seen in vCJD (PrPSc type 4). However, this could be distinguished from the typical vCJD pattern by an altered protease cleavage site in the presence of the metal ion chelator EDTA.

Conclusions
Further studies will be required to characterize the prion strain seen in this patient and to investigate its etiologic relationship with bovine spongiform encephalopathy. This case illustrates the importance of molecular analysis of prion disease, including the use of EDTA to investigate the metal dependence of protease cleavage patterns of PrPSc.

--
Communicated by:
ProMED-mail <promed@promedmail.org>

******
[The following 3 reports (3, 4, & 5), appearing during the past month (December 2007) describe new techniques for the in vitro assay of prions that promise to accelerate their characterization and epidemiology. - Mod.CP]

[3] vCJD in vitro assays
Date 11 Dec 2007
Source: PNAS, 26 Dec 2007, vol. 104, no. 52, 20908-20913 [edited]
<http://www.pnas.org/cgi/content/abstract/104/52/20908?etoc>

Prion strain discrimination in cell culture: The cell panel assay
---
By Sukhvir P. Mahal*, Christopher A. Baker*, Cheryl A. Demczyk*, Emery W. Smith*, Christian Julius, and Charles Weissmann. At the Department of Infectology, Scripps Florida, 5353 Parkside Drive, Jupiter, FL 33458; and Institute of Neuropathology, University Hospital of Zurich, Schmelzbergstrasse 12, CH-8091 Zurich, Switzerland.

Abstract:
Prions are thought to consist mainly or entirely of misfolded PrP, a constitutively expressed host protein. Prions associated with the same PrP sequence may occur in the form of different strains; the strain phenotype is believed to be encoded by the conformation of the PrP. Some cell lines can be persistently infected by prions and, interestingly, show preference for certain strains. We report that a cloned murine neuroblastoma cell population, N2a-PK1, is highly heterogeneous in regard to its susceptibility to RML and 22L prions. Remarkably, sibling subclones may show very different relative susceptibilities to the 2 strains, indicating that the responses can vary independently. We have assembled 4 cell lines, N2a-PK1, N2a-R33, LD9 and CAD5, which show widely different responses to prion strains RML, 22L, 301C, and Me7, into a panel that allows their discrimination in vitro within 2 weeks using the standard scrapie cell assay (SSCA).

--
Communicated by:
ProMED-mail <promed@promedmail.org>

******
[4] vCJD in vitro assays
Date: 20 Dec 2007
Source: Proc. Natl. Acad. Sci. USA, 10.1073/pnas.0710152105 [edited]
<http://www.pnas.org/cgi/content/abstract/0710152105v1?etoc>

Prion detection by an amyloid seeding assay

By David W. Colby, Qiang Zhang, Shuyi Wang, Darlene Groth, Giuseppe Legname, Detlev Riesner, and Stanley B. Prusiner. At the Institute for Neurodegenerative Diseases and Departments of Neurology and Biochemistry and Biophysics, University of California, San Francisco, CA 94143; and the Institut fur Physikalische Biologie, Heinrich-Heine Universitat, 40225 Dusseldorf, Germany.

Abstract:

CJD (new var.) update 2007 (03) 20070205.0455
CJD (new var.) update 2007 (02): South Korea, susp 20070115.0199
2006

CJD (new var.), blood transfusion risk 20061208.3468
CJD, transmission risk - Canada (ON) 20061207.3457
CJD (new var.) update 2006 (12) 20061205.3431
CJD (new var.) update 2006 (11) 20061106.3190
CJD (new var.) update 2006 (10) 20061002.2820
CJD (new var.) - Netherlands: 2nd case 20060623.1741
CJD (new var.) - UK: 3rd transfusion-related case 20060209.0432
CJD (new var.) update 2006 (02) 20060206.0386
CJD (new var.) update 2006 20060111.0101
2005

CJD (new var.) update 2005 (12) 20051209.3547
CJD (new var.) update 2005 (11) 20051108.3270
CJD (new var.) update 2005 (10) 20051006.2916
CJD (new var.) update 2005 (02) 20050211.0467
CJD (new var.) - UK: update 2005 (01) 20050111.0095
2004

CJD, genetic susceptibility 20041112.3064
CJD (new var.) - UK: update 2004 (14) 20041206.3242
CJD (new var.) - UK: update 2004 (10) 20040909.2518
CJD (new var.) - UK: update 2004 (02) 20040202.0400
CJD (new var.) - UK: update 2004 (01) 20040106.0064
CJD (new var.) - France: 8th case 20041022.2864
CJD (new var.) - France: 9th case 20041123.3138
CJD (new var.), blood supply - UK 20040318.0758
CJD (new var.), carrier frequency study - UK 20040521.1365
2003

CJD (new var.) - UK: update 2003 (13) 20031216.3072
CJD (new var.) - UK: update 2003 (01) 20030108.0057
2002

CJD (new var.) - UK: update Dec 2002 20021207.5997
CJD (new var.) - UK: update Jan 2002 20020111.3223
2001

CJD (new var.), incidence & trends - UK (02) 20011124.2875
CJD (new var.), incidence & trends - UK 20011115.2816
CJD (new var.) - UK: reassessment 20011029.2671
CJD (new var.) - UK: update Oct 2001 20011005.2419
CJD (new var.) - UK: regional variation (02) 20010907.2145
CJD (new var.) - UK: update Sep 2001 20010906.2134
CJD (new var.) - UK: update Aug 2001 20010808.1872
CJD (new var.) - UK: 9th Annual Report 20010628.1231
CJD (new var.) - UK: update June 2001 20010622.1188
CJD (new var.) - UK: update 3 Jan 2001 20010104.0025]
.....cp/msp/jw
##
****

****

****

##

別紙様式第 2-1
番号 10

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
				2008年3月24日	該当なし	

一般的名称	乾燥濃縮人アンチトロンビンIII	研究報告の公表状況	TRANSFUSION 2008; 48: 609-619	公表国 フランス
販売名（企業名）	①ノイアート（ベネシス） ②ノイアート静注用1500単位（ベネシス）			

研究報告の概要

＜背景＞輸血によるTSE感染のリスクはプリオンたん白の病理学的イソフォーム（PrP^{sc}）の血中濃度に依存するが、細胞PrP（PrP^{c}）の血中濃度によっても影響を受ける可能性がある。これらの濃度はPrPの血液クリアランスによって制御されるが、これについてはこれまで評価されたことはなかった。
＜研究デザイン及び方法＞ヒツジの精製された原核生物の組み換えPrP（rPrP）の血液(実際は血漿)クリアランスは、遺伝子型の異なるヒツジおよび腎摘出されたヒツジを用いて測定した。スクレイピー関連のフィブリルの静注後のプロテイナーゼK抵抗性PrP断片(PrP^{res})への曝露についても、ヒツジで調査した。
＜結果＞rPrPのARR変異型は、VRQ変異型よりもより早く除去された。感受性の高いホモ接合体のVRQヒツジのPrP^{c}血漿濃度は、ホモ接合体のARR抵抗性ヒツジのそれよりも大きく、PrP^{c}のARR変異型のクリアランスがVRQ変異型のそれよりも大きいことを示唆している。rPrPの血漿クリアランスは、両方の腎臓摘出後は52%減少し、このことはrPrP除去において腎臓が重要な寄与をしていることを示している。PrP^{res}は、スクレイピー関連断片の静注後はゆっくりと除去されることが判明した。
＜結論＞PrP宿主の遺伝型及び生理病理学的要因は、血液のPrPクリアランスを調節することでTSE感染リスクに影響する。このリスクは、静注後にPrP^{res}へ曝露が続くことによって増大する。投与された物質は実際の種と異なるが、これらはPrPクリアランスのメカニズムを調査するためのプローブとして重要である。

報告企業の意見	今後の対応
ヒツジに静注したプリオンたん白の血液クリアランスは、宿主の遺伝子的及び生理病理学的要素に影響を受けるとの報告である。 これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてノイアート（献血）の記載を示す。
2. 重要な基本的注意
(1) 略
1) 略
2) 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

37

Blood clearance of the prion protein introduced by intravenous route in sheep is influenced by host genetic and physiopathologic factors

Véronique Gayrard, Nicole Picard-Hagen, Catherine Viguié, Elisabeth Jeunesse, Guillaume Tabouret, Human Rezaei, and Pierre-Louis Toutain

BACKGROUND: The risk of transmissible spongiform encephalopathy (TSE) transmission by blood transfusion is dependent on the blood concentrations of the pathologic isoform of prion protein (PrPsc) but may also be influenced by blood concentrations of cellular PrP (PrPc). These concentrations are controlled by the blood clearance of PrP, which has never been evaluated.

STUDY DESIGN AND METHODS: The blood (actually plasma) clearance of ovine purified prokaryote recombinant PrP (rPrP) was measured in genotyped and in nephrectomized sheep. The exposure to proteinase K-resistant fragments of PrP (PrPres) after intravenous (IV) administration of scrapie-associated fibrils (SAFs) was also investigated in a sheep.

RESULTS: The ARR variant of rPrP was eliminated more rapidly than its VRQ counterpart. The PrPc plasma concentrations in homozygous highly susceptible VRQ sheep were greater than in homozygous ARR-resistant sheep, suggesting that clearance of the ARR variant of PrPc was higher than that of the VRQ variant. The plasma clearance of rPrP was decreased by 52 percent after a bilateral nephrectomy indicating the significant contribution of the kidneys in eliminating rPrP. PrPres was shown to be slowly eliminated after IV administration of scrapie-associated fibrils.

CONCLUSION: PrP host genotype and physiopathologic factors could influence the risk of TSE transmission by modulating blood PrP clearance. This risk was increased by the sustained exposure to PrPres after IV administration. It should be noted that although the materials that have been administered (rPrP and SAFs) were not the actual species of interest, they can be of value as probes for investigating PrP clearance mechanisms.

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases that occur in humans (e.g., Creutzfeldt-Jakob disease [CJD]) and animals including sheep (scrapie) and cattle (bovine spongiform encephalopathy [BSE]). These diseases are characterized by the cerebral deposition of the pathologic isoform (PrPsc) of a host-encoded cellular prion protein (PrPc) that is highly expressed in the brain.

Attempts to detect infectivity in the blood of animals naturally affected with TSE have often been inconclusive.[1,2] PrPsc or infectivity, however, has been evidenced in blood from intracerebrally inoculated rodents[3] and in

ABBREVIATIONS: MRT = mean residence time(s); Plgn = plasminogen; PrP = prion protein; PrPc = cellular PrP; PrPres = proteinase K-resistant fragment of PrP; PrPsc = pathologic isoform of PrP; rPrP = recombinant PrP; SAF(s) = scrapie-affected fibril(s); TSE(s) = transmissible spongiform encephalopathy(-ies).

From the Ecole Nationale Vétérinaire de Toulouse, UMR 181 Physiopathologie et Toxicologie Expérimentales, INRA, ENVT, UMR1225 Interactions Hôtes-Agents Pathogènes, INRA, ENVT, Toulouse; and Institut National de la Recherche Agronomique, UR892 Virologie et Immunologie Moléculaires, Jouy-en-Josas, France.

Address reprint requests to: Pierre-Louis Toutain, UMR 181 Physiopathologie et Toxicologie Expérimentales, INRA, ENVT, Ecole Nationale Vétérinaire de Toulouse, 23 chemin des Capelles, 31076 Toulouse, France; e-mail: pl.toutain@envt.fr.

This study was supported by grants from the French National Institute for Agronomical Research (INRA), from GIS prion, and DGER. The authors declare that there is no conflict of interest relative to the major sources of funding that would prejudice their impartiality relative to the results of the study.

Received for publication April 17, 2007; revision received August 2, 2007, and accepted October 20, 2007.

doi: 10.1111/j.1537-2995.2007.01628.x

TRANSFUSION 2008;48:609-619.

blood of sheep naturally[4] or experimentally infected with scrapie[5] or BSE.[4,6]

Evidence that variant CJD (vCJD) is transmitted by transfusion has accumulated, initially from animal models, including primates, in which the high efficiency of the intravenous (IV) route of infection for BSE transmission has been demonstrated.[7] There is no longer any doubt concerning the risk of blood products obtained from individuals during the presymptomatic phase of vCJD. A third case has been reported of autopsy-confirmed vCJD infection (and a second case of clinical vCJD) from a cohort of recipients who had received transfused blood products from individuals subsequently diagnosed with vCJD.[8] More recently, a fourth symptomatic case of vCJD infection has been identified in a patient who received a transfusion from the same donor as the third case.[9] This fourth incidence considerably strengthens the evidence for prion transmission by transfusion.[10]

The pathogenesis of TSE disease highlights the importance of the PrPc as a substrate for PrPsc replication in tissue. Hence, the risk of TSE transmission by blood transfusion could be affected by the level of blood PrPc. This is controlled by its own blood clearance and modified by factors affecting this variable. The transfusion risk will also depend on the ability of the body to clear the abnormal prion protein (PrPsc), that is, the plasma (blood) clearance. This clearance variable, essential to the estimation of systemic exposure, has never been evaluated.

The aim of this study was to document the exposure of sheep to the recombinant prion protein (rPrP) after IV administration and to identify genetic and pathophysiologic factors that might modulate such exposure. Sheep were chosen because of the similarity of the pathogenesis of scrapie with vCJD and because the impact of Prnp gene polymorphism on the susceptibility to scrapie infection is well documented. The $V_{136}R_{154}Q_{171}$ (VRQ) and ARR alleles have consistently been associated with high susceptibility and natural resistance to the clinical disease, respectively,[11-13] although atypical scrapie strain(s) can naturally infect sheep harboring the so-called resistant PrP genotype.[14] The ARR and VRQ genetic variants of the purified prokaryote rPrP were used as probes and only as probes (see Discussion) to test the hypothesis that the higher intrinsic stability of the VRQ variant relative to its ARR counterpart[15,16] could result in a lower in vivo clearance rate of the VRQ protein.

This approach also allowed us to examine the hypothesis that the subject's genetic background could directly influence PrP clearance and to evaluate the contribution of the kidneys to the overall clearance of plasma PrP, the kidney being a major organ for protein clearance.[17] The fate of ovine proteinase K-resistant fragment of PrP (PrPres) during the first hours after its direct entry in the blood was also evaluated in one sheep to assess the ability of the body to clear the scrapie agent.

MATERIALS AND METHODS

General

All experimental procedures were performed in accordance with French legal requirements regarding the protection of laboratory animals and under authorization number 31242 from the French Ministry of Agriculture.

Design

The objectives of Experiment 1 were 1) to compare the pharmacokinetics of the VRQ and ARR genetic variants of the purified prokaryote ovine rPrP; 2) to examine the influence of the genotype of the test animal on plasma pharmacokinetic variables of rPrP; and 3) to test the hypothesis of a first pass effect at the level of the brain, that is, direct trapping of the protein during its initial transit across the head.

The experiment was performed on eight healthy Romanov ewes: four homozygous VRQ at codons 136, 154, and 171 of the Prnp gene and four homozygous ARR. The VRQ ewes were 1 year old and weighed 42.1 ± 2.7 kg and the ARR ewes were 2 years old and weighed 51.2 ± 5.5 kg. The experiment involved two periods separated by 2 days. During the two periods, the ewes received an IV administration of rPrP (VRQ vs. ARR, 0.02 mg/kg) according to a crossover design. Ten days later, one ARR and two VRQ ewes received an intraarterial (external carotid) administration of the VRQ variant (0.02 mg/kg) to compare the pharmacokinetic variables of rPrP after arterial and IV administrations.

The ex vivo stability of the variants of rPrP in blood and plasma was compared by separately adding 145 ng of each of the ARR and VRQ variant of rPrP to 10-mL aliquots of fresh sheep blood and plasma that were incubated at 37°C under constant stirring. Samples were taken at 0.5, 1, 2, 3, 4, 5, 6, and 24 hours. The plasma was immediately separated from blood after centrifugation for 10 minutes at 1400 × *g* and all the plasma samples were stored at –20°C until PrP assay.

Experiment 2 was designed to evaluate the role of the kidneys in the clearance of rPrP and to examine the fate of PrPres after its direct entry into the blood. The first part of the experiment was performed with three Lacaune ewes aged from 5 to 8 years and weighing 38 to 55 kg. The pharmacokinetic variables of the VRQ variant of the rPrP intravenously administered (0.02 mg/kg) were determined before (control period) and immediately after a bilateral nephrectomy (experimental period). During the control period, one ewe was anesthetized according to the same protocol as that used for surgery. During the experimental period, which took place 1 to 14 days later, anesthesia was induced with sodium thiopental (Nesdonal, Merial, Lyon, France; 20 mg/kg) and maintained for 24 hours by repeated administrations of 0.2 to 0.3 mg per kg sodium

thiopental at 20- to 30-minute intervals. The ewes were bilaterally nephrectomized according to the method previously described.[18] Immediately after surgery, three control blood samples were obtained at 20-minute intervals and the VRQ variant was intravenously administered. The ewes were sacrificed in extremis. The second part of the experiment was performed with one young Lacaune ewe aged 6 months and weighing 30.5 kg that received an IV administration of 125 mL of scrapie-affected fibrils (SAFs) in 0.8 mol per L urea. The quantity of PrPres administered was evaluated at 75 µg equivalent of VRQ rPrP.

Ten-milliliter blood samples were collected at 20-minute intervals for 1 hour before administration; at 1, 2, 4, 8, 15, 30, 45, 60, 90, and 120 minutes after the variant administration; at 1-hour intervals for 12 hours; and finally at 24, 36, and 48 hours after administration, for Experiment 1 and the first part of Experiment 2, respectively. The same protocol was used for the second part of Experiment 2 except that 15-mL blood samples were also obtained every day until Day 7 after SAF administration.

For all experiments, in intact ewes, all the urine in the bladder was removed before the administrations and then at 1-hour intervals for 12 hours and at 3-hour intervals during the following 12 hours. The total volume of urine removed was measured in each case.

Brain extraction

Brain samples were obtained from three Romanov homozygous VRQ ewes naturally affected with scrapie. The frozen brain samples were homogenized to give a 20 percent (wt/vol) suspension in buffer (Bio-Rad, Marnes la Coquette, France) and PrPres was extracted with the purification protocol of the Bio-Rad TSE test (TeSeE sheep/goat purification kit, Bio-Rad) except that the final precipitate was solubilized with 4 mol per L urea (Sigma-Aldrich, Lisle d'Abeau Chesnes, France) and stored at –20°C until administration. The extract was diluted five-fold in 0.1 mol per L phosphate buffer containing 1 mg per mL bovine serum albumin (BSA) and 0.15 mol per L NaCl (Sigma-Aldrich), heated at 100°C for 5 minutes, and sonicated for 15 minutes at 560-W power setting (Transsonic 95HL, Prolabo, Fontenay sous Bois, France) in the hour preceding the administration. The PrPres content of the solution was measured by enzyme-linked immunosorbent assay (ELISA). Seventy-five micrograms of PrPres (equivalent to VRQ rPrP) was obtained from 100 g of brain tissue.

Administration and sampling

The IV administrations were performed in the right jugular vein via an indwelling catheter (Hemocath, Vygon, Ecouen, France). The intraarterial administrations were performed in the right external carotid artery via an intraarterial catheter (BD Careflow, Becton Dickinson, Le Pont-de-Claix, France) inserted in anesthetized ewes 2 days before the administrations. The VRQ and ARR variants of the ovine PrP were expressed in *Escherichia coli* and purified according to the method previously described.[15] Previous authors have shown that the recombinant proteins are monomeric in solution. The variants were kept at 4°C in solution in 20 mmol/L MOPS (Sigma-Aldrich), pH 7.25, at a concentration of approximately 1 mg per mL. The protein concentration was measured from the optical density at 280 nm with the extinction coefficient of 58718.0 mol per L per cm. ARR and VRQ solutions of rPrP were prepared in sheep plasma at a concentration of 0.2 mg per mL and kept at –20°C for all administrations.

