transfusion in sheep. *Blood*. 112(12): 4739-45.

Ironside JW, Bishop MT, Connolly K *et al* (2006): Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study. *BMJ* 332: 1186-8

Kaski D, Mead S, Hyare H *et al* (2009): Variant CJD in an individual heterozygous for *PRNP* codon 129 *Lancet*, 374: 2128

Knight R (2010): The risk of transmitting prion disease by blood or plasma products. *Transfusion and Apheresis Science* 43, 387-391.

Li J, Browning S, Mahal SP, Oelschlegel AM and Weissmann C (2009): Darwinian evolution of prions in cell culture. *Science* 12 February 2010: 869-872. Published online 31 December 2009

Llewelyn CA, Hewitt PE, Knight RS *et al* (2004): Possible transmission of vCJD by blood transfusion. *Lancet* 363: 417-421

Llewelyn CA, Wells AW, Amin M, *et al* (2009): The EASTR study: a new approach to determine the reasons for transfusion in epidemiological studies. *Transfusion Medicine*, 19: 89–98

Mackay GA, Knight RSG and Ironside JW (2011): The molecular epidemiology of variant CJD. *Int J Mol Epidemiol Genet*. 2(3): 217–227.

de Marco MF, Linehan J, Gill ON, Clewley JP and Brander S (2010): Large-scale immunohistochemical examination for lymphoreticular prion protein in tonsil specimens collected in Britain, *Jnl of Pathology* 222: 380-387

McCutcheon S, Alejo Blanco AR, Houston EF *et al* (2011): All Clinically-Relevant Blood Components Transmit Prion Disease following a Single Blood Transfusion: A Sheep Model of vCJD. PLoS One. 2011; 6(8): e23169.

McGowan CR and Viens AM (2011): Coroners and the obligation to protect public health: the case of the failed vCJD study. *Public Health* 125: 234-237

Mead S, Poulter M, Uphill J, Beck J, Whitfield J, Webb TEF, Campbell T, Adamson G, Deriziotis P, Tabrizi SJ, Hummerich H, Verzilli C, Alpers MA, Whittaker JC and Collinge J (2009): Genetic risk factors for variant Creutzfeldt-Jakob disease: a genome-wide association study. *The Lancet Neurology*, 8 (1): 57 - 66

Peden AH *et al* (2004): Preclinical vCJD after Blood Transfusion in a PRNP Codon 129 Heterozygous Patient. *Lancet* 364: 527-529

Peden A, McCardle L, Head MW *et al* (2010): Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. *Haemophilia*, 16: 296-304.

Pryer D and Hewitt P (2010): CJD: risk communication in a health care setting, in Bennett, Calman, Curtis and Fischbacher-Smith (eds), *Risk Communication and Public Health* (2nd Edition), Oxford University Press, 2010

Stevenson WJ (1992*): Introduction to Management Science.* Irwin, Homewood IL, 2nd edition

Wadsworth JDF and Collinge J (2006): Molecular Pathology of Prion Diseases. In: Turner ML, Ed: *Creutzfeldt-Jakob Disease: Managing the Risk of Transmission via Blood, Plasma and Tissues*, Bethesda MD: AABB Press 2006

Wadsworth JDF, Dalmau-Mena I, Joiner S *et al*. (2010): Effect of fixation on brain and lymphoreticular vCJD prions and bioassay of key positive specimens from a retrospective vCJD prevalence study. *J Pathol* 223: 511-518

Wallis JP (2010): Strategies to reduce transfusion-acquired vCJD. *Transfusion Medicine* 2010, doi: 10.1111/j.1365-3148.2020.01047.x

Wallis JP, Well A, Matthews JN and Chapman CE (2004): Long-term survival after blood transfusion: a population based study in the North of England. *Transfusion* 44, 1025-1032.

Watkins NA, Dobra SA, Bennett PG, Cairns J, Turner ML (2012): The management of blood safety in the presence of uncertain risk: a United Kingdom perspective *Transfus Med Rev.* 2012 Jul;26(3):238-51. doi: 10.1016/j.tmrv.2011.09.003.

Wells AW, Llewelyn CA, Casbard A, *et al* (2009): The EASTR Study: indications for transfusion and estimates of transfusion recipient numbers in hospitals supplied by the National Blood Service. *Transfusion Medicine*, 2009, 19, doi: 10.1111/j.1365-3148.2009.00933.x

Will R (2010): Variant CJD: where has it gone, or has it? *Pract Neurol*, 10: 250-251

Wroe SJ, Pal S, Siddique D et al (2006): Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. *Lancet*, 368: 2061-7

Zaman SMA, Hill FGH, Palmer B, Millar CM *et al* (2011): The risk of variant Creutzfeldt-Jakob Disease among UK patients with bleeding disorders, known to have received potentially contaminated plasma products. *Haemophilia*, 2011 doi: 10.1111/j.1365-2516.2011.02508.x