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研究報告の概要

○プリオン病はヒツジにおいて輸血により効率的に伝播する
ウシ海綿状脳症(BSE)のエピデミックに続く変異型クロイツフェルトヤコブ病(vCJD)の出現により、当該疾患の輸血による医原性伝播リスクの可能性が懸念され、血液供給を保護するために費用のかかる制御措置がとられることとなった。以前我々は、BSEおよび自然発生スクレイピーが輸血により伝播することをヒツジにおいて示した予備データを報告した。本稿で報告する当該実験の最終結果は、予想以上に高い輸血伝播率(BSE36%、スクレイピー43%)を示している。輸血によりBSE感染した受血ヒツジの一部(3/8)は、疾患の臨床症状を示すことなく、最高7年間生存した。大多数の伝播は、推定潜伏期の50%を超えたヒツジから採取された血液から生じた。この伝播率の高さ、および臨床症状を示す受血ヒツジの潜伏期が比較的短く一定であることから、血中の感染価が高いこと、および(または)輸血により効率的に伝播することが示される。当該実験により、血液製剤によるヒトでのvCJD伝播の調査に関して、ヒツジの使用が有用なモデルであることが示された。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ヒツジを用いた感染実験において、BSEは36%、スクレイピーは43%と予想以上に高い輸血伝播率を示し、TSEが輸血により効率的に伝播すること、血液製剤によるヒトでのvCJD伝播の調査に関して、ヒツジが有用なモデルであることが示されたとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

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Prion diseases are efficiently transmitted by blood transfusion in sheep

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Prion diseases are efficiently transmitted by blood transfusion in sheep.

Running title: Transmission of sheep TSEs by blood transfusion

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Abstract

The emergence of variant Creutzfeld-Jakob disease (vCJD), following on from the bovine spongiform encephalopathy (BSE) epidemic, led to concerns about the potential risk of iatrogenic transmission of disease by blood transfusion and the introduction of costly control measures to protect blood supplies. We previously reported preliminary data demonstrating the transmission of BSE and natural scrapie by blood transfusion in sheep. The final results of this experiment, reported here, give unexpectedly high transmission rates by transfusion of 36% for BSE and 43% for scrapie. A proportion of BSE-infected tranfusion recipients (3/8) survived for up to 7 years without showing clinical signs of disease. The majority of transmissions resulted from blood collected from donors at >50% of the estimated incubation period. The high transmission rates and relatively short and consistent incubation periods in clinically positive recipients suggest that infectivity titres in blood were substantial and/or that blood transfusion is an efficient method of transmission. This experiment has established the value of using sheep as a model for studying transmission of vCJD by blood products in humans.

Introduction

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases, which include Creutzfeld-Jakob disease (CJD) in man, scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle. A new variant of CJD (termed vCJD) was recognised in the United Kingdom in the mid-1990s, apparently as a result of transmission of BSE to humans[1]. To date, there have been 166 cases of vCJD recorded in the UK, as well as several cases in other countries. Human TSEs are characterised by long asymptomatic incubation periods (usually several years), and there is no reliable test for detecting infection before the onset of clinical disease. It is not known how many people in the UK harbour vCJD, although estimates based on screening of tonsil and appendix samples suggest there could be up to 4000[2]. These infected individuals pose a risk of human-to-human transmission *via* blood transfusion or contaminated surgical instruments.

In patients with vCJD there is widespread replication of the infectious agent and deposition of PrP^{Sc} (disease-associated form of prion protein) in lymphoreticular tissues such as the tonsil, spleen and lymph nodes, in contrast to sCJD, where lymphoreticular involvement is minimal[3]. The fact that lymphocytes continually recirculate between blood and lymphoreticular tissues strongly suggests that the blood of vCJD patients is likely to be infectious. Data from rodent TSE models had shown that the highest levels of infectivity in blood were associated with leukocytes and, to a lesser extent, plasma[4]. As a result, costly control measures such as leucodepletion (filtration of blood and blood products to remove leukocytes) and importation of plasma were introduced to protect UK blood supplies, despite the limited data that were then available to judge the size of the risk and the efficacy of the control measures.