Blood samples were collected from the left jugular vein via an indwelling catheter into ethylenediaminetetraacetate-containing tubes and centrifuged for 10 minutes at 1400 × *g*. The plasma was separated and stored at –20°C until assay. Urine was obtained via an indwelling closed urethral catheter (Rüsch, Teleflex Medical, Le Faget, France) that was kept in the bladder for 24 hours. Samples were stored at –20°C until analysis.

Quantification of PrP with a two-site enzyme immunoassay

Two immunometric assays were adapted from the method previously described.[19] The plasma and urine concentrations of purified prokaryote rPrP in all samples were measured by ELISA with BAR210, an anti-N terminal monoclonal antibody (MoAb)[20] recognizing residues 26 to 34 and the 12F10-AchE Spi Bio (Massy, France) antibody, an anti-C terminal MoAb recognizing residues between amino acids 154 and 171. Native plasma PrP, which lacks the BAR210 epitopes, was monitored by ELISA in plasma samples collected before administration of the rPrP, with the SAF34 and 12F10-AchE Spi Bio antibodies. Standard curves ranging from 0.5 to 20 ng per mL were established by diluting rPrP (VRQ and ARR) in ovine plasma, urine, or enzyme immunoassay (EIA) buffer (0.1 mol/L phosphate buffer, pH 7.4, 0.15 mol/L NaCl, 0.1% BSA, NaN_3 0.01%; Sigma-Aldrich).

Plasma (or urine) rPrP concentrations were calculated from the optical readings obtained by reference to the standard curve established with plasma (or urine) supplemented with the corresponding variant. Native PrP plasma concentrations were calculated from the optical readings obtained by reference to the standard curve established with EIA buffer solutions of the variants of rPrP. As an illustration, standard curves of ovine rPrP in EIA buffer or in plasma are shown in Fig. 1.

The limit of quantification of native and rPrP assays was 0.5 ng per mL. The accuracies of the PrP assay of plasma samples supplemented with the solution of VRQ

appropriate volume to consider when determining the amount of PrP in the body at equilibrium, was obtained from

$$V_{SS} = V_C[1+(k_{12}/k_{21})],$$

where V_C is the volume of the central compartment, k_{12} is the first-order rate constant of transfer from the central compartment to the peripheral compartment, and k_{21} is the first-order rate constant of transfer from the peripheral compartment to the central compartment. The total plasma clearance, which expresses the capacity of the organism to eliminate proteins (Cl_{TOT}, mL/(kg·min)), was calculated with

$$Cl_{TOT} = \text{dose}/AUC,$$

where AUC is the area under the plasma PrP concentration–time curve obtained by integrating the equation

$$AUC = A/\alpha + B/\beta.$$

The clearance of distribution was calculated with

$$Cl_D = k_{12}V_C.$$

The appropriate volume to consider when calculating the amount of PrP remaining when the pseudodistribution equilibrium has been reached, V_{area} (mL/kg) was obtained from

$$V_{area} = Cl_{TOT}/\beta,$$

where β is the slope of the terminal phase. The terminal plasma half-life ($t_{1/2}$, min) was obtained from

$$t_{1/2} = \text{Log}2/\beta.$$

Different mean residence times (MRTs) were calculated.[24] The MRT (min), that is, the mean total time taken for each PrP molecule to transit through the body, was calculated with

$$MRT = (A/\alpha^2 + B/\beta^2)/(A/\alpha + B/\beta).$$

The MRT in the central compartment (MRT_C), that is, the mean time spent by the protein within the measured compartment was obtained from

$$MRT_C = 1/k_{10},$$

where k_{10} is the first-order rate constant of elimination from the central compartment. The MRT in the peripheral compartment (MRT_T), that is, the mean time spent by the protein outside the measured compartment, was obtained from

$$MRT_P = MRT - MRT_C.$$

Statistical analysis

Results are reported as mean ± standard deviation (SD). Statistical analyses were performed with computer software (SYSTAT 8.0, SPSS, Inc., Chicago, IL). PrP concentrations below the limit of quantification of the assay were arbitrarily fixed at 0.5 ng per mL. p Values lower than 0.05 were considered as significant. The pharmacokinetic variables of PrP genetic variants were compared between homozygous ARR and VRQ ewes with repeated-measures analysis of variance (ANOVA) with ewes as a random effect factor and the genetic variant of PrP, the ewe genotype, and their interactions as fixed effect factors. The genotype effect was tested with "ewe within-group variance" as the residual term. The mean pharmacokinetic variables of the VRQ variant of rPrP obtained after IV and intraarterial administration were compared by a paired t test. The effect of genotype on basal plasma PrPc concentrations was analyzed with repeated-measures ANOVA with ewes as a random effect factor, and genotype and time as fixed effect factors. The mean pharmacokinetic variables of the rPrP obtained before and after nephrectomy were compared by a paired t test. The effect of time on plasma PrP concentrations in vitro was analyzed by ANOVA with medium (blood vs. plasma), genetic variant, and time as fixed effect factors.

RESULTS

Pharmacokinetic variables of the prokaryote rPrP

Figure 2 shows, in a representative ewe, the semilogarithmic plots of the ARR and VRQ variants of the purified prokaryote rPrP after IV administration (0.02 mg/kg).

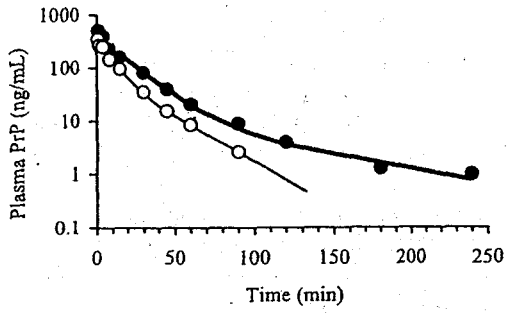

Fig. 2. Representative plasma concentrations of the genetic variants of PrP after IV administration. The observed ARR (○), VRQ (●), and corresponding fitted ARR (thin line) and VRQ (thick line) plasma concentrations were obtained in a representative homozygous VRQ sheep that received IV administrations of the ARR and VRQ genetic variants of the purified ovine prokaryote rPrP at a dose of 0.02 mg per kg.

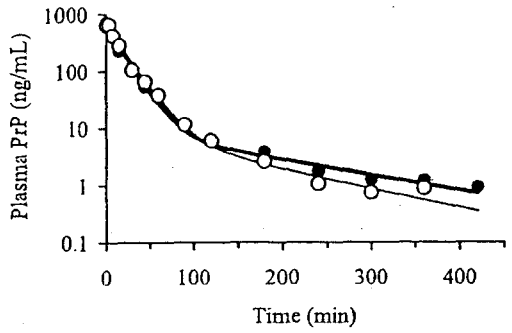

Fig. 4. Representative plasma concentrations of PrP according to its route of administration. The observed (●) and corresponding fitted (thick line) plasma concentrations of the VRQ variant of the purified ovine prokaryote rPrP were obtained after IV administration of the variant (jugular vein) at 0.02 mg per kg and the observed (○) and corresponding fitted (thin line) plasma concentrations of the VRQ variant were obtained after its administration at the same dose by the intraarterial route (external carotid) in a representative ARR sheep.

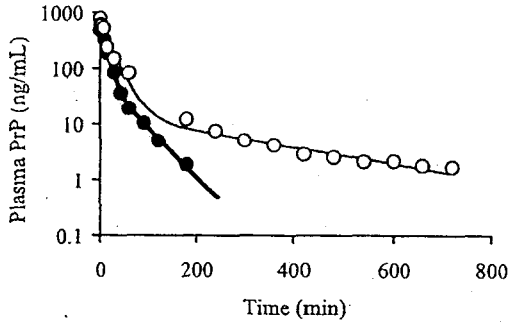

Fig. 5. Representative plasma concentrations of PrP after its administration before and after nephrectomy. The symbols represent the observed plasma concentrations of VRQ variant of the purified ovine rPrP obtained before (●) and after (○) a bilateral nephrectomy in a representative sheep intravenously administered with the VRQ variant at 0.02 mg per kg. The lines represent the corresponding fitted plasma concentrations of the VRQ variant obtained before (thick line) and after (thin line) nephrectomy.

TABLE 2. Pharmacokinetic variables (mean ± SD) of the VRQ variant of the purified prokaryote rPrP according to the route of administration*

Variables	Route of administration	
	IV	Intraarterial
Cl_{TOT}, mL/(kg·min)	1.98 ± 0.41	2.41 ± 0.90
$t_{1/2}$, min	85.8 ± 28.3	68.4 ± 18.8
V_C, min	40.6 ± 9.10	47.8 ± 12.2
V_{SS}, mL/kg	74.0 ± 26.7	72.4 ± 26.3
V_{area}, mL/kg	233.2 ± 30.1	227.6 ± 359.3
MRT, min	37.9 ± 12.9	30.8 ± 8.70
MRT_C, min	20.5 ± 1.48	19.5 ± 2.50
MRT_P, min	17.4 ± 11.5	11.3 ± 7.10

* The pharmacokinetic variables were obtained after an IV (jugular vein) or an intraarterial (external carotid) administration of the VRQ variant at a dose of 0.02 mg per kg to three sheep. Abbreviations are explained in Table 1.

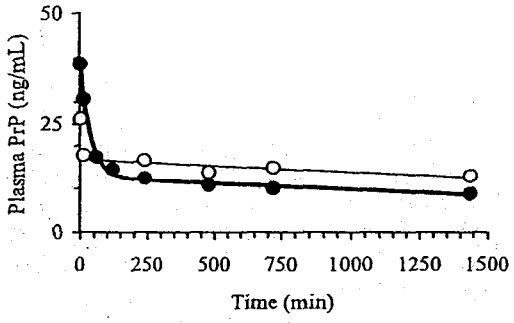

Fig. 6. Plasma PrPres concentrations after administration of SAF solution. The symbols represent the observed plasma concentrations of PrPres obtained with the PIgn-coated beads extraction method (○) or after a 60°C heat treatment (●) in a sheep intravenously administered an SAF solution containing a 75-µg equivalent of VRQ prokaryote rPrP. The lines represent the corresponding fitted plasma concentrations measured by the PIgn-coated beads method (thin line) and after the 60°C heat treatment (thick line).

Mechanisms of plasma PrP clearance

Figure 5 shows the temporal variations in plasma concentrations of the VRQ variant of rPrP after IV administration of 0.02 mg per kg to a representative sheep before and after a bilateral nephrectomy. Figure 5 shows the dramatic effect of nephrectomy, with a much slower elimination rate in the nephrectomized sheep. The mean plasma clearance of the VRQ variant was 2.1 times lower after bilateral nephrectomy (1.56 ± 0.66 mL/(kg·min) vs. 0.75 ± 0.36 mL/(kg·min); t test, $p < 0.05$). The renal PrP clearance derived from plasma clearance values obtained before and after nephrectomy (0.81 ± 0.30 mL/(kg·min)) represented 52 percent of the total clearance. The mean residence time of the protein was 2.6-fold higher (24.2 ± 7.6 min vs. 65.2 ± 2.6 min; $p < 0.05$, t test) after nephrectomy. The mean value of the volume of the central compartment obtained after nephrectomy was slightly lower than that obtained during the control period but this difference was not relevant (22.3 ± 9.82 mL/kg vs. 24.0 ± 9.69 mL/kg; t test, $p < 0.01$). No other pharmacokinetic variables were affected by nephrectomy.

Figure 6 shows the temporal decrease in plasma PrPres concentrations after the IV administration of an SAF solution containing 75 µg equivalent of VRQ rPrP. After a relatively fast distribution phase, the plasma PrPres concentrations decreased very slowly and reached the detection limit of the assay (7.5 ng/mL) 24 hours after administration. The estimated plasma clearance of PrPres

was 0.24 or 0.43 mL per (kg·min) based on the results obtained after Plgn-coated microbead capture or 60°C heating, respectively. The estimated mean terminal half-lives obtained after Plgn-coated microbead capture or by the 60°C heating method were 58 and 41 hours, respectively. The mean residence time and the steady-state distribution volumes were 5030 minutes (84 hr) versus 3435 minutes (57 hr) and 1187.0 versus 1487.4 mL per kg, respectively (Plgn-coated microbead capture method vs. 60°C plasma heating treatment). If the results obtained by Plgn-coated microbead capture are considered, then the MRT_C and MRT_P values are equivalent. PrPres could not be detected in any of the urine samples collected after administration of the SAF solution.

DISCUSSION

Before any discussion of the present experiments, we must acknowledge that we tested our different hypotheses with a rPrP of bacterial origin and not the native form of the PrP, which is not currently available for pharmacokinetic studies. The natural form is glycosylated and the glycosylation of a protein may considerably influence its fate in the body. We nevertheless believe that this pioneer work may have some value to explore the clearance pathways and the influence of the genotype and the role mediated by the conformation of the variant of PrP and/or by the clearance mechanisms. Similarly, the disease-associated isoform of PrP that was administered underwent a series of physicochemical processes before its administration (solubilization) that were able to partly denature the protein. Thus, the different test proteins used in the present experiment should be considered only as probes. We hypothesized they were able to explore, at least qualitatively, and possibly quantitatively (i.e., giving an order of magnitude), different disposition processes of physiologic interest, mainly concerning the overall plasma clearance that contributes to the systemic exposure.

Our results show that clearance of the rPrP is low and of the same order of magnitude as the glomerular filtration rate in sheep[25] (approx. 2 mL/(kg·min)). Clearance of the ARR variant of rPrP associated with resistance to scrapie was almost twice that of the VRQ variant associated with scrapie susceptibility. For a given variant, however, the genotype of the recipient had no effect on PrP plasma clearance, indicating that the protein conformation, not the animal's clearance mechanisms, was responsible for the difference between the two genotypes.

It is generally accepted that the kidney is the major organ of protein elimination[26] and this was confirmed in the present experiment where the kidney clearance of the VRQ variant of rPrP was about half the total clearance. Despite the high renal clearance, no PrP was found in urine. This is not really surprising because most of the low-molecular-weight proteins (here approx. 23 kDa for our variants) that are filtered through the glomerulus are metabolized by enzymes located in the brush border of the tubular lumen.[26] We did not investigate the renal clearance of the ARR variant. It is generally accepted, however, that the renal excretion of proteins is mainly governed by their size and that the sieving effect of glomerular filtration is independent, for small proteins, of molecular charge[27] or other conformational differences. Because ARR and VRQ are of the same molecular weight, it can be hypothesized that the observed difference in clearance of the ARR and VRQ recombinant proteins was of nonrenal origin.

Nonrenal clearance of rPrP was partly explored in our experiment and the direct metabolism of rPrP by plasma proteases can be excluded because we showed that the plasma concentrations obtained from blood or plasma supplemented with either the VRQ or the ARR variant and incubated at 37°C did not vary over a 6-hour period, that is, a time greater than that required to observe PrP elimination in vivo. Similarly, we can exclude a selective trapping of rPrP in the brain because there was no evidence of a first-pass effect when the recombinant protein was directly administered through the carotid artery. It is likely that, as for many other proteins, the main nonrenal clearance mechanism involves the reticuloendothelial system. The liver was shown to contribute significantly to protein metabolism, especially through receptor-mediated endocytosis followed by degradation in lysosomes. This mechanism, contrary to the bulk filtration of protein at the glomerular level, is likely to be a more specific process of protein elimination that could explain the difference in clearance that we observed between the two tested variants.

It is generally accepted that proteins are initially distributed into the plasma volume and then more slowly into the interstitial fluid space. This view is supported by the present experiment where the initial distribution volume of rPrP approximated to that of the plasma volume (overall mean $V_C = 43$ mL/kg); in contrast, the volume of distribution associated with the terminal disposition phase of rPrP was of the same order of magnitude as the volume of extracellular fluid ($V_{area} = 162$ mL/kg), indicating that at least a fraction of the administered rPrP gained access to the extracellular fluid. Owing to this restricted distribution, the overall mean residence time of the recombinant protein was rather short, ranging from 14.5 to 52 minutes, despite the low clearance rate. The greater mean residence time of the VRQ variant, when compared with that of the ARR variant, was mainly explained by the greater mean residence time of the VRQ variant in the central compartment as measured by the MRT_C (20 min vs. 12.5 min). By contrast the MRT_P (i.e., the overall MRT of the protein in the peripheral compart-

ment) was relatively similar for the two variants (8 min vs. 11 min).

These results obtained with the nonglycosylated purified prokaryote recombinant protein may provide useful information for plasma PrPc because some authors[28-33] have shown that the three-dimensional structure and the thermal stability of rPrP produced in *E. coli* are essentially identical to those of the natural glycoprotein. Thus, as expected, the glycosylation and the GPI anchor did not affect the folding of the PrP protein and transgenic mice expressing a nonglycosylated PrPc as well as transgenic mice harboring a PrP without a GPI anchor[34,35] are able to replicate the infectious agent. Furthermore, we have shown that the basal plasma PrPc concentrations of homozygous VRQ ewes were almost twice those of homozygous ARR ewes. This suggests that the plasma clearance of the ARR variant of PrPc is, like the same variant of rPrP, nearly twice that of its VRQ counterpart assuming that there is no difference in the synthesis of the protein and that rPrP may be a relevant probe for studying the fate of PrPc. Our results are in agreement with those of Halliday and coworkers[36] who showed that the level of PrPc expressed on the cell surface of peripheral blood mononuclear cells was influenced by the genotype, with the highest levels found in scrapie-susceptible homozygous VRQ sheep and the lowest in scrapie-resistant homozygous sheep. The level of PrP expression by blood cells was correlated with the level of ovine plasma PrPc by Thackray and colleagues[37] who showed genotypic differences in the level of ovine plasma PrPc, with the highest and lowest levels being observed in plasma from homozygous VRQ and ARR sheep, respectively. The higher plasma PrPc concentrations of dogs affected by renal insufficiency when compared to healthy ones (unpublished observations) together with previous observations in humans with extensive renal insufficiency[38,39] strongly suggest that the kidneys contribute highly to PrPc clearance.

Our results raise the question of the significance of host genetic and pathophysiologic (renal insufficiency)-caused variations of plasma PrPc levels with respect to TSE susceptibility. The implication of PrPc plasma levels for peripheral pathogenesis of scrapie is still debated because blood transmissions can occur in species such as hamsters where blood levels of normal PrP are exceedingly low. It cannot be ruled out, however, that some knowledge of the influence of genetic and pathophysiologic factors on plasma concentrations or clearance of the normal protein provides a ground for future investigations aimed at a better understanding of the role of plasma PrPc in the transmissibility of the infectious agent by the IV route.

For the single sheep that we investigated, the kinetics of the temporal decrease in plasma PrPres concentrations after the IV administration indicated that the disease-associated isoform of PrP (clearance of 0.24 mL/(kg·min)) is eliminated much more slowly than the recombinant genetic variants of PrP (2-3 mL/(kg·min)), resulting in a greater mean residence time (approx. 84 hr vs. 14-52 min). In addition, we observed a high steady-state volume of distribution for PrPres (1.2-1.5 L/kg) suggesting that PrPres is more widely distributed than rPrP. It should be stressed that our pharmacokinetic approach required the solubilization of PrPres recovered as pellets though we cannot exclude the persistence of insoluble aggregated forms of PrPres in the administered SAF preparation despite the ultrasonic and heating treatments. Despite such a limitation, the difference in clearance between the recombinant and pathologic isoforms of PrP is so great that we have no doubt that the disease associated isoform of PrP is eliminated much more slowly than the recombinant protein. The limit of our analytical method prevented us from evaluating PrPres concentrations below the level of quantification (7.5 ng/mL) and from ensuring that PrPres did not persist for a longer time at low concentrations.

Considerable uncertainty exists about the relevant spiking form of prion to document the risk of TSE transmission by blood transfusion.[40] Data from a rodent experimental model of TSE suggest that the infectious agent in plasma is very small, unsedimentable, and poorly aggregated,[41,42] but many attempts to solubilize PrPSc under nondenaturing conditions have been unsuccessful[43,44] until recently.[45] In the present clearance study, it must be assumed that the method used to prepare the prion material from sheep brain must be efficient and safe enough to obtain a PrPres dose that can be administered intravenously to a sheep. The purpose of this single PrPres infusion was no more than to obtain a first estimate of the order of magnitude of the clearance of the disease-associated form of PrP.

In conclusion, we have shown, by use of purified prokaryote rPrP, that the clearance of the ARR variant associated with resistance to scrapie is greater than the clearance of the VRQ variant associated with sensitivity to scrapie. This, together with the higher basal plasma PrPc concentrations observed in homozygous highly susceptible VRQ ewes compared with homozygous resistant ARR ewes, suggests that the ARR variant of PrPc is eliminated more rapidly than the VRQ variant. The 52 percent decrease in clearance of the prokaryote rPrP in nephrectomized ewes suggests that the kidneys contribute considerably to the elimination of the prion protein and that renal insufficiency could represent a risk factor for TSE disease transmission. The pathologic isoform of PrP was shown to be cleared very slowly from the blood, leading to sustained exposure after its direct IV administration.

ACKNOWLEDGMENT

We thank J. Grassi for providing genetic BAR210 anti-PrP MoAb.

REFERENCES

1. Schmerr MJ, Jenny AL, Bulgin MS, Miller JM, Hamir AN, Cutlip RC, Goodwin KR. Use of capillary electrophoresis and fluorescent labeled peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy. J Chromatogr A 1999;853:207-14.
2. Herrmann LM, Baszler TV, Knowles DP, Cheevers WP. PrP(Sc) is not detected in peripheral blood leukocytes of scrapie-infected sheep: determining the limit of sensitivity by immunohistochemistry. Clin Diagn Lab Immunol 2002; 9:499-502.
3. Brown P, Cervenakova L, McShane LM, Barber P, Rubenstein R, Drohan WN. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. Transfusion 1999;39:1169-78.
4. Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, Eaton S, MacKenzie C, Houston F. Transmission of prion diseases by blood transfusion. J Gen Virol 2002;83:2897-905.
5. Houston EF, Halliday SI, Jeffrey M, Goldmann W, Hunter N. New Zealand sheep with scrapie-susceptible PrP genotypes succumb to experimental challenge with a sheep-passaged scrapie isolate (SSBP/1). J Gen Virol 2002;83: 1247-50.
6. Houston F, Foster JD, Chong A, Hunter N, Bostock CJ. Transmission of BSE by blood transfusion in sheep. Lancet 2000;356:999-1000.
7. Herzog C, Sales N, Etchegaray N, Charbonnier A, Freire S, Dormont D, Deslys JP, Lasmezas I. Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. Lancet 2004;363:422-8.
8. Wroe SJ, Pal S, Siddique D, Hyare H, MacFarlane R, Joiner S, Linehan JM, Brandner S, Wadsworth JD, Hewitt P, Collinge J. Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. Lancet 2006;368:2061-7.
9. Editorial team. Fourth case of transfusion-associated vCJD infection in the United Kingdom. Euro Surveill 2007;12: E070118.4.
10. Wilson K, Ricketts MN. A third episode of transfusion-derived vCJD. Lancet 2006;368:2037-9.
11. Hunter N, Goldmann W, Foster JD, Cairns D, Smith G. Natural scrapie and PrP genotype: case-control studies in British sheep. Vet Rec 1997;141:137-40.
12. Elsen JM, Amigues Y, Schelcher F, Ducrocq V, Andreoletti O, Eychenne F, Khang JV, Poivey JP, Lantier F, Laplanche JL. Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. Arch Virol 1999;144:431-45.
13. Baylis M, Chihota C, Stevenson E, Goldmann W, Smith A, Sivam K, Tongue S, Gravenor MB. Risk of scrapie in British sheep of different prion protein genotype. J Gen Virol 2004; 85:2735-40.
14. Le Dur A, Beringue V, Andreoletti O, Reine F, Lai TL, Baron T, Bratberg B, Vilotte JL, Sarradin P, Benestad SL, Laude H. A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. Proc Natl Acad Sci U S A 2005;102:16031-6.
15. Rezaei H, Marc D, Choiset Y., Takahashi M, Hui Bon Hoa G, Haertlé T, Grosclaude J, Debey P. High yield purification and physico-chemical properties of full-length recombinant allelic variants of sheep prion protein linked to scrapie susceptibility. Eur J Biochem 2000;267:2833-9.
16. Rezaei H, Choiset Y, Eghiaian F, Treguer E, Mentre P, Debey P, Haertle T, Grosclaude J. Amyloidogenic unfolding intermediates differentiate sheep prion protein variants. J Mol Biol 2002;322:799-814.
17. Maack T, Johnson V, Kau ST, Figueiredo J, Sigulem D. Renal filtration, transport, and metabolism of low-molecular weight proteins. A review. Kidney Int 1979;16: 251-70.
18. Horney D, Archibald J. Nephrectomy in cattle and sheep. J Am Vet Med Assoc 1961;138:551-2.
19. Grassi J, Creminon C, Frobert Y, Fretier P, Turbica I, Rezaei H, Hunsmann G, Comoy E, Deslys JP. Specific determination of the proteinase K-resistant form of the prion protein using two-site immunometric assays. Application to the post-mortem diagnosis of BSE. Arch Virol Suppl 2000;16: 197-205.
20. Feraudet C, Morel N, Simon S, Volland H, Frobert YF, Creminon C, Vilette D, Lehmann S, Grassi J. Screening of 145 anti-PrP monoclonal antibodies for their capacity to inhibit PrPSc replication in infected cells. J Biol Chem 2005;280:11247-58.
21. Fischer MB, Roeckl C, Parizek P, Schwarz HP, Aguzzi A. Binding of disease-associated prion protein to plasminogen. Nature 2000;408:479-83.
22. Maissen M, Roeckl C, Glatzel M, Goldmann W, Aguzzi A. Plasminogen binds to disease-associated prion protein of multiple species. Lancet 2001;357:2026-8.
23. Gibaldi M, Perrier D. Pharmacokinetics. 2nd ed. New York: Marcel Dekker; 1982.
24. Kong AN, Jusko WJ. Definitions and application of mean residence times in reference to the two-compartment mammillary plasma clearance model. J Pharm Sci 1988;77: 157-65.
25. Nesje M, Flaoyen A, Moe L. Estimation of glomerular filtration rate in normal sheep by the disappearance of iohexol from serum. Vet Res Commun 1997;21:29-35.
26. Rowland M, Tozer TN. Clinical pharmacokinetics. Concepts and applications. 3rd ed. New York: Lea & Febiger; 1995.
27. Maack T, Park CH, Camargo MJ. Renal filtration, transport and metabolism of proteins. In: Seldin DW, Giebish G, editors. The kidney physiology and pathophysiology. New York: Raven Press; 1992. p. 3005-38.

28. Calzolai L, Zahn R. Influence of pH on NMR structure and stability of the human prion protein globular domain. J Biol Chem 2003;278:35592-6.
29. De Simone A, Dodson GG, Verma CS, Zagari A, Fraternali F. Prion and water: tight and dynamical hydratation sites have a key role in structural stability. Proc Natl Acad Sci U S A 2005;102:7535-40.
30. Eghiaian F, Grosclaude J, Lesceu S, Debey P, Doublet B, Treguer E, Rezaei H, Knossow M. Insight into the $PrP^{C} \rightarrow PrP^{Sc}$ conversion from the structures of antibody-bound ovine prion scrapie-susceptibility variants. Proc Natl Acad Sci U S A 2004;101:10254-9.
31. Haire LF, Whyte SM, Vasisht N, Gill AC, Verma C, Dodson EJ, Dodson GG, Bayley PM. The crystal structure of the globular domain of sheep prion protein. J Mol Biol 2004; 336:1175-83.
32. Hornemann S, Schorn C, Wüthrich K. NMR structure of the bovine prion protein isolated from healthy calf brains. EMBO Rep 2004;5:1159-64.
33. Zahn R, Güntert P, Von Schroetter C, Wüthrich K. NMR structure of a variant human prion protein with two sulfide bridges. J Mol Biol 2003;326:225-34.
34. Chesebro B, Trifilo M, Race R, Meade-White K, Teng C, LaCasse R, Raymond L, Favara C, Baron G, Priola S, Caughey B, Masliah E, Oldstone M. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. Science 2005;308:1435-9.
35. Legname G, Baskakov IV, Nguyen HO, Riesner D, Cohen FE, De Armond SJ, Prusiner SB. Synthetic mammalian prions. Science 2004;305:673-6.
36. Halliday S, Houston F, Hunter N. Expression of PrPC on cellular components of sheep blood. J Gen Virol 2005;86: 1571-9.
37. Thackray AM, Fitzmaurice TJ, Hopkins L, Bujdoso R. Ovine plasma prion protein levels show genotypic variation detected by C-terminal epitopes not exposed in cell-surface PrPc. Biochem J 2006;400:349-58.
38. Starke R, Drummond O, MacGregor I, Biggerstaff J, Camilleri R, Gale R, Lee CA, Nitu-Whalley S, Machin SJ, Harrison P. The expression of prion protein by endothelial cells: a source of the plasma form of prion protein? Br J Haematol 2002;119:863-73.
39. Starke R, Mackie I, Drummond O, MacGregor I, Harrison P, Machin S. Prion protein in patients with renal failure. Transfus Med 2006;16:165-8.
40. Foster PR. Selection of spiking materials for studies on the clearance of agents of transmissible spongiform encephalopathy during plasma fractionation. Biologicals 2007;Mar 30:1-2.
41. Brown P, Cervenakova L, McShane LM, Barber P, Rubenstein R, Drohan WN. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. Transfusion 1999;39:1169-78.
42. Yakovleva O, Janiak A, McKenzie C, McShane L, Brown P, Cervenakova L. Effect of protease treatment on plasma infectivity in variant Creutzfeldt-Jakob disease mice. Transfusion 2004;44:1700-5.
43. Post K, Brown DR, Groschup M, Kretzschmar HA, Riesner D. Neurotoxicity but not infectivity of prion proteins can be induced reversibly in vitro. Arch Virol Suppl 2000;16: 265-73.
44. Wille H, Prusiner SB. Ultrastructural studies on scrapie prion protein crystals obtained from reversed micellar solutions. Biophys J 1999;76:1048-62.
45. Berardi VA, Cardone F, Valanzano A, Lu M, Pocchiari M. Preparation of soluble infectious samples from scrapie-infected brain: a new tool to study the clearance of transmissible spongiform encephalopathy agents during plasma fractionation. Transfusion 2006;46:652-8.

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-4

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 2 月 28 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Progress and limits of TSE diagnostic tools. Grassi, J. et al, Vet. Res. 39, 33 (2008).	公表国 フランス	
販売名（企業名）						

研究報告の概要

本稿では，1990 年代にウシ海綿状脳症（BSE）が英国で流行したことを受けて，ヒト及び動物において伝染性海綿状脳症（TSE）を検出するために開発された様々な技法について焦点をあてている。脳組織中で典型的な病変及び異常プリオンタンパク PrP^{Sc} の蓄積を検出する従来の組織学的及び免疫組織学的方法に加えて，感受性の高いげっ歯類に疾患を実験的に伝播させることも確認手段としての機能を果たしてきた。さらに，ウェスタンブロット法も組織抽出物中の PrP^{Sc} 検出において高感度であることが実証されている。しかしながら，早急にハイスループットスクリーニングに適した手法が必要性であったことから，ウシ及び小型反芻動物（ヒツジ，ヤギ）の屠畜後に TSE を診断する，いわゆる「迅速な検査」が導入された。これらの多くは，感染動物又は非感染動物の脳組織を検討した大規模試験（対象は数百万検体）で妥当性が確認された後に，EU 保健・消費者保護総局（European Directorate General for Health and Consumer Protection）の承認を受けている。検査法の大半は ELISA 法であり，プロテイナーゼ K による PrPSc 消化とその後の変性及び特異的抗体による検出に依存している。現在，発症前での早期診断が可能となり得る屠畜前検査法が必要となっている。蛋白折りたたみ異常反復増幅法（Protein Misfolding Cyclic Amplification：PMCA）と呼ばれるアプローチ法は有望であると考えられていたが，最近では当初考えられていたほど特異的ではないことが明らかになっている。ヒトにおいて輸血により vCJD が伝播し得ることが示されていることから，プリオン研究分野では信頼性の高い血液検査法の開発が最優先事項となっている。様々な戦略が実施されているものの，いずれも未だ満足のいく結果にはつながっておらず，当該分野は依然として活発な研究領域である。

報告企業の意見	今後の対応
著者は，これまでに開発された動物及びヒトにおける TSE 検出法について詳しく調査している。本研究分野は，大規模な調査に基づいたスクリーニング方法の開発に成功したと考えられるが，依然として，血液における迅速な生前診断の必要性は高い。これが可能になれば，血液ドナーのスクリーニングにも使用でき，血液由来製剤の安全性も高まるであろう。	今後も引き続き，血漿分画製剤の製造に適用できるプリオンスクリーニング検査の有用性の情報収集に努める。

使用上の注意記載状況・その他参考事項等

BYL-2008-0313

38

Vet. Res. (2008) 39:33
DOI: 10.1051/vetres:2008009

www.vetres.org

Review article

Progress and limits of TSE diagnostic tools

Jacques GRASSI*, Séverine MAILLET, Stéphanie SIMON, Nathalie MOREL

CEA, iBiTecS, Service de Pharmacologie et d'Immunoanalyse, bâtiment 136, CEA/Saclay, 91191 Gif-sur-Yvette, France

(Received 2 October 2007; accepted 5 February 2008)

Abstract – Following the two "mad cow" crises of 1996 and 2000, there was an urgent need for rapid and sensitive diagnostic methods to identify animals infected with the bovine spongiform encephalopathy (BSE) agent. This stimulated research in the field of prion diagnosis and led to the establishment of numerous so-called "rapid tests" which have been in use in Europe since 2001 for monitoring at-risk populations (rendering plants) and animals slaughtered for human consumption (slaughterhouse). These rapid tests have played a critical role in the management of the mad cow crisis by allowing the removal of prion infected carcasses from the human food chain, and by allowing a precise epidemiological monitoring of the BSE epizootic. They are all based on the detection of the abnormal form of the prion protein (PrP^{Sc} or PrP^{res}) in brain tissues and consequently are only suitable for post-mortem diagnosis. Since it is now very clear that variant Creutzfeldt-Jakob disease (vCJD) can be transmitted by blood transfusion, the development of a blood test for the diagnosis of vCJD is a top priority. Although significant progress has been made in this direction, including the development of the protein misfolding cyclic amplification (PMCA) technology, at the time this paper was written, this objective had not yet been achieved. This is the most important challenge for the years to come in this field of prion research.

TSE diagnosis / PrP / blood test / PMCA

Table of contents

* Corresponding author: jacques.grassi@cea.fr

Article available at http://www.vetres.org or http://dx.doi.org/10.1051/vetres:2008009

1. INTRODUCTION

Humans have a long history of contact with animals affected by a transmissible spongiform encephalopathy (TSE), without apparent problems. Scrapie in sheep has been described since the 18th century and the available epidemiological data do not indicate a detectable risk for humans under natural conditions. The huge epizootic of bovine spongiform encephalopathy (BSE) in Great Britain that was first detected in 1986 (over 200 000 confirmed cases to date) and, above all, the announcement in 1996 of possible transmission of BSE to humans in the form of the variant Creutzfeldt-Jakob disease (vCJD), created enormous concern among European consumers and triggered the first European-wide mad cow crisis. In 1999, testing of at-risk populations was introduced in some European countries and subsequently identified cases of BSE in countries previously believed to be untouched by the epizootic (Germany, Italy, Spain). This realisation triggered a further mad cow crisis at the end of 2000 and prompted the European authorities to take a whole series of measures to stop the spread of the epizootic, and to protect consumers from possible contamination by BSE. In particular, there was a total ban on meat and bone meal (held to be the main reason for BSE propagation in cattle) in livestock feed. Consumer protection was essentially ensured by removal of organs most likely to contain prions (specified risk material), and the implementation of systematic testing of all cattle aged between 24 and 30 months, depending on the carcass category and country[1]. The BSE epizootic has clearly receded since 2001 in Western Europe, but the situation is less clear in Eastern Europe.

Although arguments have accumulated since 1996 to confirm a link between BSE and vCJD [10, 11], fewer people are affected by vCJD than might have been feared (201 as of August 2007, with 163 in the UK and 22 in France) all of them carrying the Met/Met genotype at codon 129 of the prion protein (PrP). It is possible, however, that affected carriers of other genotypes (Val/Val and Met/Val) may appear in the future. Furthermore, a risk of secondary transmission within the human species is now clearly identified, following the detection in Great Britain of four cases linked to blood transfusion [41, 56, 84].

In the late 1990s, there was a pressing need for rapid and sensitive diagnostic methods to identify animals infected by the BSE agent in order to define the extent of the epizootic and avoid transmission to humans. Surprisingly successful rapid tests have been developed since the mid-1990s and, as we shall see, have proven to be very useful.

The development of a blood test for the diagnosis of vCJD is now a priority, first to make blood transfusions safe, and secondly to identify affected individuals early so that treatment (which at present does not exist) can be initiated before neuroinvasion and onset of the first clinical signs. At the time this paper was written, this objective had not yet been achieved.

2. THE CURRENT STATUS OF TSE DIAGNOSIS

Before the mad cow epizootic, the diagnosis of prion diseases was not a public health or economic issue. For humans and live animals, it was essentially based on the analysis of clinical signs and post-mortem histological analysis.

2.1. Conventional methods

Historically, techniques used to diagnose TSE were designed to detect, in appropriate tissue samples, lesions characteristic of TSE, or disease associated forms of the prion protein (PrP^{Sc}), or the transmissible agent itself.

2.1.1. Histological and immunohistological techniques

Histological detection of lesions typical of TSE (spongiosis, astrogliosis, amyloid plaques), essentially in the tissue of the central nervous system, is the reference method

[1] This does not hold for the United Kingdom since, between 1996 and 2005, cattle over 30 months were not eligible for human consumption (certainly the more efficient protection), and consequently not systematically tested. The same active surveillance scheme as in other European countries has been in place since November 2005.

for confirming a clinical diagnosis [23]. It is very specific, since it allows direct observation of the signs of the disease, notably symmetrical spongiform lesions, but is less sensitive than other techniques [24, 83]. The sensitivity of microscopic observation can be increased by immunohistochemical techniques that use antibodies specific to PrP to detect accumulation of PrP^{Sc} in amyloid deposits [79, 80]. This technique's efficiency depends greatly on sample preparation and on the nature of the antibodies used. Although these methods are ill-suited to rapid, routine analysis, they are excellent for confirmation. They are also effective for the analysis of samples of lymphoid tissues (tonsils, Peyer's patches, lymph nodes) and can be utilised, for example, in preclinical diagnosis of scrapie in sheep [2, 79] and chronic wasting disease [73], i.e., diseases characterised by marked replication of the prion in the lymphoid organs during the presymptomatic phase. The same observation has been reported for the diagnosis of vCJD and retrospective examination of over 8 000 tonsil and appendix samples by immuno-histochemistry (IHC) identified one case of vCJD in Great Britain in a person presenting no clinical signs [32, 33].