The potential for using sheep as a model for studying the risks of vCJD transmission by blood transfusion was highlighted by the similarity between the distribution of infectivity and PrP^{Sc} in sheep infected with TSEs and humans infected with vCJD[5-7]. One factor limiting the successful transmission of TSEs by blood in rodent models

was the small volumes of blood that could be injected. In contrast, the relative similarity in size of sheep and humans means that volumes of blood comparable to those used in human transfusion practice can be collected from and transfused into sheep. Using this model, we previously reported preliminary results showing that both BSE and natural scrapie could be transmitted between sheep by blood transfusion[8,9]. Although scrapie is not thought to be transmissible to humans, it was included as a representative of infection acquired under field conditions, which may give different results to those obtained from experimentally infected animals. Our blood transfusion experiment in sheep is complete after nine years, and this paper presents the full data from the study. The overall transmission rates for both scrapie and BSE are surprisingly high when factors such as the stage of infection and genetic background are taken into account, suggesting that blood transfusion represents an efficient route of transmission.

Materials and Methods

Donor and recipient sheep

The animal work was reviewed and approved by internal Ethical Review procedures at the Institute for Animal Health, UK, and carried out under the authority of Home Office Project Licences.

PrP genotypes of all sheep were confirmed by sequencing the coding region of the PrP gene[10], and are represented by single letter amino acid code for codons 136, 154 and 171, which have been linked to scrapie susceptibility (e.g. ARQ represents alanine, arginine and glutamine respectively at codons 136, 154 and 171).

All donor sheep were from the Edinburgh NPU Cheviot flock, which has endemic natural scrapie. The recipient sheep (including scrapie negative control donors) were Cheviots derived from the DEFRA scrapie-free (DEFRA/SF) flock of New Zealand origin. Transfusion recipients, positive and negative controls were housed in a purpose-built isolation unit on a different site to the donors, with strict procedures in place to minimise the risk of cross-contamination between groups, as described[9]. The sheep were scored at weekly intervals for clinical signs of TSEs, and killed when they reached humane end points agreed with the Home Office. For experimentally inoculated animals (BSE donors, positive controls and transfusion recipients), the incubation period (IP) in clinically positive sheep was defined as the period between the date of inoculation and the date of death. For scrapie-exposed donors, the IP in clinically positive sheep was defined as the age at death (i.e. they were assumed to have become infected immediately after birth).

Blood collection and transfusion

Procedures for blood collection/transfusion were as previously described[9]. Briefly, venous blood (450-500ml = 1 unit) was collected into sterile collection bags (NBPI-Fresenius, Emmer-Compascuum, NL) containing citrate phosphate dextrose adenine solution as anticoagulant. From donors that were about to be euthanased, 2 units were collected just before post-mortem, while from donors that were to be left alive, separate collections of 1 unit were made at least 28 days apart. However, for practical reasons it was not always possible to collect 2 units of blood from every donor sheep.

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In most cases where 2 units of blood were obtained, one was transfused as whole blood (without leucodepletion) and the other was used to prepare a buffy coat fraction.

BSE blood transfusions

Fifteen sheep experimentally inoculated either orally (14) or intracerebrally (1) with 5g or 0.05g respectively of BSE-infected cattle brain homogenate were used as blood donors. The donor PrP genotypes were ARQ/ARQ (n = 3), ARQ/AHQ (n = 5) or AHQ/AHQ (n = 7), which are resistant to natural scrapie in the NPU flock, but produce the shortest IPs after inoculation with BSE. Two sheep previously reported as donors[9] were excluded from the study (along with their recipients) when re-genotyping showed them to be ARQ/ARR and VRQ/AHQ respectively, genotypes which result in relative resistance to oral infection with BSE.