2.1.2. Experimental infection tests

The most sensitive and specific method of diagnosing TSE is unquestionably experimental infection in laboratory animals. The animal is injected (usually by the intracranial route) with a homogenate prepared from the potentially infected tissue and is watched for the appearance of clinical signs. After the death of the experimentally infected animal, disease development is confirmed using classic techniques (histology, immunohistology, Western blot). For obvious practical reasons, these experiments are generally performed in rodents (mice, hamsters, bank voles). Recently, the availability of transgenic mice that overexpress the same PrP as that of the donor species has significantly increased the efficiency of experimental transmission and shortened incubation periods [71]. However, these methods are too labour-intensive and time-consuming for use in routine high-throughput screening.

2.1.3. Western blotting

Western blotting has been used to detect PrP^{Sc} in tissue extracts for 20 years now [6]. Since all samples also always contain PrP^{C}, the protease-sensitive prion protein, they are systematically treated with proteinase K. After denaturation of the tissue extract by heating with sodium dodecyl sulfate (SDS), it is analysed by polyacrylamide gel electrophoresis (PAGE) and the denatured protein is transferred to a solid support and detected with an enzyme-labelled antibody. The specificity of Western blotting stems, among other things, from the fact that proteolysis with proteinase K characteristically alters the molecular weight of the PrP^{res}, because of the partial degradation of the N-terminal part of the protein. As a consequence, in addition to the residual signal observed, the gel bands shift in a manner typical of PrP^{res}. This technique has enabled highly sensitive detection of PrP^{Sc} in various tissues from vCJD patients [81]. Western blotting is also commonly used to characterise prion strains. The characteristics of the molecular pattern (size and relative intensity of the bands) of the three glycoforms of PrP^{res}, and the reaction of certain antibodies directed against the N-terminal part of the PrP can, in some cases, be used to identify the molecular signature of the prion strain [75, 76].

2.2. Rapid tests for post-mortem diagnosis of TSE

2.2.1. General characteristics

None of the methods mentioned above are really suited to high-throughput screening, and cannot be automated. After the 1996 mad cow crisis, and the fear of possible transmission to humans, it became clear that there was a need to develop new simpler and faster diagnostic tests for large-scale epidemiological studies, and more accurate assessment of the characteristics of the epizootic, or for routine testing to warrant safety of animal meat, for instance, of all cattle before they enter the food chain or industrial circuits. A new generation of so-called "rapid" diagnostic tests emerged, all based on the immunological detection of PrP^{Sc}, the only identified reliable marker of TSE.

It has long been perfectly apparent that antibodies can distinguish between the different conformations of the same protein, and the abnormal form of the prion protein is known to differ from the normal cellular form by its conformation, which contains a much higher proportion of β sheets, and less α helices [57, 58]. Yet, despite several promising publications [17, 37, 54, 87], there are, as yet, no clearly identified antibodies that, under practical conditions, specifically recognise PrP^{Sc} with satisfactory affinity in its native form. This is not the least of the paradoxes encountered in this field of research, but is beyond the scope of the present review.

In the absence of antibodies that specifically recognise PrP^{Sc}, it was necessary to resort to indirect approaches to distinguish between PrP^{Sc} and PrP^{C} in tissue extracts, which is generally present at a higher concentration. In almost all the rapid tests developed hitherto, this distinction is based on the distinct biochemical properties of the two forms of the protein. Most tests utilise the relative resistance of PrP^{Sc} to degradation by proteolytic enzymes, particularly proteinase K. Other tests are based on the aggregation properties of PrP^{Sc} when extracted using detergents. Note that the extraction of PrP (PrP^{C} or PrP^{Sc}) is an indispensable step in all tests, because it is hard to envisage detecting PrP without extracting it from its neighbouring membrane structures. This is generally achieved by treating a tissue homogenate with one or more detergents. Lastly, all the rapid tests include a step in which PrP^{res} is denatured, to permit its detection by antibodies that recognise PrP^{C} or denatured PrP (whether from PrP^{C} or PrP^{Sc}).

2.2.2. *European validation campaigns*

In May 1999, the Directorate General XXIV (Consumer Policy and Consumer Health Protection) of the European Commission validated, under very strict conditions (blind testing in a limited time overseen by a European Commission representative), three tests (from Enfer Technology Ltd (Newbridge, Ireland), Prionics (Zurich, Switzerland), and CEA (Saclay, France)) that were suitable for rapid industrial development.

The Prionics test uses an industrialised format of Western blotting that enables large-scale analysis [49, 65], and was the first rapid test used in large-scale epidemiological studies, first in Switzerland and then in France [12].

A diagnostic test developed by the CEA since 1998 is another example of a rapid test based this time on a conventional immunoenzyme approach (enzyme-linked immunosorbent assay, ELISA). This test is now marketed by Bio-Rad (Hercules, CA, USA, TeSeE tests). In the first step of the test, PrP^{res} is selectively purified using proteinase K, centrifugation, and denaturation. In the second step, the solubilised and denatured PrP^{res} is measured by a two-site (so-called sandwich) immunoassay that uses two monoclonal antibodies [27].

Enfer Technology Ltd developed an ELISA [46] in which PrP^{Sc} is directly immobilised on a solid support in the presence of proteinase K, denatured, and then detected using a polyclonal antibody directed against a peptide sequence characteristic of PrP.

The evaluation of these three tests was performed on more than 1 600 brain stem samples from uninfected animals (1 000 animals from New Zealand) and from animals at the clinical stage of the disease (300 animals from the UK). Brain homogenates were also diluted to test the analytical sensitivity of the tests [46]. A fourth test (from Wallac, Bucks, UK) gave unsatisfactory results and was subsequently re-evaluated in a substantially different format in 2001. The Enfer Technology, Prionics, and CEA tests were found to have 100% sensitivity and specificity on the series studied. Later work demonstrated that the CEA test, and its industrial version developed by Bio-Rad, were as sensitive as intracerebral inoculation tests in conventional RIII mice [20, 27]. It was also shown that rapid tests can also detect the accumulation of PrP^{res} in nerve tissue before the appearance of clinical signs [3, 26].

In 2002 and 2004, 15 new tests were evaluated by the Directorate General for Health and Consumer Protection by a similar

procedure, albeit on fewer samples[2]. Nine of the tests were approved for the post-mortem diagnosis of BSE: Prionics-Check LIA and Prionics-Check Prio-Strip (both from Prionics), TSE Kit Version 2.0 (Enfer), CDI-5 (InPro, San Francisco, USA), Ceditect BSE (Cedi, Lelystad, The Netherlands), HerdChek BSE Test Kit (IDEXX, Westbrook, USA), Speed'it BSE (Institut Pourquier, Montpellier, France), Beta Prion BSE EIA (Roboscreen, Leipzig, Germany) and PrionScreen (Roche, Basel, Switzerland).

Most of these new validated tests work in an ELISA format except Prio-Strip (lateral flow technology). However, three are based on markedly different principles. InPro's CDI-5 and Cedi's Ceditect BSE detect PrP^{Sc} by taking advantage of the fact that its immunoreactivity increases upon denaturation due to the unmasking of cryptic epitopes [63, 64]. HerdChek BSE from IDEXX is unique in two aspects, in that it does not use proteinase K digestion, and uses an aggregate specific capture ligand on a dextran polymer (Seprion ligand technology, Microsens Biotechnologies, London, UK) of PrP^{Sc}, which after denaturation is detected using an anti-PrP antibody.

Today, virtually all testing of cattle is done with the tests from Bio-Rad, Prionics, IDEXX, and Enfer. Some of these tests have also proved effective in diagnosing chronic wasting disease in wild ruminants [31].

From 2002 to 2004, five tests validated for the post-mortem diagnosis of BSE in cattle were provisionally approved for the post-mortem diagnosis of TSE in small ruminants[3]: TeSeE (Bio-Rad), TSE Kit (Enfer), CDI-5 (InPro), Prionics-Check LIA and Prionics-Check Western (both Prionics). Between 2004 and 2005, the European Commission specifically assessed nine tests for application to small ruminants, and recommended eight of them: TeSeE and TeSeE sheep/goat (Bio-Rad), TSE post-mortem test (IDEXX), Prionics-Check Western SR and Prionics-Check LIA SR (both from Prionics), Enfer TSE test Version 2.0 (Enfer), CDI-5 (InPro), Institut Pourquier Scrapie ELISA test (Institut Pourquier). It should be noted, however, that only the first three of these effectively detect atypical scrapie (Nor98) in brain stem samples. In practice, almost all testing on small ruminants is now done with the Bio-Rad, Prionics, IDEXX, and Enfer tests.

Note too that all results recorded using the rapid tests are confirmed in national reference laboratories, essentially using histopathology, immunohistochemistry, and Western blotting.

2.2.3. *Large-scale use of rapid tests*

Between 1st of January 2001 and 31st of December 2006, nearly 60 million tests on cattle within the European Community (almost 90% at the slaughterhouse) detected over 4 800 cases of BSE, approximately 1 170 at the slaughterhouse and about 3 700 in at-risk animals collected in rendering plants[4]. Over the same period, passive surveillance detected only 2 361 cases of BSE.

Rapid tests have therefore contributed significantly to consumer protection, first by providing a basis for confidence in meat safety, and secondly, because they led to the withdrawal of over 1 000 infected carcasses from human consumption. In addition to the increased safety they provide, these large-scale analyses have detected BSE in

[2] The evaluation of five rapid tests for the diagnosis of spongiform encephalopathy in bovines (2nd study), 27 March 2002, http://ec.europa.eu/food/food/biosafety/bse/sci_advice_en.htm, and scientific report of the European Food Safety Authority on the evaluation of seven new rapid post mortem BSE tests, 16 November 2004, http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620780462.htm [consulted January 2008].

[3] Scientific report of the European Food Safety Authority on the evaluation of rapid post mortem TSE tests intended for small ruminants, adopted on 17 May 2005, http://efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620780483.htm [consulted on January 2008].

[4] Reports on the monitoring and testing for the presence of TSE in the EU, 2001–2006, http://ec.europa.eu/food/food/biosafety/bse/annual_reps_en.htm, and monthly report of Member States on BSE and TSE, http://ec.europa.eu/food/food/biosafety/bse/mthly_reps_en.htm [consulted on January 2008].

numerous European countries (Austria, Czech Republic, Finland, Germany, Greece, Holland, Italy, Luxembourg, Poland, Slovakia, Slovenia, Spain), as well as in Japan, Canada, and the USA. They have also shown that many countries with no recorded case up to 2000 (e.g. Germany, Italy, and Spain), and which denied the presence of BSE, had an incidence equivalent to or higher than that of France and Switzerland, which have been recording cases since the early 1990s. Lastly, rapid tests allow much more precise epidemiological follow-up, allowing the measurement of trends at low prevalence, and which has more clearly shown a spectacular decline in the BSE epizootic in Europe.

Active monitoring of TSE in small ruminants (sheep and goats) was set up in Europe in March 2002, essentially to gather epidemiological data, and obliges member states of the European Community to test a quota of animals slaughtered normally or from at-risk populations. Between 2002 and 2006, nearly three million tests were performed, which led to the detection of over 13 000 cases of scrapie[4]. Note that this active surveillance resulted in the detection of a great many cases of so-called atypical scrapie among European livestock. This form of scrapie, which very likely corresponds to strain Nor98 [7] identified in 1998 in Norway, now accounts for over 50% of TSE cases in small ruminants in many countries (France, Germany, Portugal, UK, etc.). The PrP^{Sc} associated with this strain is characterised by increased sensitivity to proteinase K, which makes its detection more difficult and explains why numerous rapid tests perform poorly in diagnosis. In practice, the vast majority of cases of atypical scrapie were identified using the tests from Bio-Rad (TeSeE since 2002) and IDEXX (post-mortem test, since 2005).

In view of the diversity of TSE strains present in small ruminants, the European Commission set up biochemical typing in 2005, mainly designed to identify the BSE strain in small ruminant populations[5]. Testing, performed exclusively in national reference laboratories, is based on Western blotting techniques [74, 76]. It is worth noting that, to date, only a single case of BSE, in a goat, was observed in France [21] before the implementation of this new regulation.

3. NEW APPROACHES TO ANTE-MORTEM TESTS

As we have seen, diagnosis of prion diseases depends principally on the detection of the abnormal form of PrP (PrP^{Sc} or PrP^{res}). This approach has been very useful in reacting to the BSE epizootic and in setting up active surveillance for TSE in ruminants, but to date has not met all the requirements of the diagnosis of prion diseases. These tests are only applicable to tissues collected after the death of the animal and so cannot be used for early preclinical diagnosis. So far, no test can give a reliable diagnosis using a readily available sample from a living animal or person, such as blood or urine. The problem is particularly acute for blood transfusion, insofar as it is now well established that vCJD can be transmitted by blood. Considerable effort has been devoted to the search for alternative markers enabling earlier diagnosis of TSE (for a review see Parveen et al. [55]).

3.1. The search for new markers

The search for alternative markers has grown greatly in recent years, boosted by the development of postgenomic approaches, which can be used for large-scale parallel analysis of the transcriptome, proteome, and metabolome of tissues. Attention naturally first turned to neuronal markers, which include protein 14-3-3 [28, 78], neurone-specific enolase [1], the protein S100B [5, 29], glial acidic fibrillar protein [44, 50], Tau protein [51], and prionins [59]. However, none of these markers has proved usable as a basis for a sufficiently sensitive and specific test allowing early preclinical diagnosis.

[5] Commission Regulation (EC) No 36/2005 of 12 January 2005 amending Annexes III and X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards epidemio-surveillance for transmissible spongiform encephalopathies in bovine, ovine and caprine animals: http://ec.europa.eu/food/food/biosafety/bse/legisl_en.htm [consulted 11 January 2008].

Metabolic markers, such as fatty acid-binding proteins, interferon γ, prostaglandin E2, C-reactive protein, interleukin 6, cystatin C, and corticosteroids, have also been studied, but with no more success (for a review see Parveen et al. [55]).

Transcriptomic studies have revealed potential markers [70, 85], but to date none has proved of practical use in the diagnosis of prion diseases. Erythroid differentiation-related factor, for example, initially seemed highly promising (downregulation [43]), but its value was not confirmed in subsequent work [25].

Finally, some research groups have developed an approach based on serum analysis by Fourier transform infrared spectroscopy combined with data processing by the neural network method [13, 39, 40, 69, 77]. This approach has shown high (> 90%) sensitivity and specificity in cattle populations, but it remains to be seen whether it is usable under routine conditions, and can be used to make an early diagnosis of TSE.

3.2. Protein misfolding cyclic amplification

To facilitate preclinical detection of prions in peripheral tissues, notably blood, Claudio Soto's group developed an original approach in which the PrP^{Sc} in a sample is amplified by means of protein misfolding cyclic amplification (PMCA) [62]. In this approach, which seeks to mimic pathological processes and is akin to the polymerase chain reaction used to amplify DNA (but without addition of exogenous polymerase enzyme), PrP^{Sc} is incubated in the presence of excess PrP^{C} to allow expansion of aggregates of PrP^{Sc} which are then dispersed by sonication to generate smaller units and to encourage the formation of new aggregates. The quantity of PrP^{Sc} formed depends on the number of expansion/sonication cycles performed. In early articles [62, 72], amplification was modest (10- to 50-fold), but optimisation and automation subsequently enabled amplifications of several million fold [61]. In most studies, amplification is achieved by using as a source of PrP^{C}, a brain extract from the same species as that which produced the PrP^{Sc} to be amplified. Recent works [18, 19] have shown that PrP^{Sc} can be replicated in a more controlled "minimal" system in the presence of highly purified PrP^{C} (the only identified contaminant being lipids) and polyanions (polyA RNA in these studies).

Although most of the work by Soto's group concerns a hamster model infected by strain 263K, significant amplification has been achieved with the PrP^{Sc} produced by various mammalian species, including mice [47, 72], sheep, goats and cattle [72], cervids [38] and humans [36]. The PrP^{Sc} newly formed by PMCA has all the properties of the original PrP^{Sc}, notably its infectious character [14, 82]. Lastly, early detection of PrP^{Sc} in hamster blood fractions (buffy coats) was achieved at a sensitivity ranging between 0 and 89% and a specificity of 100% [15, 60].

PMCA has great potential and is certainly the most promising approach from the viewpoint of developing a blood test. It is, though, hampered by various fundamental and technical difficulties. Given the requirements imposed by a blood test (see paragraph below), notably in terms of practicability, sensitivity, and specificity, several technical improvements are needed. For adaptation to routine analysis, there is a need for simplification, reduction of the duration, and better control. Moreover, the obligatory requirement for a concentrated source of PrP^{C} (brain extract or purified PrP^{C}) of the same species as the target to be amplified constitutes an important practical handicap. This specific problem could be resolved by the use of recombinant PrP and accelerated procedures as recently shown [4], assuming the results obtained with the hamster model can be extended to other mammalian species. However, PMCA must also prove effective in terms of diagnosis (sensitivity and specificity close to 100%) using blood sample series more representative than those obtained with the hamster model. Finally, recent results from Supattapone's group show that infectious PrP^{Sc} can be generated de novo and stochastically by PMCA [18] in the absence of pre-existing prions, and this raises concerns about the specificity of this approach when used in routine conditions.

3.3. Blood tests: state of the art

As we have emphasised several times in this review, the development of a blood test is the top priority in prion disease diagnosis, notably to ensure the safety of blood transfusion in humans. Numerous difficulties, however, have to be overcome, which explains why no test is yet operational. Whereas blood from vCJD infected patients is clearly infectious [41, 56, 84], its concentration of infectious material is very likely much lower than that in the central nervous system, and its concentrations of PrP^{Sc} are estimated to be in the range of pg/mL [8, 9]. Given the efficacy of disease transmission by the intravenous route, and the large volume (commonly > 400 mL) of packed red blood cells transfused in humans, transmission can occur with very low levels of infectious material, and, as a consequence, candidate tests must have excellent analytical sensitivity. Also, blood is a complex tissue rich in cells and proteins, and little is known of the distribution of prions (and of PrP^{Sc}). Several studies indicate that the bulk of the infectious material is in the white blood cells, but the plasma is also clearly infectious [8, 9]. In a healthy individual, significant levels of PrP^{C} are present in white blood cells, red blood cells, platelets, and plasma, probably at much higher concentrations than PrP^{Sc}. A candidate test must therefore also be very selective. Also, we know very little about the biochemical properties of the PrP^{Sc} in the different blood fractions. Given its low concentration and its environment, it is not certain, for instance, that it can form aggregates resistant to proteinase K, the treatment on which most current rapid tests are based.

In terms of the risk of vCJD infection by blood transfusion, because the incidence of the disease is assumed to be very low, a highly specific test is needed, or it could lead to more false-positive results than detection of real cases. Such a situation would be very difficult to manage ethically, given that vCJD is a fatal disease for which at present there is no treatment. There is clearly a great need for at least one very specific confirmation test, which does not exist today.

Due to the above mentioned difficulties it is not surprising that very few publications report on blood tests for TSE.

The first promising results were obtained, as early as 1996, by the group of Mary-Jo Schmerr, which combined capillary electrophoresis with a competitive immunoassay to detect a PK resistant C-terminal sequence of PrP in the blood of sheep infected with scrapie [66–68]. The technique was subsequently improved and applied to more relevant series of scrapie infected sheep [34, 35, 42, 86], but despite achieved improvement, the method appeared insufficiently robust for routine use [22].

In recent years, many research groups or companies have developed original strategies to try to overcome the intrinsic difficulties associated with the blood test. These include:

- The use of ligands for a specific capture of PrP^{Sc} possibly present in blood fractions, which include the 15B3 antibody produced by Prionics [37, 48] and the Seprion resin of the Microsense company (already used in the IDEXX test for post-mortem diagnosis). In both cases, the idea is to concentrate abnormal PrP by immunoprecipitation, taking advantage of its polymerisation state (aggregate? polymers? oligomers?) to allow a more sensitive and more specific detection by ELISA or flow cytometry. Another approach developed by the bioMérieux company (Marcy l'Étoile, France) involves binding and aggregation of abnormal PrP in plasma by streptomycin [45], followed by a specific capture on calyx-Arenes "molecular basket" immobilised onto a solid phase, and final detection with an appropriate anti-PrP antibody.
- The development of immunoassays designed to detect polymerised PrP (AS-ELISA, for aggregate specific ELISA) and based on the use of the same monoclonal antibody for capture and detection [52]. The sensitivity of AS-ELISA was increased by combining signal amplification (fluorescence) and target amplification (prion amplification using a simplified PMCA like procedure). Using

this approach (named Am-A-FACTT) the group of Man-Sun Sy succeeded in detecting prion aggregates in plasma from mice or deer infected with scrapie or CWD respectively [16]. A similar approach has been developed by the Korean company PeopleBio (Seoul, Korea, Multimer Detection System (MDS)) without amplification of signal and target but details remain unpublished.

- The use of fluorescence labelled palindromic PrP peptides to detect misfolded PrP (MPD for misfolded protein diagnostic). In this approach, when the labelled peptide is in contact with PrP^{Sc}, it undergoes a large coil to a β-sheet conformational change which largely modifies the fluorescence properties of the pyrene label [30]. This method allowed discrimination between TSE infected and uninfected animals, albeit on a rather small series of blood samples [53].

However, even if some of these approaches seem promising, for the moment none of these tests has fulfilled the very strict analytical and diagnostic requirements described above. With the passage of time (some of these approaches were initially described a few years ago) it becomes apparent that they are facing real difficulties in establishing routine and robust assays, and that much more time and development is needed to achieve the goal of an operational blood test for TSE.

4. CONCLUSIONS

The successive "mad cow" crises of 1996 and 2000 have clearly boosted very significantly research in the field of prion diseases, and more data have been accumulated during the last ten years than during the previous century. This has considerably improved our knowledge on prion biology, but also provided much more relevant tools including: transgenic mice ($PrP^{0/0}$ or over-expressing various forms of wild-type or mutated PrP), cellular models of TSE infection, a large series of well characterised monoclonal antibodies and, of course, much more relevant analytical methods and diagnostic tests. As far as diagnosis is concerned, very significant progress has been made in the post-mortem detection of PrP^{Sc}, with the development of reliable and very sensitive methods suitable for routine analysis (results available within less than three hours, more than 20 000 tests performed every day throughout the world), having the capacity of diagnosing TSE before the onset of clinical signs. These tests have been used efficiently for managing the mad cow crisis, and are still very useful for monitoring the BSE epizootic as well as the various forms of TSE in small ruminants and cervids. The analytical sensitivity of these tests can now be considerably improved by coupling PMCA amplification with the appropriate detection techniques (ELISA, CDI, Western-blot), and this allows detection of minute amounts of PrP^{Sc} in the brain or in peripheral lymphoid tissues. However, so far, there is no test that delivers an early and specific diagnosis of TSE in live animals or patients, i.e. a test which can be easily applied to a body fluid like blood or urine. This is particularly critical for ensuring the safety of blood transfusion in countries that have experienced a large BSE epizootic (UK and Western Europe). We have seen that PMCA has shown a good potential, in terms of sensitivity, for achieving such an aim but its use in routine conditions and its actual specificity are questionable. There is thus a place for another approach, and the development of a blood test for TSE diagnosis remains the most important challenge for the years coming in this field of prion research.

REFERENCES

[1] Aksamit A.J. Jr., Preissner C.M., Homburger H.A., Quantitation of 14-3-3 and neuron-specific enolase proteins in CSF in Creutzfeldt-Jakob disease, Neurology (2001) 57:728–730.

[2] Andréoletti O., Berthon P., Marc D., Sarradin P., Grosclaude J., van Keulen L., et al., Early accumulation of PrP(Sc) in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie, J. Gen. Virol. (2000) 81:3115–3126.

[3] Arnold M.E., Ryan J.B., Konold T., Simmons M.M., Spencer Y.I., Wear A., et al., Estimating the temporal relationship between PrPSc detection and incubation period in experimental bovine spongiform encephalopathy of cattle, J. Gen. Virol. (2007) 88:3198–3208.

[4] Atarashi R., Moore R.A., Sim V.L., Hughson A.G., Dorward D.W., Onwubiko H.A., et al., Ultrasensitive

detection of scrapie prion protein using seeded conversion of recombinant prion protein, Nat. Methods. (2007) 4:645–650.

[5] Beekes M., Otto M., Wiltfang J., Bahn E., Poser S., Baier M., Late increase of serum S100 beta protein levels in hamsters after oral or intraperitoneal infection with scrapie, J. Infect. Dis. (1999) 180:518–520.

[6] Bendheim P.E., Bolton D.C., A 54-kDa normal cellular protein may be the precursor of the scrapie agent protease-resistant protein, Proc. Natl. Acad. Sci. USA (1986) 83:2214–2218.

[7] Benestad S.L., Sarradin P., Thu B., Schonheit J., Tranulis M.A., Bratberg B., Cases of scrapie with unusual features in Norway and designation of a new type, Nor98, Vet. Rec. (2003) 153:202–208.

[8] Brown P., Blood infectivity, processing and screening tests in transmissible spongiform encephalopathy, Vox Sang. (2005) 89:63–70.

[9] Brown P., Cervenakova L., Diringer H., Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease, J. Lab. Clin. Med. (2001) 137:5–13.

[10] Brown P., Will R.G., Bradley R., Asher D.M., Detwiler L., Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease: background, evolution, and current concerns, Emerging Infect. Dis. (2001) 7:6–16.

[11] Bruce M.E., Will R.G., Ironside J.W., McConnell I., Drummond D., Suttie A., et al., Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent, Nature (1997) 389:498–501.

[12] Calavas D., Ducrot C., Baron T., Morignat E., Vinard J.L., Biacabe A.G., et al., Prevalence of BSE in western France by screening cattle at risk: preliminary results of a pilot study, Vet. Rec. (2001) 149:55–56.

[13] Carmona P., Monleon E., Monzon M., Badiola J.J., Monreal J., Raman analysis of prion protein in blood cell membranes from naturally affected scrapie sheep, Chem. Biol. (2004) 11:759–764.

[14] Castilla J., Saa P., Hetz C., Soto C., In vitro generation of infectious scrapie prions, Cell (2005) 121:195–206.

[15] Castilla J., Saa P., Soto C., Detection of prions in blood, Nat. Med. (2005) 11:982–985.

[16] Chang B., Cheng X., Yin S., Pan T., Zhang H., Wong P., et al., Test for detection of disease-associated prion aggregate in the blood of infected but asymptomatic animals, Clin. Vaccine Immunol. (2007) 14:36–43.

[17] Curin S., V. Bresjanac M., Popovic M., Pretnar H.K., Galvani V., Rupreht R., et al., Monoclonal antibody against a peptide of human prion protein discriminates between Creutzfeldt-Jacob's disease-affected and normal brain tissue, J. Biol. Chem. (2004) 279:3694–3698.

[18] Deleault N.R., Harris B.T., Rees J.R., Supattapone S., From the cover: formation of native prions from minimal components in vitro, Proc. Natl. Acad. Sci. USA (2007) 104:9741–9746.

[19] Deleault N.R., Lucassen R.W., Supattapone S., RNA molecules stimulate prion protein conversion, Nature (2003) 425:717–720.

[20] Deslys J.P., Comoy E., Hawkins S., Simon S., Schimmel H., Wells G., et al., Screening slaughtered cattle for BSE, Nature (2001) 409:476–478.

[21] Eloit M., Adjou K., Coulpier M., Fontaine J.J., Hamel R., Lilin T., et al., BSE agent signatures in a goat, Vet. Rec. (2005) 156:523–524.

[22] Everest D.J., Waterhouse S., Kelly T., Velo-Rego E., Sauer M.J., Effectiveness of capillary electrophoresis fluoroimmunoassay of blood PrpSc for evaluation of scrapie pathogenesis in sheep, J. Vet. Diagn. Invest. (2007) 19:552–557.

[23] Fraser H., The pathology of a natural and experimental scrapie, Front. Biol. (1976) 44:267–305.

[24] Gavier-Widen D., Stack M.J., Baron T., Balachandran A., Simmons M., Diagnosis of transmissible spongiform encephalopathies in animals: a review, J. Vet. Diagn. Invest. (2005) 17:509–527.

[25] Glock B., Winter M., Rennhofer S.O., Brunholzl E., Troscher D., Reisacher R.B., Mayr W.R., Transcript level of erythroid differentiation-related factor, a candidate surrogate marker for transmissible spongiform encephalopathy diseases in blood, shows a broad range of variation in healthy individuals, Transfusion (2003) 43:1706–1710.

[26] Grassi J., Comoy E., Simon S., Creminon C., Frobert Y., Trapmann S., et al., Rapid test for the preclinical postmortem diagnosis of BSE in central nervous system tissue, Vet. Rec. (2001) 149:577–582.

[27] Grassi J., Creminon C., Frobert Y., Fretier P., Turbica I., Rezaei H., et al., Specific determination of the proteinase K-resistant form of the prion protein using two-site immunometric assays. Application to the post-mortem diagnosis of BSE, Arch. Virol. Suppl. (2000) 16:197–205.

[28] Green A.J., Cerebrospinal fluid brain-derived proteins in the diagnosis of Alzheimer's disease and Creutzfeldt-Jakob disease, Neuropathol. Appl. Neurobiol. (2002) 28:427–440.

[29] Green A.J., Jackman R., Marshall T.A., Thompson E.J., Increased S-100b in the cerebrospinal fluid of some cattle with bovine spongiform encephalopathy, Vet. Rec. (1999) 145:107–109.

[30] Grosset A., Moskowitz K., Nelsen C., Pan T., Davidson E., Orser C.S., Rapid presymptomatic detection of PrPSc via conformationally responsive palindromic PrP peptides, Peptides (2005) 26:2193–2200.

[31] Hibler C.P., Wilson K.L., Spraker T.R., Miller M.W., Zink R.R., DeBuse L.L., et al., Field validation and assessment of an enzyme-linked immunosorbent assay for detecting chronic wasting disease in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*), J. Vet. Diagn. Invest. (2003) 15:311–319.

[32] Hilton D.A., Ghani A.C., Conyers L., Edwards P., McCardle L., Penney M., et al., Accumulation of prion protein in tonsil and appendix: review of tissue samples, BMJ (2002) 325:633–634.

[33] Hilton D.A., Ghani A.C., Conyers L., Edwards P., McCardle L., Ritchie D., et al., Prevalence of lymphoreticular prion protein accumulation in UK tissue samples, J. Pathol. (2004) 203:733–739.

[34] Jackman R., Everest D.J., Schmerr M.J., Khawaja M., Keep P., Docherty J., Evaluation of a preclinical blood test for scrapie in sheep using immunocapillary electrophoresis, J. AOAC Int. (2006) 89:720–727.

[35] Jackman R., Schmerr M.J., Analysis of the performance of antibody capture methods using fluorescent peptides with capillary zone electrophoresis with laser-induced fluorescence, Electrophoresis (2003) 24:892–896.

[36] Jones M., Peden A., Prowse C., Groner A., Manson J., Turner M., et al., In vitro amplification and detection of variant Creutzfeldt-Jakob disease PrP(Sc), J. Pathol. (2007) 213:21–26.

[37] Korth C., Stierli B., Streit P., Moser M., Schaller O., Fischer R., et al., Prion (PrPSc)-specific epitope defined by a monoclonal antibody, Nature (1997) 390:74–77.

[38] Kurt T.D., Perrott M.R., Wilusz C.J., Wilusz J., Supattapone S., Telling G.C., et al., Efficient in vitro amplification of chronic wasting disease PrPres, J. Virol. (2007) 81:9605–9608.

[39] Lasch P., Beekes M., Schmitt J., Naumann D., Detection of preclinical scrapie from serum by infrared spectroscopy and chemometrics, Anal. Bioanal. Chem. (2007) 387:1791–1800.

[40] Lasch P., Schmitt J., Beekes M., Udelhoven T., Eiden M., Fabian H., et al., Antemortem identification of bovine spongiform encephalopathy from serum using infrared spectroscopy, Anal. Chem. (2003) 75:6673–6678.

[41] Llewelyn C.A., Hewitt P.E., Knight R.S., Amar K., Cousens S., Mackenzie J., Will R.G., Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion, Lancet (2004) 363:417–421.

[42] Lourenco P.C., Schmerr M.J., MacGregor I., Will R.G., Ironside J.W., Head M.W., Application of an immunocapillary electrophoresis assay to the detection of abnormal prion protein in brain, spleen and blood specimens from patients with variant Creutzfeldt-Jakob disease, J. Gen. Virol. (2006) 87:3119–3124.

[43] Miele G., Manson J., Clinton M., A novel erythroid-specific marker of transmissible spongiform encephalopathies, Nat. Med. (2001) 7:361–364.

[44] Missler U., Wiesmann M., Wittmann G., Magerkurth O., Hagenstrom H., Measurement of glial fibrillary acidic protein in human blood: analytical method and preliminary clinical results, Clin. Chem. (1999) 45:138–141.

[45] Moussa A., Coleman A.W., Bencsik A., Leclere E., Perret F., Martin A., Perron H., Use of streptomycin for precipitation and detection of proteinase K resistant prion protein (PrPsc) in biological samples, Chem. Commun. (Camb.) (2006) 9:973–975.

[46] Moynagh J., Schimmel H., Tests for BSE evaluated. Bovine spongiform encephalopathy, Nature (1999) 400:105.

[47] Murayama Y., Yoshioka M., Horii H., Takata M., Yokoyama T., Sudo T., et al., Protein misfolding cyclic amplification as a rapid test for assessment of prion inactivation, Biochem. Biophys. Res. Commun. (2006) 348:758–762.

[48] Nazor K.E., Kuhn F., Seward T., Green M., Zwald D., Purro M., et al., Immunodetection of disease-associated mutant PrP, which accelerates disease in GSS transgenic mice, EMBO J. (2005) 24:2472–2480.

[49] Oesch B., Doherr M., Heim D., Fischer K., Egli S., Bolliger S., et al., Application of prionics Western blotting procedure to screen for BSE in cattle regularly slaughtered at Swiss abattoirs, Arch. Virol. Suppl. (2000) 16:189–195.

[50] Otto M., Wiltfang J., Differential diagnosis of neurodegenerative diseases with special emphasis on Creutzfeldt-Jakob disease, Restor. Neurol. Neurosci. (2003) 21:191–209.

[51] Otto M., Wiltfang J., Cepek L., Neumann M., Mollenhauer B., Steinacker P., et al., Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease, Neurology (2002) 58:192–197.

[52] Pan T., Chang B., Wong P., Li C., Li R., Kang S.C., et al., An aggregation-specific enzyme-linked immunosorbent assay: detection of conformational differences between recombinant PrP protein dimers and PrP(Sc) aggregates, J. Virol. (2005) 79:12355–12364.

[53] Pan T., Sethi J., Nelsen C., Rudolph A., Cervenakova L., Brown P., Orser C.S., Detection of misfolded prion protein in blood with conformationally sensitive peptides, Transfusion (2007) 47:1418–1425.

[54] Paramithiotis E., Pinard M., Lawton T., LaBoissiere S., Leathers V.L., Zou W.Q., et al., A prion protein epitope selective for the pathologically misfolded conformation, Nat. Med. (2003) 9:893–899.

[55] Parveen I., Moorby J., Allison G., Jackman R., The use of non-prion biomarkers for the diagnosis of transmissible spongiform encephalopathies in the live animal, Vet. Res. (2005) 36:665–683.

[56] Peden A.H., Head M.W., Ritchie D.L., Bell J.E., Ironside J.W., Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient, Lancet (2004) 364:527–529.

[57] Prusiner S.B., Prions, Proc. Natl. Acad. Sci. USA (1998) 95:13363–13383.

[58] Prusiner S.B., Scott M.R., DeArmond S.J., Cohen F.E., Prion protein biology, Cell (1998) 93:337–348.

[59] Ruth L., The quest for new prion tests, Anal. Chem. (2003) 75:32A–36A.

[60] Saa P., Castilla J., Soto C., Presymptomatic detection of prions in blood, Science (2006) 313:92–94.

[61] Saa P., Castilla J., Soto C., Ultra-efficient replication of infectious prions by automated protein misfolding cyclic amplification, J. Biol. Chem. (2006) 281:35245–35252.

[62] Saborio G.P., Permanne B., Soto C., Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding, Nature (2001) 411:810–813.

[63] Safar J., Wille H., Itri V., Groth D., Serban H., Torchia M., et al., Eight prion strains have PrP(Sc) molecules with different conformations, Nat. Med. (1998) 4:1157–1165.

[64] Safar J.G., Scott M., Monaghan J., Deering C., Didorenko S., Vergara J., et al., Measuring prions causing bovine spongiform encephalopathy or chronic wasting disease by immunoassays and transgenic mice, Nat. Biotechnol. (2002) 20:1147–1150.

[65] Schaller O., Fatzer R., Stack M., Clark J., Cooley W., Biffiger K., et al., Validation of a western immunoblotting procedure for bovine PrP(Sc) detection and its use as a rapid surveillance method for the diagnosis of bovine spongiform encephalopathy (BSE), Acta Neuropathol. (1999) 98:437–443.

[66] Schmerr M.J., Cutlip R.C., Jenny A., Capillary isoelectric focusing of the scrapie prion protein, J. Chromatogr. A (1998) 802:135–141.

[67] Schmerr M.J., Goodwin K.R., Cutlip R.C., Jenny A.L., Improvements in a competition assay to detect scrapie prion protein by capillary electrophoresis, J. Chromatogr. B, Biomed. Appl. (1996) 681:29–35.

[68] Schmerr M.J., Jenny A.L., Bulgin M.S., Miller J.M., Hamir A.N., Cutlip R.C., Goodwin K.R., Use of capillary electrophoresis and fluorescent labeled peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy, J. Chromatogr. A (1999) 853:207–214.

[69] Schmitt J., Beekes M., Brauer A., Udelhoven T., Lasch P., Naumann D., Identification of scrapie infection from blood serum by Fourier transform infrared spectroscopy, Anal. Chem. (2002) 74:3865–3868.

[70] Schutz E., Urnovitz H.B., Iakoubov L., Schulz-Schaeffer W., Wemheuer W., Brenig B., Bov-tA short interspersed nucleotide element sequences in circulating nucleic acids from sera of cattle with bovine spongiform encephalopathy (BSE) and sera of cattle exposed to BSE, Clin. Diagn. Lab Immunol. (2005) 12:814–820.