Eleven donor sheep provided blood for transfusion at the preclinical stage of infection. Eight of these were culled at the time of donation as part of a separate time course pathogenesis experiment. The remaining three pre-clinical donors went on to develop clinical signs of BSE, with respective IPs of 629, 761 and 2131 days post infection. Four sheep were used as blood donors once they had developed clinical signs of BSE at 561-671 days post infection. PrP^{Sc} deposits in brain and/or in peripheral tissues were confirmed in all clinically affected donors by immunohistochemistry (IHC). In two donors culled at the pre-clinical stage, sparse PrP^{Sc} deposits were found in only one tissue in each sheep: Peyer's patch (58x81) and dorsal root ganglion (60x49). However, a negative result was obtained when the same tissues were immunostained in another laboratory. There were 15 ARQ/ARQ recipients of whole blood and 7 ARQ/ARQ recipients of buffy coat from BSE-infected donors. Figure 1 gives a summary of the experimental design, while details of the donor and recipient sheep are in Table 1.

Scrapie blood transfusions

The donors for this experiment were ten VRQ/VRQ and one VRQ/ARQ Cheviot sheep from the Edinburgh NPU flock, where sheep of these genotypes show a disease incidence approaching 100%. Epidemiological and pathological evidence suggests that infection occurs around the time of birth. Blood collections were made from animals in 3 different age groups (200-250 days, 450-500 days, 700-850 days) to represent donors at different pre-clinical stages of disease, as well as from one clinical case. Seven donors were culled after developing clinical signs of scrapie at ages ranging from 1081 to 1556 days, and were confirmed positive by histopathology and IHC. Two donors were culled before the onset of clinical signs at 1197 and 1350 days of age respectively, but PrP^{Sc} was detected in their tissues by IHC. Two donors died prematurely at 349 and 974 days of age: one was IHC negative, in the other, the tissues were too decomposed to allow analysis. There were 21 recipients (all VRQ/VRQ PrP genotype) of blood from scrapie-exposed donors; eleven were transfused with buffy coat and ten with whole blood. See Figure 1 for a summary of the experimental design, and Table 2 for details of donor and recipient sheep.

Positive and negative controls

Seven ARQ/AHQ and three ARQ/ARQ sheep were infected intravenously with 0.2g of the same BSE-infected cattle brain homogenate as given orally to the blood donors, and served as positive controls. No positive controls were used in the scrapie transfusion experiment. As negative controls for the BSE transfusion experiment, 12 ARQ/ARQ recipients were given transfusions of whole blood (6) or buffy coat (6) from 7 uninfected donors (6 ARQ/AHQ, 1 ARQ/ARR). Two recipients died at 633 days and 1181 days post transfusion respectively, and the remaining 10 recipients were culled between 2462 and 2586 days post transfusion. As negative controls for the scrapie experiment, 16 VRQ/VRQ sheep received either whole blood (8) or buffy coat (8) collected from 8 uninfected VRQ/VRQ donors. There were two intercurrent deaths at 397 days and 464 days post transfusion, and the other 14 animals were culled between 2052 and 2409 days post transfusion. None of the negative controls for the BSE or scrapie experiments showed clinical signs of TSEs and all were IHC negative for PrP^{Sc}.

PrP^{Sc} detection by immunohistochemistry (IHC)

Tissue samples from the brain, spleen, mesenteric lymph node and palatine tonsil of the sheep under study were fixed in formaldehyde and processed according to standard procedures. Sections were immunolabelled for PrP^{Sc} detection by IHC with primary antibody R145, which recognizes the 222-226 amino acid sequence of ovine PrP[11], as described previously[12,13].

Results

1) BSE transfusion experiment

A total of five transfusion recipients showed clinical signs of TSEs, and were confirmed positive by IHC and/or Western blot (see Table 1 & Figure 2). These included two (F19 and D505) out of twelve sheep transfused with whole blood from donors in the pre-clinical phase of infection (at 45% and 50% of estimated IP, respectively), as reported previously[8,9]. Two out of three recipients of whole blood and one out of two recipients of buffy coat from donors clinically affected by BSE developed clinical BSE. The IPs in the five clinically positive recipient sheep ranged from 531 to 610 days post transfusion (mean ± SD = 565 ± 35 days), and there was no obvious difference in the IPs of those that received blood from pre-clinical or clinical donors.

One recipient (D452) of whole blood from a pre-clinical donor died of unrelated causes at 1139 days post transfusion, but had PrP^{Sc}-positive IHC labelling in brain and other tissues. One of three recipients of whole blood (G92) and one of two recipients of buffy coat (G61) from clinical donors showed weak PrP^{Sc} deposition in the brain and lymphoid tissues after being culled at 2003 and 2497 days post transfusion respectively, in the absence of clinical signs. Full sequencing of the PrP gene of these sheep revealed that they carried an additional proline (P) to leucine (L) substitution at codon 168[14,15], which appears to be associated with the prolonged survival of these infected sheep. The polymorphism was also identified in two recipients of blood from a pre-clinical BSE-challenged donor, neither of which showed evidence of infection.

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Taking the results for all 22 recipients of blood from BSE-exposed donors, five clinical cases and three sheep showing evidence of infection in the absence of clinical signs were identified, giving an overall transmission rate of 36%.

One recipient was culled for health reasons at 1444 days post transfusion, two were culled with suspected TSE clinical signs at 2480 and 2160 days post transfusion respectively, and the remaining clinically negative sheep were culled between 2239 and 3068 days post transfusion. With one exception, examination of the tissues by IHC did not find evidence of infection. The exception (D337) was culled at 3018 days post transfusion and showed positive PrP^{Sc} labelling in the brain, but with a pattern distinct from that observed in other BSE-infected sheep. The brain PrP^{Sc} distribution involving major white matter tracts and sparing the dorsal motor nucleus of the vagus was similar to that of Nor98 (or "atypical" sheep scrapie) and therefore unlikely to be transfusion-related. No other sheep in the present study showed evidence of being infected with atypical scrapie.

Out of the ten sheep that were infected intravenously with BSE as positive controls, eight developed clinical signs confirmed by IHC, with an average IP of 702 days (± 61 days standard deviation). The remaining two animals were culled at 2591 days post infection and, although not demonstrably clinically affected, IHC showed PrP^{Sc} deposition in the brains and lymphoid tissues of both animals. These two sheep were heterozygous (PL_{168}) for the PrP polymorphism P168L (see above), while the other eight were homozygous (PP_{168}).

The PrP^{Sc} profile obtained by IHC from BSE positive recipients was the same as that found in the orally inoculated donors and in the positive controls[16]. In addition, characteristic BSE glycoform patterns were obtained by Western blot analysis of PrP^{Sc} positive donor and recipient sheep (data not shown; see[9]), and inoculation of brain homogenates from infected donors and recipients into a panel of inbred mouse strains produced IPs and lesion profiles characteristic of BSE (data not shown). Taken together, these results confirm that the strain characteristics were not altered following transmission *via* blood.

2) Scrapie transfusion experiment

Four out of ten recipients of whole blood and four out of ten recipients of buffy coat from donors in the pre-clinical phase of scrapie infection developed clinical signs of scrapie, which were confirmed by positive IHC results. One sheep transfused with buffy coat from the single clinical donor was also clinically affected and IHC positive (see Table 2 & Figure 2). Four of these cases (F144, F153, F141 & F143) were reported previously[9]. There were four intercurrent deaths at 354, 753, 1237 and 1615 days post transfusion respectively, and the eight remaining recipients were culled between 2329 and 2484 days post transfusion. These twelve animals were clinically negative at the time of death, and showed no detectable PrP^{Sc} by IHC. Thus, nine out of 21 recipients of blood from scrapie-exposed sheep developed clinical scrapie, giving an overall transmission rate of 43%.

The majority of confirmed scrapie cases in recipients (n = 7) occurred in the groups that received transfusions from donors in the late pre-clinical (>50% of estimated IP) or clinical phase of infection. Only 2 out of 9 recipients in these groups remained free

of infection. The other two positive recipients were in the group of 6 sheep that received transfusions from donors at 28-37% of estimated IP, and their IPs were much longer than the rest (1101 and 1138 days post transfusion compared to a range of 575-853 days in recipients of blood from donors at >50% of estimated IP). No disease was confirmed in the 6 recipients that received blood from donors at ≤20% of estimated IP.