[71] Simmons M.M, Spiropoulos J., Hawkins S.A.C., Bellworthy S.J., Tongue S.C., Approaches to investigating transmission of spongiform encephalopathies in domestic animals using BSE as an exemple, Vet. Res. (2008) 39:34.

[72] Soto C., Anderes L., Suardi S., Cardone F., Castilla J., Frossard M.J., et al., Pre-symptomatic detection of prions by cyclic amplification of protein misfolding, FEBS Lett. (2005) 579:638–642.

[73] Spraker T.R., O'Rourke K.I., Balachandran A., Zink R.R., Cummings B.A., Miller M.W., Powers B.E., Validation of monoclonal antibody F99/97.6.1 for immunohistochemical staining of brain and tonsil in mule deer (*Odocoileus hemionus*) with chronic wasting disease, J. Vet. Diagn. Invest. (2002) 14:3–7.

[74] Stack M., Jeffrey M., Gubbins S., Grimmer S., Gonzalez L., Martin S., et al., Monitoring for bovine spongiform encephalopathy in sheep in Great Britain, 1998–2004, J. Gen. Virol. (2006) 87:2099–2107.

[75] Stack M., Jeffrey M., Gubbins S., Grimmer S., Gonzalez L., Martin S., et al., Monitoring for bovine spongiform encephalopathy in sheep in Great Britain, 1998–2004, J. Gen. Virol. (2006) 87:2099–2107.

[76] Stack M.J., Chaplin M.J., Clark J., Differentiation of prion protein glycoforms from naturally occurring sheep scrapie, sheep-passaged scrapie strains (CH1641 and SSBP1), bovine spongiform encephalopathy (BSE) cases and Romney and Cheviot breed sheep experimentally inoculated with BSE using two monoclonal antibodies, Acta Neuropathol. (2002) 104:279–286.

[77] Thomzig A., Spassov S., Friedrich M., Naumann D., Beekes M., Discriminating scrapie and bovine spongiform encephalopathy isolates by infrared spectroscopy of pathological prion protein, J. Biol. Chem. (2004) 279:33847–33854.

[78] Tyler J.W., Lakritz J., Weaver D., Johnson G., VanMetre D., Smith K., et al., The 14-3-3 cerebrospinal fluid immunoassay lacks utility in the diagnosis of clinical scrapie, J. Vet. Diagn. Invest. (2001) 13:537–539.

[79] van Keulen L.J., Schreuder B.E., Meloen R.H., Mooij-Harkes G., Vromans M.E., Langeveld J.P., Immunohistochemical detection of prion protein in lymphoid tissues of sheep with natural scrapie, J. Clin. Microbiol. (1996) 34:1228–1231.

[80] van Keulen L.J., Schreuder B.E., Meloen R.H., Poelen-van den B.M., Mooij-Harkes G., Vromans M.E., Langeveld J.P., Immunohistochemical detection and localization of prion protein in brain tissue of sheep with natural scrapie, Vet. Pathol. (1995) 32:299–308.

[81] Wadsworth J.D., Joiner S., Hill A.F., Campbell T.A., Desbruslais M., Luthert P.J., Collinge J., Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay, Lancet (2001) 358:171–180.

[82] Weber P., Giese A., Piening N., Mitteregger G., Thomzig A., Beekes M., Kretzschmar H.A., Generation of genuine prion infectivity by serial PMCA, Vet. Microbiol. (2007) 123:346–357.

[83] Wells G.A., Wilesmith J.W., The neuropathology and epidemiology of bovine spongiform encephalopathy, Brain Pathol. (1995) 5:91–103.

[84] Wroe S.J., Pal S., Siddique D., Hyare H., Macfarlane R., Joiner S., et al., Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report, Lancet (2006) 368:2061–2067.

[85] Xiang W., Windl O., Wunsch G., Dugas M., Kohlmann A., Dierkes N., et al., Identification of differentially expressed genes in scrapie-infected mouse brains by using global gene expression technology. J. Virol. (2004) 78:11051–11060.

[86] Yang W.C., Schmerr M.J., Jackman R., Bodemer W., Yeung E.S., Capillary electrophoresis-based noncompetitive immunoassay for the prion protein using fluorescein-labeled protein A as a fluorescent probe, Anal. Chem. (2005) 77:4489–4494.

[87] Zou W.Q., Zheng J., Gray D.M., Gambetti P., Chen S.G., Antibody to DNA detects scrapie but not normal prion protein, Proc. Natl. Acad. Sci. USA (2004) 101:1380–1385.

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-3

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008 年 2 月 28 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Approaches to investigating transmission of spongiform encephalopathies in domestic animals using BSE as an example. Simmons, M. M. et al, Vet. Res., 39, 34 (2008).	公表国 英国	
販売名（企業名）						

研究報告の概要

本稿では，伝染性海綿状脳症（TSE）の伝播性を調べるための実験的アプローチ法を要約し，実験における所見と自然発生する TSE〔主にウシ海綿状脳症（BSE）及びスクレイピー〕との関連性を考察している。BSE はこれまで影響を受けなかった動物種における新規の海綿状脳症であり，点感染源の特徴を有する。本実験では，人工的感染経路（脳内接種）及び自然感染経路（経口）を用いて伝播の効率ならびに宿主の感受性を，特に食用動物種に焦点を当てて特定した。実験的伝播が認められても，曝露時の動物の年齢等，種々のパラメータの影響を受けることから結果の解釈は常に困難であった。しかしながら，ヒツジでは，BSE 陽性ウシの脳を経口投与した雌ヒツジから，その仔ヒツジへの BSE 伝播が示された。

これとは対照的に，スクレイピーは英国で数世紀にわたりヒツジ個体群において地域固有のものであった。それにもかかわらず，スクレイピーの真の垂直（子宮内）伝播は確認されておらず，一方で水平伝播が確認されている。すなわち，疾患を引き起こすには汚染された環境に曝露するだけで十分であると考えられる。特に胎盤はスクレイピーの自然伝播の原因とされており，感染性の PrP^{Sc} プリオンを含むことが立証されている。現時点では多くの疑問が依然として解明されておらず，結論として著者らは，様々な分野の研究者らに対して TSE の特性をより理解するため協力及び支援を強く呼び掛けている。

使用上の注意記載状況・その他参考事項等

BYL-2008-0312

報告企業の意見	今後の対応
本概論は，TSE 研究の複雑さを明らかにしており，反芻動物でない，生物学上遠隔種のトランスジェニックマウスを用いた研究であっても，全ての研究結果は有益であり，疾患管理の向上及び公衆衛生を守る上で役立つであろう。	ヒトに影響するプリオン関連疾患伝播のメカニズムの更なる理解に関連した調査の情報を収集する以外，現時点で新たな安全対策上の措置を講じる必要はないと考える。

39

Vet. Res. 39 (2008) 39:34
DOI: 10.1051/vetres:2008011

www.vetres.org

Review article

Approaches to investigating transmission of spongiform encephalopathies in domestic animals using BSE as an example

Marion Mathieson SIMMONS[1]*, John SPIROPOULOS[1], Stephen Anthony Charles HAWKINS[1], Susan Jane BELLWORTHY[1], Susan Carol TONGUE[2]

[1] Pathology Department, Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB, UK
[2] Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB, UK

(Received 29 October 2007; accepted 12 February 2008)

Abstract – Bovine spongiform encephalopathy was a novel spongiform encephalopathy, in an hitherto unaffected species, that had characteristics of a point source epidemic, with an agent that could have been incorporated into a wide variety of feedstuffs and iatrogenically administered to naïve populations, and there was early evidence that it was not restricted to bovines. It was vital to establish, albeit experimentally, which other species might be affected, and whether the epidemic could be maintained by natural transmission, if the source was removed. In contrast, scrapie has been endemic throughout Great Britain for centuries, is maintained naturally (even if we don't know exactly how) and has a known host range. The principles, process and integration of evidence from different types of studies, however, are similar for both of these transmissible spongiform encephalopathies (TSE) and can be applied to any emerging or suspected spongiform encephalopathy. This review discusses the experimental approaches used to determine TSE transmissibility and infectivity and how they relate to natural disease and control measures.

TSE / transmission / natural / experimental / domestic animals

Table of contents

* Corresponding author:
m.m.simmons@vla.defra.gsi.gov.uk

Article available at http://www.vetres.org or http://dx.doi.org/10.1051/vetres:2008011

1. INTRODUCTION

1.1. Spongiform encephalopathies of animals

The spongiform encephalopathies of animals include scrapie, chronic wasting disease (CWD), transmissible mink encephalopathy (TME), bovine spongiform encephalopathy (BSE), feline spongiform encephalopathy (FSE), the spongiform encephalopathies seen in non-domestic captive ungulate species such as eland, oryx and greater kudu, and captive ostriches [85–87]. Spongiform change can also be seen in other diseases, such as rabies, other viral diseases [14, 29, 88], and hepatic encephalopathies. They may be encountered as a genetic or congenital problem [62, 63, 102], as an incidental finding in normal sheep [126], or even as an artefact [108].

However, the only observed natural animal-to-animal transmission of a spongiform encephalopathy occurs in ruminants: scrapie in small ruminant species, CWD in deer and elk, and possibly BSE in small ruminants (although this latter example has only been observed in an experimental flock [8]). Natural spongiform encephalopathies in other species, including humans, are either genetic in origin (e.g. Gerstmann-Sträussler-Scheinker disease, fatal familial insomnia) or have been linked predominantly with an idiopathic transmission mechanism i.e. exposure to contaminated feedstuffs (TME, BSE, FSE, and kuru in humans). There is no recorded occurrence of spongiform encephalopathies being able to transmit effectively within non-ruminant species.

The naturally occurring transmissible spongiform encephalopathies (TSE) are invariably fatal, have long incubation periods and provoke no overt immune response in the host. In some, such as scrapie, there are known genetic effects on whether exposure leads to the development of clinical disease [98, 100]. Additional factors that may affect host susceptibility have been proposed [25, 45, 93] and there could be other unconfirmed, or even as yet unidentified, factors that might affect host susceptibility.

1.2. Aim and objectives

An integral part of the classification of spongiform encephalopathies is whether they are transmissible or not. If it is possible to experimentally transmit "to pass or hand on" [4] i.e. transfer the disease, then it has the potential to be naturally infectious. An infectious disease is one that is due to the "transmission of a specific agent, or its toxic products from an infected person, animal, or reservoir to a susceptible host, either directly or indirectly through an intermediate plant or animal host, vector, or the inanimate environment" [71]. This has implications for disease control strategies; different approaches will be needed if there is an infectious component than if the disease was purely due to a nutritional or genetic cause. It should also be noted that an infectious disease may not be contagious – where contagious is defined as "the communication of disease by direct or indirect contact" i.e. it is communicable to other individuals [3].

Experimental approaches to the investigation of whether transmission occurs have become more sophisticated since the start of the 20th century when Cuillé and Chelle first achieved experimental transmission of sheep scrapie via the conjunctival route in France in 1936 [17, 18]. This experimental evidence of transmissibility was confirmed, somewhat unintentionally, by the iatrogenic transmission of scrapie from sheep to sheep via the medium of

a louping ill vaccine, which led to outbreaks in Great Britain during the 1930s [41].

In this review on the transmission of TSE in animals our first objective is to illustrate the route to the designation of a spongiform encephalopathy as "transmissible", through the example of BSE in the 20th century. The knowledge that a spongiform encephalopathy is transmissible then leads to the question of the relevance of experimental findings to the field situation, where the required outcomes are public health protection, disease control and, ultimately, disease eradication. This then is our second objective; to put transmissibility into a "real-world" context. Scrapie and BSE are our main examples, with other TSE of animals referred to where appropriate. We also aim to briefly highlight some of the challenges and unanswered (or unanswerable) questions that are inevitably raised when a novel spongiform encephalopathy is encountered, and its ability to transmit is investigated.

1.3. Definitions

- PrP^{Sc}: "Prion protein". An abnormal isoform of a naturally occurring host protein (PrP^{C}) which is resistant to proteolysis.
- End-stage/clinical disease: presence of clinical signs and PrP^{Sc} in brainstem and/or lymphoreticular system (LRS).
- Positive animal: PrP^{Sc} detectable, regardless of location (i.e. central nervous system (CNS), peripheral nervous system, lymphoreticular system) or clinical status.
- Exposed animal: known challenge with positive material, or contact with positive animals or a contaminated environment. May or may not also be in one of the categories above.
- Negative animal: no detectable PrP^{Sc} in any tissue tested (must include CNS (if animal dead) and/or LRS).
- Negative control: animal from a flock or farm with good records, no recorded TSE and a feeding history which does not include meat and bone meal supplements.
- Vertical transmission: transmission from one generation to the next via the germline or in utero [11].
- Horizontal transmission: lateral spread to others in the same group and at the same time; spread to contemporaries [11].
- Maternal transmission: there is some difficulty in separating possible horizontal and vertical components to transmission involved with the dam-offspring relationship, and so the term "maternal transmission" is often used in discussion of the transmission of scrapie, maternal transmission being defined as transmission before or immediately after birth.

2. CONFIRMATION OF DISEASE AND/OR INFECTION

The absolute nature of the infectious agent poses a unique challenge and is still a contentious issue. Accumulations of disease-specific prion protein (PrP^{Sc}) in the CNS can be demonstrated in all cases of clinical disease, so the detection of PrP^{Sc} is now used to confirm the disease status of a clinically suspect case at post-mortem [76]. PrP^{Sc} accumulations in a variety of tissues can also be seen in the absence of clinical signs and the demonstration of their presence is generally considered as evidence of exposure and infection. However, such PrP^{Sc} accumulation occurs relatively late in the incubation period of the disease [6, 117], so this reliance on the presence of PrP^{Sc} limits in vivo diagnosis of disease, and surveillance for evidence of exposure or infection, with current diagnostic tools [76]. The currently accepted paradigm is that accumulations of PrP^{Sc} are not only associated with disease, but are also associated with transmission and infectivity [92]. Whether it is the sole infectious component is still a subject of some dispute. Firstly, naturally occurring PrP^{Sc}, when used for transmission experiments, is inevitably contained in a suspension of the tissue in which it originated, and therefore the existence of another factor, or factors, coexisting with PrP^{Sc}, and responsible for infectivity cannot be unequivocally excluded. Secondly, disease has been experimentally produced by tissue suspensions from potentially infected animals in which no PrP^{Sc} was demonstrable with current diagnostic tools [69]. However, in order to investigate

transmission of spongiform encephalopathies, all studies currently use the presence of PrP^{Sc} as a confirmatory marker of disease or exposure/infection.

In experimental studies of TSE, the prolonged incubation periods and the availability of resources coupled with welfare considerations may not allow for individual animals to be followed up to the ultimate fatal endpoint. For this reason there is a lexicon of terms that are applied both in experimental studies and surveillance (see Section 1.3.).

3. THE SEARCH FOR EVIDENCE OF TRANSMISSION OF BOVINE SPONGIFORM ENCEPHALOPATHY

Experimental transmission studies in a wide range of recipient species have established that many species are susceptible to parenteral exposure with positive tissue from TSE cases under experimental circumstances (e.g. cattle, sheep, goats, cats, mink, deer, elk, exotic ungulates, primates, laboratory rodents). Detailed reviews of these transmissions have been published recently [52] and will not be repeated here.

3.1. Bovine spongiform encephalopathy – a TSE?

Following the identification of BSE in cattle [107] and its epidemiological link to contaminated feed [118, 119], the major transmission questions to be addressed, as in any other new disease, were:

- Can it be transmitted?
- Who or what can it be transmitted to in order to determine the potential host range, which food animal species are susceptible, and if there is a public health risk?
- How much is required to achieve transmission/infection, to define infectious dose and host susceptibility?

Then, if and when transmission is achieved:

- What is the pathogenesis of the resulting disease, what is the earliest time at which evidence of exposure can be detected and in which tissue(s)?
- What are the possible routes and mechanisms of transmission under natural as well as experimental conditions?
- What is the relative importance of identified routes and mechanisms in the transmission of the disease under natural conditions in the original host and other species?

Only then can fully effective steps be taken to intervene and minimise any risks to public or animal health that may arise.

3.2. Experimental transmission studies

3.2.1. Artificial exposure – artificial routes

Some of these questions were addressed for BSE initially by experimental transmission studies (see Tab. I for details [6, 8, 9, 20, 21, 26, 32, 35, 46, 49, 54, 58–60, 72, 83, 97, 109–114, 116, 117, 124]). In the case of BSE, a sense of urgency accompanied these investigations, partly as a consequence of the subsequent emergence of similar disease in a range of other species [57, 64, 125], and partly because infected animals would have entered the human food chain. Historically the most efficient transmission route to use to provide an indication of potential host range susceptibility was that of intracerebral inoculation (i.c.). Initial studies established that transmission of disease to food animal species using CNS tissues from natural cases of BSE in cattle was possible to cattle, sheep, goats and pigs but not chickens.

Table I summarises the experimental challenges that have been undertaken using cattle BSE as a source, and food animal species as recipients. A similar range of studies could be listed for other donor species/strains (in particular scrapie and CWD), and indeed for BSE challenges into non-food animal recipients, but exhaustively listing these is considered to be beyond the scope of this paper.

3.2.2. Artificial exposure – natural routes

The next stage was to establish if susceptibility could also be demonstrated by more natural routes of infection. The natural route(s) for the transmission of TSE in the field is still not known, but for most experimental purposes the oral route is considered appropriate.

Table I. Food animal species susceptibility to BSE – summary of experimental transmissions from bovine tissue.

Recipient species/ genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Cattle	i.c./i.v.	Brain	0.1/0.5	N/A	16	4–5 months	74–90 weeks	Proof of transmissibility within species[1] [20]. End stage disease looks the same as natural disease regardless of route [110]
Cattle	Oral	Brain	100	$10^{3.46}$	30	4 months	Timed kills	Pathogenesis of BSE in original host [111]. BSE infectivity identified in the CNS, ileum [109] and bone marrow [112] of pre-clinical cases. Endstage disease after experimental challenge is the same as natural disease [46, 110]
Cattle	Oral	Brain	3 × 100, 100, 10, 1, 0.1, 0.01, 0.001	$10^{3.5}$	10–15 per group (total n = 90)	4–6 months	34–98 months	Determination of LD_{50} and minimum infectious dose of BSE in cattle [117]. Establish attack rate for interpretation of pathogenesis study [6]. Establish minimum effective dose for epidemiological modelling. Confirm that experimental endstage disease looks the same regardless of dose and incubation period (Simmons, unpublished data)
Cattle	i.c.	Brain	Log dilutions	N/A	24 (4 per group)	4 months	20–39 months	Comparative titration BSE in cattle and mice showed that cattle approx. 500 times more sensitive than mice (Cattle 10^6, mice $10^{3.3}$)[2]
Cattle	i.c.	Range of tissues from initial pathogenesis study time kills	0.1	N/A	325 in groups of 5	4–6 months	23–45 months depending on tissue	In addition to CNS, palatine tonsil [114] and nictitating membrane (Wells, Hawkins, unpublished data) harbour BSE infectivity in cattle. The majority of peripheral tissues assayed were negative (Hawkins, unpublished data)
Cattle	Oral	Brain	100 – 3 × 100	$10^{2.86}$	24	6 months	Time kills	Early pathogenesis and the involvement of Peyer's patches in the distal ileum [97]

Table I. (continued).