The PrP^{Sc} profile obtained from brains of donors and recipients highlighted some differences in terms of presence of vascular plaques or glia-associated PrP^{Sc} in donors but not in recipients, or *vice versa* (unpublished data). Such discrepancies were interpreted as presence of more than one natural scrapie strain in the flock of origin.

Discussion

The outcome of the blood transfusion experiments showed that two different TSE agents, scrapie and BSE, could be efficiently transmitted between sheep by blood transfusion, using volumes similar to those employed in human transfusions. The overall transmission rates (percentage of all recipients that became infected) were 36% for BSE and 43% for scrapie. For BSE, the figure was much higher than anticipated because three of the eight BSE-infected recipients survived for long periods without showing clinical signs, whereas all the scrapie-infected recipients identified by IHC were also clinically positive. The greater probability of sub-clinical infection in recipients of blood from BSE-exposed donors is largely due to variability in the genetic susceptibility to infection among sheep used in the BSE experiment, which will be discussed below. The results are consistent with the known facts about transmission of vCJD by blood transfusion in humans[17]. Sixty-six individuals known to have received labile blood products from 18 donors who subsequently developed vCJD were followed up in an on-going study. Three of these recipients have been confirmed clinically and pathologically as vCJD cases, with intervals between transfusion and the development of clinical signs ranging from approximately 6½ years to 8½ years[18-20]. Another individual, who died of unrelated causes 5 years post transfusion, showed PrP^{Sc} deposits in lymphoid tissues but not brain at post mortem, and is thought to represent pre-clinical or sub-clinical infection[21]. These four individuals represent 6% of the total recipients, or 12.5% of recipients surviving longer than 5 years.

Various factors influence the transmission rate by transfusion in both sheep and humans, including: (i) the interval between blood donation and the onset of clinical signs in the donors, (ii) genetic variation in susceptibility of donors and recipients, and (iii) the blood component transfused.

1) Stage of incubation period of the donors at the time of blood donation.

The effect of the stage of incubation can best be deduced from the results of the scrapie transfusion experiment, since the PrP genotype of the sheep used (VRQ/VRQ) renders them almost 100% susceptible to natural and experimental infection[22]. The stage of incubation of the donor has a strong influence on the probability of transmission to the recipient (Figure 2). When donations were made at ≤20% of the estimated IP, there was no disease transmission, while donations made at >50% of the estimated IP produced an 80% transmission rate, with a mean IP of 729 days (SD ±

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99) in the recipients. Blood collected at 28-37% of the estimated IP transmitted infection at a lower rate of approximately 33%, and with longer IPs in the recipients of >1000 days. The data are consistent with a gradual increase in infectivity in the blood, from approximately 30-50% of IP until the clinical phase.

In the BSE transfusion experiment, the correlation between stage of infection and transmission is not clear-cut, but shows the same general trend of increasing probability of transmission to recipients as infection progresses in the donors (Figure 2). Possible explanations for the lower transmission rates from pre-clinical BSE-infected blood donors compared to pre-clinical scrapie-infected donors include:

a) Variation in susceptibility to infection of both donor and recipient sheep. This will be discussed below.
b) Differences in the pathogenesis of natural scrapie and experimental BSE. VRQ/VRQ sheep naturally infected with scrapie have detectable PrP^{Sc} deposits in lymphoid tissues early after infection (i.e. <50% estimated IP)[23,24]. Time course studies of ARQ/ARQ sheep orally infected with BSE showed that PrP^{Sc} was not consistently detected in lymphoid tissues before at least 65% of the average IP[7]. If infectivity in blood correlates with its presence in lymphoid tissues, this could explain the differences observed in the two transfusion experiments.