Recipient species/genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Cattle	Oral	Brain		N/A	56	4–6 months	Time kills (ongoing)	Pathogenesis in original host [49]
Cattle	Oral	Brain	100 or 1	$10^{3.1}$	200	4 months	Timed kills up to a clinical end-point of 30–78 months	Pathogenesis of BSE in original host. Distribution of tissue infectivity in cattle using a range of statutory screening tests to ensure that SRM controls remain appropriate [6]. Provision of tissue bank (including milk) for future test evaluation. End-stage experimental disease looks like end-stage natural disease (Hawkins and Simmons, unpublished data). No PrP^{Sc} in milk from affected animals [26]
Cattle	Oronasal	Foetal membranes	90 mL oral, 5 mL nasal of a 50% homogenate	N/A	12	2–3 months	Animals survived to 7 years	No demonstrable infectivity in foetal membranes[2] [20]
Cattle	Embryo transfer	Live embryos from clinically affected donors	N/A	N/A	347	Young adult	N/A	BSE cannot be transmitted through embryo transfer [124]
Sheep (positive and negative line Cheviots)	i.c.	Brain	0.5 mL of 10% homogenate	N/A	11	6–18 months	440–994 days	Sheep are susceptible to BSE, including sheep not universally susceptible to scrapie [32]
Sheep (positive and negative line Cheviots)	Oral	Brain	50 mL of 1% homogenate	N/A	12	6–18 months	538–994 days	Sheep are susceptible to BSE by this route [32]
Sheep ARQ/ARQ	Oral	Brain	5 g	$10^{3.97}$	20	6 months	664-909 days	Distribution of infectivity in positive sheep [59]. Important for SRM and risk analysis. Verification that the BSE/scrapie discriminatory tests work in the ARQ/ARQ genotype [58]

Table I. (continued).

Recipient species/genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Sheep ARQ/ARQ	Oral	Brain	5, 0.5, 0.05, 0.005, 0.0005	$10^{3.97}$	120	3–6 months	Ongoing at VLA	Establishes minimum infectious dose of BSE in sheep by oral route, contributing to epidemiology and risk models. Endstage disease is the same regardless of dose (Bellworthy, Jeffrey, unpublished data)
Sheep ARQ/ARR ARR/ARR	Oral	Brain	5 g	$10^{3.97}$	20 each	6 months	Ongoing at VLA	Are these genotypes susceptible by the oral route? Relevant for genotype-based disease control strategies. What is distribution of infectivity if they are? Is there any evidence of carrier state?
Sheep ARQ/ARQ	Oral	Brain	5 g	$10^{3.97}$	30	6 months	569–1 058 days	Provision of material for statutory controls and other requests. Provision of milk from sheep with BSE. Create a BSE affected flock to establish if transmission could occur to in-contact animals and lambs [8]
Sheep ARQ/ARQ	Oral	Brain	5 g	$10^{3.97}$	8	2 weeks	535–824 days	Lower age at challenge reduces spread of incubation period, but does not shorten the minimum incubation period (Bellworthy, unpublished data)
Sheep AHQ/AHQ	Oral	Brain	5 g	$10^{3.97}$	5	6 months	568–665 days	Susceptibility and end-stage disease in particular genotype. Relevant for genotype-based disease control strategies. Verification that discriminatory tests work in the genotype. Contribution to BSE "flock" [8]
Sheep VRQ/VRQ	Oral	Brain	5 g	$10^{3.97}$	5	6 months	2 clinical supects at 1 570 days	Susceptibility of particular genotype. Relevant for genotype-based disease control strategies. Verification that discriminatory tests work in the genotype. Contribution to BSE "flock" [8]
Sheep ARQ/ARQ VRQ/VRQ VRQ/ARQ ARQ/ARR ARR/ARR	i.c.	Brain	0.05 g	N/A	19 (ARQ/ARQ) 10 (VRQ/VRQ) 10 (VRQ/ARQ) N/A (ARQ/ARR) 19 (ARR/ARR)	N/A	495–671 days (ARQ/ARQ) 881–1 092 days (VRQ/ARQ and VRQ/VRQ) 1 008–1 127 days (ARR/ARR)	The ARQ/ARQ, VRQ/VRQ, VRQ/ARQ and ARR/ARR genotypes of sheep are all susceptible to infection with BSE, with shorter incubation period (by this route) in ARQ/ARQ than other genotypes challenged [54]. There were survivors in all genotype groups at the time of publication. Sheep with resistant PrP genotypes are susceptible to BSE [54]. Potentially relevant for genotype-based disease control strategies

Table I. (continued).

Recipient species/genotype (where relevant)	Route of inoculation	Donor tissue	Amount (g)	Titre in RIII mice* (if known)	No. of animals challenged	Age at dosing	Incubation period	Key policy outcomes/questions addressed
Sheep ARQ/ARQ	Intraperitoneal/ intrasplenic	Brain	N/A	N/A	1 for each route	N/A	672 days and 1 444 days	Widespread peripheral tissue involvement, including muscle [9, 72]
Sheep ARR/ARR	Intraperitoneal/ intrasplenic	Brain	5 mL of 10% homogenate	N/A	1 for each route	N/A	No clinical disease	BSE-related PrP can accumulate in tissues of "scrapie resistant" sheep without any clinical signs. Evidence of potential carrier state? [9, 83]
Goats	i.c.	Brain	0.5 mL of 10% homogenate	N/A	3	4–6 years	506–570 days	Species susceptible [32]. Define end-stage disease [32, 35]
Goats	Oral	Brain	50 mL of 1% homogenate	N/A	3	2–5 years	941–1 501 days	Species susceptible by oral route (one survivor) [32]. Define end-stage disease [32, 35]. Discriminatory tests work in this species [60]
Pigs	i.c./i.v./i.p.	Brain	0.5 mL/1.2 mL/ 8–9 mL	N/A	10	1–2 weeks	69–150 weeks	Species susceptible [21]. Define end-stage disease [113]
Pigs	Oral	Brain	3 × MBM ration equivalent for an 8 week old pig	$10^{2.4}$	10	7–14 weeks	Time kills at 2 and 7 years	Species not susceptible to experimental challenge by this route [113]
Chicken	i.c./i.p.	Brain	50 µL/1 mL of 10% homogenate	N/A	12	1–14 days	N/A Survived up to 5years	Species not susceptible [116]
Chicken	Oral	Brain	5 g on 3 occasion	N/A	11	4–6 weeks	N/A Survived up to 5years	Species not susceptible to experimental challenge by this route [116]
Deer	i.c.	Brain	0.05 g	$10^{3.3}$	6	10–12 months	794–1 060 days (one still alive)	Species susceptible. Define endstage disease (Jeffrey M., personal communication)
Deer	Oral	Brain	25 g	$10^{3.1}$	18	4–6 weeks	Time kills	Is this species susceptible by this route? Ongoing. Negative to date – 4 years post challenge (Jeffrey M., personal communication)

* Mouse (i.c./i.p.) units LD_{50}/g.

[1] Dawson M., Wells G.A., Parker B.N.J., Scott A.C., Transmission studies of BSE in cattle, hamsters, pigs and domestic fowl, in: Bradley R., Savey M., Marchant B. (Eds.), Subacute spongiform encephalopathies, Proc. of a seminar in the CEC Agricultural Research Programme held in Brussels, 12–14 November 1990, Kluwer Academic Publishers, 1991, pp. 25–32.

[2] Hawkins S., Wells G., Austin A. et al., Comparative efficiencies of the bioassay of BSE infectivity in cattle and mice, in: Proc. of the Cambridge Healthtech Institute's 2nd Int. Transmissible Spongiform Encephalopathies Conference, 2–3 October 2000, Alexandria, VA, USA, 2000.

For BSE, epidemiological studies indicated that the oral ingestion of food contaminated with infected ruminant-derived protein, in the form of meat and bone meal (MBM), by cattle was likely to be a major route of transmission [118, 120]. Oral challenge studies showed that transmission of BSE was possible to sheep, goats and cattle by this route [32, 59, 111] and with very low challenge doses [117]. Transmission to pigs or chickens however was not achieved by this experimental route [113, 116].

One difficulty that arises with transmission experiments is the interpretation of a negative result; does it mean that transmission does not occur, or just that in this particular scenario it hasn't? The latter then raises questions as to why it may not have occurred. Is it the dose, route, or are there other factors involved such as species barriers or genetic influences? Given that BSE could be transmitted to pigs by the i.c. route, the absence of BSE transmission to pigs by the oral route indicated that there may be an effective species barrier, but a particular confounding factor in this type of study is that the infectious "dose" of any challenge inoculum is difficult to establish objectively. In most cases, inoculum titre (if known) is quoted as mouse LD50/g, using conventional inbred mice. However, we know that different hosts are differently susceptible [88] and that some TSE isolates do not transmit to particular species (including mice). Any experimentally-established titre is inevitably relative, and not necessarily informative for the recipient species in a particular experiment. Attempts to determine PrP^{Sc} concentration biochemically as a measure of titre are also limited by the assumption that PrP^{Sc} is an accurate and quantifiable marker for infectivity.

Conversely, a positive result in a transmission experiment only means that transmission can occur, not that it *does* in field conditions. It also leads to further questions. One is the relevance of such experiments to the field situation. There are a number of fundamental differences between natural exposure and experimental studies which should not be overlooked when comparing disease models with field cases. In natural disease, the incidence of TSE can be low but in experimental disease the aim is to achieve 100% morbidity, especially if the study contains a time-kill element. Very high doses can be given orally and such experimental exposures result in much higher attack rates than are observed naturally [117]. Time-kill approaches can then be used to study the disease pathogenesis in an experimental model, although it is not known what effect dose may have on pathogenesis. It is reassuring, therefore, that the end-stage disease resulting from such experimental exposure of cattle with BSE is virtually indistinguishable from natural cases in all but morbidity [46].

This experimental approach also assumes an oral route of transmission in the field, which is a reasonable assumption for BSE, given the clear epidemiological links with contaminated feed. However, the infectious material in feed has been subjected to a range of manufacturing processes and heat treatments in the course of MBM production, and experimental studies often use "neat" brain material (untreated) to achieve the best morbidity, since rendering has been shown to reduce titre [27, 91, 94–96]. The possible effects of rendering on the basic biological properties of any given TSE strain are very difficult to define, and almost impossible to control for in any experimental design.

It has also been suggested that age at exposure can affect susceptibility [5], but most experimental designs have focussed on a restricted range of ages at challenge from a logistical point of view.

None of these factors negates the data emerging from such experimental studies, as the studies provide a starting point. Once transmission has been achieved, further experimental protocols can be used to investigate aspects such as minimum effective doses [117], and inoculum can be treated to mimic more closely what is occurring in the field [95]. Data derived from transmission experiments can also be used in risk analyses and mathematical models, both of which may be used to inform the development of appropriate control strategies for TSE in animals, and thus to protect public and animal health. Further studies can also be implemented, as they were with BSE, to investigate hypotheses of the origin of

the disease (for example scrapie to cattle [66]), but countless variables prevent this approach from being comprehensive.

3.2.3. *Natural transmission*

With experimental confirmation that transmission is possible by a particular route, further investigation of the contribution of that route to natural transmission is vital.

For BSE it was clear that the principal driver of the epidemic was the feeding of contaminated MBM [120] – once relevant control measures were introduced the epidemic in Great Britain began to decline [50, 122] – but it was not initially known whether the disease could be sustained within an affected population by other natural or management means.

Evidence from a cohort study did not rule out the possibility of a maternal component to transmission [121]. The risk of developing BSE was slightly increased by being born to a dam approaching the clinical phase of the disease. Whether it represented genetic susceptibility, transmission or a combination of the two could not be determined. However, mathematical modelling indicated that if maternal transmission did occur, then it was highly unlikely to be at a rate that could sustain an epidemic [23]. The route through which such exposure might take place, whether it was due to true vertical transmission, or horizontal transmission through close contact also could not be established from the cohort study. A long-term large-scale experimental study to investigate the possibility of vertical transmission indicated that, "when appropriate sanitary protocols" were followed, "embryos derived from BSE-affected cattle did not transmit the disease" [124].

Ultimately for BSE in cattle the relative importance of the role of feed contaminated with infected MBM was confirmed, and the relative absence of evidence for maternal transmission [23] has enabled effective disease control interventions to be implemented.

4. BSE IN SMALL RUMINANTS

The positive results of oral transmission experiments to sheep and goats [32, 59], and the identification of a single natural case of BSE in a French goat [24] have, however, raised a new challenge: that of BSE in small ruminants. For Great Britain, this raised a concern about the national sheep population. With, hopefully, no naturally-occurring disease to study there remain only three alternatives.

Firstly, to set up small-scale animal experiments (as previously described) to investigate potential routes and mechanisms of transmission; secondly to set-up larger-scale natural transmission investigations, such as an experimental sheep flock; and thirdly, to find an alternative natural disease model that can be studied in the field.

4.1. Direct experimental exposure

Transmission of BSE in small ruminants by blood transfusion has been studied by the first method. Whilst experimental BSE can be transmitted by whole blood transfusion [53], this probably has more relevance in the establishment of a precedent for the protection of public health in the context of human-to-human transmission [1], rather than as a potential iatrogenic route in small ruminants.

4.2. Natural transmission experiments

The second method (the experimental sheep flock) has been used by both the Veterinary Laboratories Agency (VLA) and the Institute for Animal Health Neuropathogenesis Unit (NPU) in Edinburgh. The VLA has an experimental BSE-in-sheep flock in which lambs born to ewes that were orally dosed with 5 g of BSE-positive cattle brain have succumbed to clinical disease [8]. The age at onset for these lambs ranged from 654 to 968 days old. In all cases the birth of the lambs occurred within a few months prior to the onset of clinical disease in their dams. Thus we have evidence of natural transmission of BSE from sheep to sheep, albeit in experimental circumstances. Whether this represents true vertical or perinatal infection cannot be ascertained from this study. A similar but slightly different NPU study [36] did not result in transmission, however it could not be statistically ruled out.

4.3. Alternative disease models

The third method, to find an alternative natural disease model that can be studied in the field, is more problematic. Studies of the natural transmission of the only known naturally-occurring TSE of small ruminants, scrapie, might provide a model for BSE in sheep, should it occur under field conditions. Both scrapie and experimental BSE in sheep have similar clinical signs and they have similar diffuse tissue distributions of PrP^{Sc} [34, 35, 59, 115]. If natural ovine BSE is similar to experimental ovine BSE, then ovine BSE may potentially behave in a similar manner to scrapie as far as routes and mechanisms of transmission are concerned.

4.3.1. Scrapie

This is the most extensively studied TSE model. Several institutions have established, maintained and recorded naturally infected flocks of sheep in order to study various aspects of scrapie, including its transmission. These include the INRA Langlade flock of Romanov sheep, various Institute for Animal Health flocks and the VLA scrapie-affected flock.

Analyses of data collected over more than a decade from the first of these have provided epidemiological evidence for both a maternal and lateral component of transmission [22, 99]. Higher relative risks of clinical scrapie were observed associated with lambing periods. There was also a reduced risk of clinical scrapie in artificially-reared lambs from healthy dams, and an increased risk in maternally-reared lambs from scrapie-affected dams. They proposed that transmission may occur within the first 24 h of life with additional risk for those that then continue to share the maternal environment (all lambs remained on their dams for the first intake of colostrum and then for 24 h).

The Institute for Animal Health flocks have established that, despite earlier contradictory findings [30, 31, 33], true vertical transmission of ovine scrapie (via the germ-line or in utero) is improbable [36, 37]. A scrapie-free flock has been established by embryo-transfer (ET) from one with a long-standing scrapie problem. The ET-derived flock has remained scrapie-free since its establishment in 1996, even though it has a similar *PrP* genetic profile to the original flock. Of interest to mechanisms of horizontal/lateral transmission is the fact that the "clean" flock was established and maintained in a scrapie naïve environment; a parallel ET-derived flock that was maintained in close proximity to, but separate from, the original scrapie-affected flock did experience clinical scrapie cases [37]. Lateral transmission has also been shown to occur in the absence of lambing [38].

In the VLA flock it has been shown that lateral transmission occurs [84] and that exposure to a contaminated environment only is sufficient to produce disease (Dexter, Tongue, Bellworthy, unpublished data).

These flocks are managed in a way that maintains high frequencies of sheep with PrP genotypes at high risk of developing clinical disease. Thus with a high incidence of clinical disease and high infectious load, they also provide controlled environments in which to study the pathogenesis of naturally acquired disease. They effectively counter the difficulties of studying a disease that occurs at a low flock-level incidence, however it must be recognised that whilst they provide evidence for routes and mechanisms of natural transmission and estimates of transmission parameters, they are probably not representative of any but the most heavily affected (worst-case scenario) commercial flocks. They are also limited in the range of breeds present, and (potentially) in the number of different scrapie isolates/strains present. These flocks may mimic natural exposure, but at a level that no commercial flock-owner would be able to tolerate and remain as an economically viable unit. Because of this the relative importance of different components of transmission may vary in commercial field flocks and therefore intervention measures may have different outcomes. These institutionalised research flocks, therefore, act as an important bridge between the artificial exposure – natural route transmission experiments – and the true field situation.

A variety of experimental studies using the approaches outlined above have provided

evidence for possible routes of transmission of scrapie. PrP^{Sc} has been found in tissues that could be involved in the natural dissemination of the infectious agent i.e., routes that could lead to exit of the infectious agent from the animal, and result in either environmental contamination or direct transmission. These tissues include the lympho-reticular system of the gut [40, 103, 115], chronically inflamed mammary tissue associated with lymphocyctic mastitis [73], kidney tissue [90], salivary glands [104], nictitating membrane [77], and placentae [2, 81, 101].

For the majority of these tissues, evidence of infectivity or the presence of PrP^{Sc} in associated secretions and excretions is still elusive for scrapie in small ruminants. The exception is blood [55]. Although experimental blood transfusions have resulted in clinical scrapie [55], just as with BSE, it is unlikely to play a major role: blood transfusions are not regular occurrences in sheep veterinary practice.

On the other hand, not only has PrP^{Sc} and infectivity been demonstrated in placentae [3, 81, 101], but it has also been shown to produce clinical scrapie when administered orally to sheep [78, 79]. This was proposed by the authors as a mechanism for lateral transmission from ewe to ewe at lambing time. Placenta has also been cited as a possible explanation for some of the epidemiological findings thought to be associated with mechanisms of maternal transmission [74], although much of the epidemiological evidence may also be interpreted as a contribution to transmission via the lateral route, especially that of environmental contamination. For example, there are reduced odds of ever becoming a scrapie-affected flock if the flock sometimes lambs in different places, compared to those flocks that always lamb in the same place [74]; there is decreased risk of disease associated with lambing in individual pens [75], and there were increased odds for scrapie-positive status of a flock that was found to be associated with failure to remove placenta from bedding along with its disposal in compost.

Epidemiological cross-sectional [74, 75] and case-control studies [47, 51, 80] have provided supporting evidence for the role of various allied management practices in the transmission of scrapie in the field. So far they lack the consistency and specifics necessary for the development of appropriate intervention measures. The scrapie literature does however illustrate how the different types of investigations into aspects of transmission, and the different disciplines, are complementary. Experimental studies of transmission routes and epidemiological studies of risk factors are intrinsically linked in a positive feedback loop, each informing the other.

4.3.2. Chronic wasting disease

The other naturally occurring TSE, CWD of deer is probably less relevant as a model for BSE in small ruminants, has been recently reviewed elsewhere [123] and is covered by Sigurdson in this special issue [89].