The probability of transmission from pre-clinical donors is of greatest relevance to the human situation. In the case of the four transfusion-related transmissions of vCJD, the donors developed clinical signs between 17-42 months after donation. The mean IP for vCJD has been estimated to be 16.7 years, with a lower 95% confidence interval of approximately 12.4 years[25]. Therefore, it is likely that the transfusion-related vCJD cases resulted from donations made at least half-way through the IP, which is in agreement with the data from the sheep experiments. In vCJD cases, the timing of detectable lymphoid replication in the pre-clinical stages of disease is unknown; therefore it is not clear whether the peripheral pathogenesis more closely resembles BSE or natural scrapie in sheep.

2) Effect of genetic variation in susceptibility.

A small proportion of sheep with $A_{136}Q_{171}/A_{136}Q_{171}$ PrP genotypes do not succumb to infection following natural or experimental exposure to scrapie and BSE, or have very prolonged incubation periods[26-28]. The reasons for this variability in response are not clearly understood, but it can be predicted to reduce infection rates in both donor and recipient sheep in the BSE transfusion experiment. The majority of pre-clinical donor sheep (8/11) in the BSE transfusion experiment were killed at, or shortly after, the time of donation, and none showed conclusive evidence of infection, although two transmitted infection to their respective transfusion recipients. It is potentially significant that donors that failed to transmit infection were heterozygous at PrP codon 154, while those that did transmit infection were homozygous. Thus, variable susceptibility to infection among the donor sheep may be the result of a protective effect of codon 154 heterozygosity to oral challenge with BSE, although more data are required to confirm this association.

A novel polymorphism, resulting in a proline to leucine substitution at codon 168 of the PrP gene, was identified in four BSE transfusion recipients and two positive

control sheep inoculated intravenously with BSE[14]. All six survived >2000 days without developing clinical signs of BSE, but on post mortem examination four showed PrP^{Sc} deposition in brain and lymphoid tissues. This suggests that the P168L polymorphism can protect against clinical disease, but does not prevent infection by the intravenous route. This polymorphism has not been identified in the Edinburgh NPU Cheviots used as donors in the BSE experiment, nor in sheep with the VRQ/VRQ genotype.

Although the genetic basis of susceptibility to BSE infection in sheep and humans is not directly comparable, the variability in response to BSE found in ARQ/ARQ sheep provides a more realistic reflection of the situation with vCJD in the human population than the very uniform susceptibility of VRQ/VRQ sheep to scrapie infection. In addition, the survival of BSE-infected transfusion recipients for up to 7 years without clinical signs demonstrates that prolonged secondary incubation periods and/or a sub-clinical/"carrier" state are possible following transfusion in sheep. The existence of such sub-clinical or prolonged pre-clinical infection states in humans is recognised as one of the important factors influencing the probability of onward transmission, and thus the potential size of the vCJD epidemic[29]. Susceptibility to human TSEs has been linked to codon 129 of the PrP gene, which can encode either methionine (M) or valine (V). Until recently, all clinical cases of vCJD (including the 3 transfusion-related cases) that have been tested have been homozygous for methionine at 129 (129MM). Interestingly, the "pre-clinical" individual believed to have been infected by transfusion was heterozygous (129MV)[21]. There is accumulating evidence to suggest that all human 129 genotypes may be susceptible to vCJD infection, with apparently greater likelihood of sub-clinical infection in 129MV and 129VV individuals[30-32].

3) Effect of blood component.

The four transfusion-related vCJD infections occurred in individuals who received transfusions of red cells that had not been leucodepleted. Leucodepletion was introduced in the UK in 1999 to control the risk of transmission of vCJD by blood transfusion, because previous studies in rodents had shown that infectivity appeared to be concentrated in the buffy coat, which contains most of the blood leukocytes[4]. Subsequently, leucodepletion of blood from scrapie-infected hamsters was shown to remove up to 72% of infectivity[33,34]. In the sheep experiments, only whole blood and buffy coat were transfused, because we were seeking to establish proof of principle of transmission of TSEs by blood transfusion, and assessing whether infectivity appeared to be concentrated in the buffy coat. The effect of leucodepletion was not investigated, but is being addressed in a follow-up study, along with estimates of the distribution of infectivity among other blood components, including plasma, platelets and red cells.

In our experiments, transmission rates did not appear to be significantly different in recipients receiving whole blood compared to recipients transfused with buffy coat. The number of sheep transfused with buffy coat in the BSE experiment was too small to allow statistical analysis. In the scrapie experiment, five of the positive recipients were transfused with buffy coat, and four with whole blood. The similarity in transmission rates for both components suggests that they contain approximately equivalent amounts of infectivity.

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We have shown that, for sheep infected with scrapie and BSE, high transmission rates can be achieved using blood transfusion, particularly when donors are at >50% of incubation period. The results also revealed the possibility of prolonged incubation periods and/or sub-clinical infections in some recipients of BSE-infected blood, which is at least partly due to genetic variation in the sheep PrP gene. The suggestion of relatively high titres of infectivity in blood is perhaps surprising in view of the need for ultra-sensitive methods of detection for PrP^{Sc} in blood[35,36]. It may be that, in blood, infectivity is not closely correlated with levels of protease-resistant PrP, but comparative titrations of brain and blood-borne infectivity in sheep will be required to further define the relationship. The results of our sheep transfusion experiments are consistent with what is known about transfusion-associated vCJD transmission in man, and support the use of sheep as an experimental model in which to study the risks associated with different blood products, the effectiveness of control measures and the development of diagnostic and screening tests.

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Author contributions

F.H. designed the study, performed transfusions and post-mortems on recipient sheep, analyzed data and wrote the paper. A.C. and S.McC. performed Western blots, and S.McC. reviewed the report. J.F. coordinated collection of blood and post-mortems on donor sheep. W.G. analyzed and interpreted PrP genotype data and reviewed the report. S.S. and L.G. examined tissues, interpreted IHC results, analyzed data and reviewed the report. M.J. contibuted to the interpretation of IHC results and reviewed the report. N.H. designed the study, analyzed data, and reviewed the report.

The authors have no financial conflicts of interest to declare.

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Figure 1. Overview of experimental design.

Figure 2. Outcome of transfusions as a function of the stage of disease incubation in the donor. A. BSE-infected donors. B. Scrapie-infected donors. For each stage of infection in the donor sheep, the number of uninfected (open bars), clinically positive/ IHC positive (solid bars) and clinically negative/IHC positive (cross-hatched bars) recipients are shown.

Table 1. Outcome of transfusions from BSE-exposed donor sheep.

Donor sheep details							Recipient sheep details				
Donor sheep ID	Donor genotype	Clinical status at donation	% actual or average incubation period at donation[a]	Clinical outcome	IHC result	Incubation period (days)	Component transfused	Recipient sheep ID	Recipient PrP 168 codon genotype	Clinical outcome	IHC result	Incubation period (days)
58x51	ARQ/ARQ	Preclinical	12	+	+	2131	WB	D529	PP	+[c]	-	-
60x49	ARQ/ARQ	Preclinical	22	-	+/- (DRG)[b]	-	WB	D433	PL	-	-	-
			44				WB	F14	PL	-	-	-
J2747	ARQ/AHQ	Preclinical	42	-	-	-	BC	F182	PP	-	-	-
			44				WB	F181	PP	-	-	-
61x24	ARQ/AHQ	Preclinical	42	-	-	-	BC	F238	PP	+[c]	-	-
		Preclinical	43				WB	F234	PP	-	-	-
J2746	AHQ/AHQ	Preclinical	45	-	-	-	WB	F19	PP	+	+	536
J2559	AHQ/AHQ	Preclinical	51	+	+	629	WB	D505	PP	+	+	610
58x81	ARQ/AHQ	Preclinical	61	-	+/- (IPP)[b]	-	BC	D358	PP	-	-	-
58x28	ARQ/AHQ	Preclinical	61	-	-	-	WB	D421	PP	-	-	-
			61				BC	D384	PP	-	-	-
58x27	AHQ/AHQ	Preclinical	61	-	-	-	WB	D452	PP	-	+[d]	-
			61				BC	D318	PP	-	-	-
58x39	ARQ/AHQ	Preclinical	62	-	-	-	WB	D337	PP	-	+[c]	-
			62				WB	D386	PP	-	-	-
J2499	AHQ/AHQ	Preclinical	86	+	+	761	WB	D341	PP	-	-	-
J2771	AHQ/AHQ	Clinical	100	+	+	561	BC	G61	PL	-	+	-
J2770	AHQ/AHQ	Clinical	100	+	+	589	WB	G74	PP	+	+	594
60x69	AHQ/AHQ	Clinical	100	+	+	660	WB	G78	PP	+	+	556
							BC	G49	PP	+	+	531
D383	ARQ/ARQ	Clinical	100	+	+	671	WB	G92	PL	-	+	-

Key: WB = whole blood, BC = buffy coat, DRG = dorsal root ganglion, IPP = ileal Peyer's patch

[a] Calculated from the days post-infection at the time of donation, as a percentage either of the final incubation period (in sheep kept alive until the development of clinical signs), or of the average incubation period in orally-infected donors (640 days), excluding the out-lying incubation period of 2131 days for 58x51.

[b] These tissues were initially scored weakly positive by IHC, but the results were not reproducible in two laboratories and can therefore be considered as inconclusive.

[c] No evidence of infection was found on post mortem examination of tissues from these clinical suspects, therefore it is most likely they were clinically misdiagnosed.

[d] This sheep died of unrelated causes (i.e. without showing clinical signs of BSE) at 1139 days post transfusion, but was positive by IHC.

[a] This apparently healthy sheep was culled 3018 days post transfusion and found to be positive by IHC; however further analysis suggested this was a case of "atypical" scrapie, and therefore unlikely to be transfusion related (see text for details).

Table 2: Outcome of transfusions from scrapie-exposed donor sheep

Donor sheep details								Recipient sheep details			
Donor sheep ID	Donor genotype	Clinical status at donation	% actual or average incubation period at donation[a]	Clinical outcome	IHC result	Incubation period (days)	Component transfused	Recipient sheep ID	Clinical outcome	IHC result	Incubation period (days)
67x42	VRQ/VRQ	Preclinical	17	+	+	1274	BC	G247	-	-	-
			19				WB	G230	-	-	-
66x45	VRQ/VRQ	Preclinical	17	-	-	-	WB	G267	-	-	-
			19				BC	G265	-	-	-
67x23	VRQ/VRQ	Preclinical	18	+	+	1207	BC	G241	-	-	-
			20				WB	G228	-	-	-
65x13	VRQ/VRQ	Preclinical	28	+	+	1556	WB	F275	-	-	-
			30				BC	F273	-	-	-
65x02	VRQ/VRQ	Preclinical	34	-	+	-	WB	F310	-	-	-
			37				BC	F309	+	+	1101
65x03	VRQ/VRQ	Preclinical	34	-	+	-	WB	F277	+	+	1138
			37				BC	F276	+[b]	-	-
61x75	VRQ/ARQ	Preclinical	53	+	+	1324	BC	F149	+	+	782
			57				WB	F144	+	+	672
61x68	VRQ/VRQ	Preclinical	64	+	+	1113	BC	F152	+	+	853
			69				WB	F153	+	+	660
61x66	VRQ/VRQ	Preclinical	62	-	ND	-	WB	F286	-	-	-
			64				BC	F284	-	-	-
59x27	VRQ/VRQ	Preclinical	73	+	+	1137	BC	F126	+	+	826
			77				WB	F141	+	+	575
59x28	VRQ/VRQ	Clinical	100	+	+	1081	BC	F143	+	+	737

[a] Calculated from the age at the time of donation, as a percentage either of the final incubation period (for sheep that survived until the development of clinical signs), or of the average incubation period (1296 days) for sheep that died or were culled before developing clinical signs.
[b] No evidence of infection was found on post mortem examination of tissues from this clinical suspect; therefore it is most likely it was clinically misdiagnosed.