4.3.3. Other disease models

Host-specific experimental studies in large animals are expensive and do take time to produce results. The former means that they are difficult to fund. The latter means that they may have to be run in parallel with other experiments, often with more start-up assumptions than desirable, rather than in a logical step-wise order following on from previous findings. They are, however, of paramount importance. They provide an opportunity to study the disease in the original host species; they can be comparable across studies, if standardised protocols are used, and they eliminate the noise of variability, the difficulties of loss to follow-up and the potential biases that are experienced with epidemiological studies. To counter the time and resource limitations, other models have been sought.

The role of hamsters, mice, the burgeoning range of murine transgenes and other models such as voles is a large subject in its own right, and is covered by Groschup and Buschmann in this special issue [44] and elsewhere [28, 43]. In the past such models have been useful [12, 13], but they also have limitations. For example, laboratory wild-type mice cannot replace the original donor species due to the species-transmission barrier and to their different biology and physiology compared

to ruminants. The former has been addressed with the advent of transgenic mice, the latter is insurmountable. Even these do not replicate reality, and the interpretation and extrapolation of any results back to the donor/host-species needs to be a considered, objective process. For example, data from different transgenic mouse lines are not directly comparable, even between lines which have a common transgene [16, 105].

5. PUBLIC HEALTH

The ultimate question of whether a TSE has implications for public health – i.e. is transmissible to man – is difficult to address in the absence of transmission experiments on people. The most appropriate alternative is to use non-human primates [48, 67, 68, 70] which have indicated that BSE transmits with a end-stage disease indistinguishable from variant Creutzfeldt-Jacob disease (vCJD). However these experiments are limited by ethical constraints. Here the development of transgenic mice has been of prospective value, but at the same time, can be misleading. For example, mice with a single copy of the human *PrP* gene were not susceptible experimentally to BSE [10] while at the same time, epidemiological and strain-typing studies were producing a very strong body of circumstantial evidence that vCJD was a consequence of BSE infection in man. The inevitable limitation of such transgenic mice is that only one human gene is present in the model, and disease susceptibility and incubation period are inevitably multi-factorial. Transgenic mouse models which overexpress human *PrP* are also available, and they are highly susceptible to BSE [7, 15, 65, 106] but these may not be a true indicator of susceptibility in humans. Detailed discussion of these models is outwith the scope of this paper and is covered in detail by Groschup and Buschmann in this special issue [44].

6. REMAINING CHALLENGES

Many challenges remain even when a spongiform encephalopathy has been identified as transmissible, and when routes and mechanisms have been proposed.

What are the effects of repeated low dose exposure? What happens when there is intercurrent disease? How do *PrP* genetics influence the transmission process? Is any apparent reduction in susceptibility actually an effect of incubation period prolongation to beyond the natural lifespan? What is the implication of carrier state/subclinical disease for disease control and health? How can we detect animals in the early stage of disease incubation – a phase "silent" to current investigative tools?

For BSE and scrapie some of these questions have been addressed partially [39, 42, 45, 49, 56, 61]. It is possible that for novel TSE many of these questions will remain unanswered or unpursued, except by the most determined of researchers after the funding, stimulated by the public health and political aspects of BSE and vCJD, has dwindled.

Perhaps the greatest conundrum for researchers faced with a new TSE in a species, or a TSE in a species in which it has not previously been described, is whether it is "new", or merely "newly observed". This is a particular issue for BSE, should it be found in the sheep population. With much speculation over the years that scrapie could be the origin of BSE, it might not be too surprising if a detailed study of scrapie isolates revealed one with BSE-like characteristics. A number of studies in the UK and elsewhere [19, 66, 82] have taken a direct approach to this question by looking at the experimental phenotype in cattle experimentally challenged with scrapie isolates, but the diversity of scrapie isolates precludes this approach being exhaustive.

Given that no one type of study can provide all the details or all the answers required, and because of the constraints implicit in each type of study, it is important that researchers respect and integrate the work from other areas, are rigorous, do not overestimate their findings despite various pressures to do so, and are honest: both in the presentation of their findings and in the value of the outcomes. Some of those interested in pure science may disparage studies that they deem to be of low scientific merit, but which are actually of high value to those involved in policy and decision-making: equally some work of high scientific merit may

be extremely interesting in its own right, but not actually necessary to advance disease control and protect public health.

7. CONCLUSION

The approaches to the investigation of the transmission of BSE and scrapie, outlined above, differ only slightly. Those differences are due to the nature of the two diseases. BSE was a novel spongiform encephalopathy, in a hitherto unaffected species, that had characteristics of a point source epidemic, with an agent that could have been incorporated into a wide variety of feedstuffs and iatrogenically administered to naïve populations, and there was early evidence that it was not restricted to bovines. It was vital to establish, albeit experimentally, which other species might be affected, and whether the epidemic could be maintained by natural transmission, if the source was removed. In contrast, scrapie has been endemic throughout Great Britain for centuries, is maintained naturally (even if we don't know exactly how) and has a known host range. The principles, process and integration of evidence from different types of studies, however, are similar for both of these TSE and can be applied to any emerging or suspected spongiform encephalopathy.

REFERENCES

[1] Aguzzi A., Glatzel M., vCJD tissue distribution and transmission by transfusion – a worst case scenario coming true?, Lancet (2004) 363:411–412.

[2] Andréoletti O., Lacroux C., Chabert A., Monnereau L., Tabouret G., Lantier F., et al., PrP^{Sc} accumulation in placentas of ewes exposed to natural scrapie: influence of foetal PrP genotype and effect on ewe-to-lamb transmission, J. Gen. Virol. (2002) 83:2607–2616.

[3] Anonymous, Encyclopaedic word dictionary, Hanks P. (Ed.), Paul Hamlyn, London, New York, Sydney, Toronto, 1971.

[4] Anonymous, The concise Oxford English dictionary of current English, 9th ed., Thomson D. (Ed.), Oxford university press, 1995.

[5] Arnold M., Wilesmith J.W., Estimation of the age-dependent risk of infection to BSE of dairy cattle in Great Britain, Prev. Vet. Med. (2004) 66:35–47.

[6] Arnold M.E., Ryan J.B.M., Konold T., Simmons M.M., Spencer Y.I., Wear A., et al., Estimating the temporal relationship between PrP^{Sc} detection and incubation period in experimental bovine spongiform encephalopathy (BSE) of cattle, J. Gen. Virol. (2007) 88:3198–3208.

[7] Asante E.A., Linehan J.M., Desbruslais M., Joiner S., Gowland I., Wood A.L., et al., BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein, EMBO J. (2002) 21:6358–6366.

[8] Bellworthy S.J., Dexter G., Stack M., Chaplin M., Hawkins S.A.C., Simmons M.M., et al., Natural transmission of BSE between sheep within an experimental flock, Vet. Rec. (2005) 157:206.

[9] Bencsik A., Baron T., Bovine spongiform encephalopathy agent in a prion protein $(PrP)^{ARR/ARR}$ genotype sheep after peripheral challenge: complete immunohistochemical analysis of disease-associated PrP and transmission studies to ovine-transgenic mice, J. Infect. Dis. (2007) 195:989–996.

[10] Bishop M.T., Hart P., Aitchison L., Baybutt H.N., Plinston C., Thomson V., et al., Predicting susceptibility and incubation time of human-to-human transmission of vCJD, Lancet Neurol. (2006) 5:393–398.

[11] Blood D.C., Studdert V.P., Baillière's comprehensive veterinary dictionary, Baillière Tindall, London, UK, 1988.

[12] Bruce M.E., Strain typing studies of scrapie and BSE, in: Baker H.F., Ridley R.M. (Eds.), Methods in molecular medicine – Prion diseases, Humana Press, Totowa, USA, 1996, pp. 223–236.

[13] Bruce M.E., Boyle A., Cousens S., McConnell I., Foster J., Goldmann W., Fraser H., Strain characterization of natural sheep scrapie and comparison with BSE, J. Gen. Virol. (2002) 83:695–704.

[14] Bundza A., Charlton K.M., Comparison of spongiform lesions in experimental scrapie and rabies in skunks, Acta Neuropathol. (1988) 76:275–280.

[15] Collinge J., Palmer M.S., Sidle K.C., Hill A.F., Gowland I., Meads J., et al., Unaltered susceptibility to BSE in transgenic mice expressing human prion protein, Nature (1995) 378: 779–783.

[16] Cordier C., Bencsik A., Philippe S., Betemps D., Ronzon F., Calavas D., Crozet C., Baron T., Transmission and characterization of bovine spongiform encephalopathy sources in two ovine transgenic mouse lines (TgOvPrP4 and TgOvPrP59), J. Gen. Virol. (2006) 87:3763–3771.

[17] Cuillé J., Chelle P.L., La maladie dite "tremblante" du mouton ; est-elle inoculable ?, C. R. Acad. Sci. Paris (1936) 203:1552–1554.

[18] Cuillé J., Chelle P.L., La maladie dite "tremblante" du mouton est bien inoculable, C. R. Acad. Sci. Paris (1938) 206:78–79.

[19] Cutlip R.C., Miller J.M., Race R.E., Jenny A.L., Katz J.B., Lehmkuhl H.D., et al., Intracerebral transmission of scrapie to cattle, J. Infect. Dis. (1994) 169:814–820.

[20] Dawson M., Wells G.A., Parker B.N., Preliminary evidence of the experimental transmissibility of bovine

spongiform encephalopathy to cattle, Vet. Rec. (1990) 126:112–113.

[21] Dawson M., Wells G.A., Parker B.N., Scott A.C., Primary parenteral transmission of bovine spongiform encephalopathy to the pig, Vet. Rec. (1990) 127:338.

[22] Diaz C., Vitezica Z.G., Rupp R., Andréoletti O., Elsen J.M., Polygenic variation and transmission factors involved in the resistance/susceptibility to scrapie in a Romanov flock, J. Gen. Virol. (2005) 86:849–857.

[23] Donnelly C.A., Ferguson N.M., Ghani A.C., Anderson R.M., Implications of BSE infection screening data for the scale of the British BSE epidemic and current European infection levels, Proc. Biol. Sci. (2002) 269:2179–2190.

[24] Eloit M., Adjou K., Coulpier M., Fontaine J.J., Hamel R., Lilin T. et al., BSE agent signatures in a goat, Vet. Rec. (2005) 156:523–524.

[25] Elsen J.M., Amigues Y., Schelcher F., Ducrocq V., Andréoletti A., Eychenne F., et al., Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov, Arch. Virol. (1999) 144:431–445.

[26] Everest S.J., Thorne L.T., Hawthorn J.A., Jenkins R., Hammersley C., Ramsay A.M., et al., No abnormal prion protein detected in the milk of cattle infected with the bovine spongiform encephalopathy agent, J. Gen. Virol. (2006) 87:2433–2441.

[27] Fernie K., Steele P.J., Taylor D.M., Somerville R.A., Comparative studies on the thermostability of five strains of transmissible-spongiform-encephalopathy agent, Biotechnol. Appl. Biochem. (2007) 47:175–183.

[28] Flechsig E., Hegyi I., Raeber A.J., Cozzio A., Aguzzi A., Weissmann C., Knockouts and transgenic mice in prion research, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 134–150.

[29] Foley G.L., Zachary J.F., Rabies-induced spongiform change and encephalitis in a heifer, Vet. Pathol. (1995) 32:309–311.

[30] Foote W.C., Clark W., Maciulis A., Call J.W., Hourrigan J., Evans R.C. et al., Prevention of scrapie transmission in sheep using embryo transfer, Am. J. Vet. Res. (1993) 54:1863–1868.

[31] Foster J.D., McKelvey W.A., Mylne M.J., Williams A., Hunter N., Hope J., Fraser H., Studies on maternal transmission of scrapie in sheep by embryo transfer, Vet. Rec. (1992) 130:341–343.

[32] Foster J.D., Hope J., Fraser H., Transmission of bovine spongiform encephalopathy to sheep and goats, Vet. Rec. (1993) 133:339–341.

[33] Foster J.D., Hunter N., Williams A., Mylne M.J., McKelvey W.A., Hope J., Fraser H., Bostock C., Observations on the transmission of scrapie in experiments using embryo transfer, Vet. Rec. (1996) 138:559–562.

[34] Foster J.D., Parnham D.W., Hunter N., Bruce M., Distribution of the prion protein in sheep terminally affected with BSE following experimental oral transmission, J. Gen. Virol. (2001) 82:2319–2326.

[35] Foster J.D., Parnham D., Chong A., Goldmann W., Hunter N., Clinical signs, histopathology and genetics of experimental transmission of BSE and natural scrapie to sheep and goats, Vet. Rec. (2001) 148:165–171.

[36] Foster J.D., Goldmann W., McKenzie C., Smith A., Parnham D.W., Hunter N., Maternal transmission studies of BSE in sheep, J. Gen. Virol. (2004) 85:3159–3163.

[37] Foster J., McKenzie C., Parnham D., Drummond D., Goldmann W., Stevenson E., Hunter N., Derivation of a scrapie-free sheep flock from the progeny of a flock affected by scrapie, Vet. Rec. (2006) 159:42–45.

[38] Foster J., McKenzie C., Parnham D., Drummond D., Chong A., Goldmann W., Hunter N., Lateral transmission of natural scrapie to scrapie-free New Zealand sheep placed in an endemically infected UK flock, Vet. Rec. (2006) 159:633–634.

[39] Goldmann W., Houston F., Stewart P., Perucchini M., Foster J., Hunter N., Ovine prion protein variant A(136)R(154)L(168)Q(171) increases resistance to experimental challenge with bovine spongiform encephalopathy agent, J. Gen. Virol. (2006) 87:3741–3745.

[40] González L., Dagleish M.P., Bellworthy S.J., Sisó S., Stack M.J., Chaplin M.J., et al., Postmortem diagnosis of preclinical and clinical scrapie in sheep by the detection of disease-associated PrP in their rectal mucosa, Vet. Rec. (2006) 158:325–331.

[41] Gordon W.S., Advances in veterinary research. Louping-ill, tick borne fever and scrapie, Vet. Rec. (1946) 58:516–520.

[42] Gravenor M.B., Stallard N., Curnow R., McLean A.R., Repeated challenge with prion disease: the risk of infection and impact on incubation period, Proc. Natl. Acad. Sci. USA (2003) 100:10960–10965.

[43] Groschup M.H., Geissen M., Buschmann A., The experimental transmissibility of prions and infectivity distribution in the body, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 473–478.

[44] Groschup M.H., Buschmann A., Rodent models for prion diseases, Vet. Res. (2008) 39:32.

[45] Gruner L., Elsen J.M., Vu Tein Khang J., Eychenne F., Caritez J.C., Jacquiet P., et al., Nematode parasites and scrapie: experiments in sheep and mice, Parasitol. Res. (2004) 93:493–498.

[46] Hawkins S.A.C., Wells G.A.H., Simmons M.M., Blamire I.W.H., Meek S.C., Harris P., The topographic distribution pattern of vacuolation in the central nervous system of cattle infected orally with bovine spongiform encephalopathy, Bovine Practitioner (1997) 31.2:73–76.

[47] Healy A.M., Hannon D., Morgan K.L., Weavers E., Collins J.D., Doherty M.L., A paired case-control study of risk factors for scrapie in Irish sheep flocks, Prev. Vet. Med. (2004) 64:73–83.

[48] Herzog C., Salès N., Etchegaray N., Charbonnier A., Freire S., Dormont D., et al., Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection, Lancet (2004) 363:422–428.

[49] Hoffmann C., Ziegler U., Buschmann A., Weber A., Kupfer L., Oelschlegel A., Hammerschmidt B., Groschup M.H., Prions spread via the autonomic nervous system from the gut to the central nervous system in catle incubating bovine spongiform encephalopathy, J. Gen. Virol. (2007) 88:1048–1055.

[50] Hoinville L.J., Decline in the incidence of BSE in cattle born after the introduction of the "feed ban", Vet. Rec. (1994) 134:274–275.

[51] Hopp P., Ulvund M.J., Jarp J., A case-control study on scrapie in Norwegian sheep flocks, Prev. Vet. Med. (2001) 51:183–198.

[52] Hornlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter Berlin, New York, 2007.

[53] Houston F., Foster J.D., Chong A., Hunter N., Bostock C.J., Transmission of BSE by blood transfusion in sheep, Lancet (2000) 356:999–1000.

[54] Houston F., Goldmann W., Chong A., Jeffrey M., González L., Foster J., et al., Prion diseases: BSE in sheep bred for resistance to infection, Nature (2003) 423:498.

[55] Hunter N., Foster J., Chong A., McCutcheon S., Parnham D., Eaton S., et al., Transmission of prion diseases by blood transfusion, J. Gen. Virol. (2002) 83:2897–2905.

[56] Jackman R., Everest D.J., Schmerr M.J., Khawaja M., Keep P., Docherty J., Evaluation of a preclinical blood test for scrapie in sheep using immunocapillary electrophoresis, J. AOAC Int. (2006) 89:720–727.

[57] Jeffrey M., Wells G.A.H., Spongiform encephalopathy in a nyala (*Tragelaphus angasi*), Vet. Pathol. (1988) 25:398–399.

[58] Jeffrey M., Martin S., Gonzalez L., Ryder S.J., Bellworthy S.J., Jackman R., Differential diagnosis of infections with the bovine spongiform encephalopathy (BSE) and scrapie agents in sheep, J. Comp. Pathol. (2001) 125:271–284.

[59] Jeffrey M., Ryder S., Martin S., Hawkins S.A., Terry L.M., Berthelin-Baker C., Bellworthy S.J., Oral inoculation of sheep with the agent of bovine spongiform encephalopathy (BSE). 1. Onset and distribution of disease-specific PrP accumulation in brain and viscera. J. Comp. Pathol. (2001) 124:280–289.

[60] Jeffrey M., Martin S., Gonzalez L., Foster J., Langeveld J.P., van Zijderveld F.G., et al., Immunohistochemical features of PrP(d) accumulation in natural and experimental goat transmissible spongiform encephalopathies, J. Comp. Pathol. (2006) 134:171–181.

[61] Kao R.R., Houston F., Baylis M., Chihota C.M., Goldmann W., Gravenor M.B., et al., Epidemiological implications of the susceptibility to BSE of putatively resistant sheep, J. Gen. Virol. (2003) 84:3503–3512.

[62] Kelly D.F., Gaskell C.J., Spongy degeneration of the central nervous system in kittens, Acta Neuropathol. (1976) 35:151–158.

[63] Kinney H.C., Sidman R.L., Pathology of the spongiform encephalopathy in the gray tremor mutant mouse, J. Neuropathol. Exp. Neurol. (1986) 45:108–126.

[64] Kirkwood J.K., Cunningham A.A., Epidemiological observations on spongiform encephalopathies in captive wild animals in the British Isles, Vet. Rec. (1994) 135:296–303.

[65] Kong Q., Huang S., Zou W., Vanegas D., Wang M., Wu D., et al., Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models, J. Neurosci. (2005) 25:7944–7949.

[66] Konold T., Ryder S.J., Lee Y.H., Stack M.J., Horrocks C., Green R., et al., Different prion disease phenotypes result from inoculation of cattle with two temporally separated sources of sheep scrapie from Great Britain, BMC Vet. Res. (2006) 2:31.

[67] Lasmézas C.I., Comoy E., Hawkins S., Herzog C., Mouthon F., Konold T., et al., Risk of oral infection with bovine spongiform encephalopathy agent in primates, Lancet (2005) 365:781–783.

[68] Lasmézas C.I., Deslys J.P., Demaimay R., Adjou K.T., Lamoury F., Dormont D., et al., BSE transmission to macaques, Nature (1996) 381:743–744.

[69] Lasmezas C.I., Deslys J.P., Robain O., Jaegly A., Béringue V., Peyrin J.M., et al., Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein, Science (1997) 275:402–405.

[70] Lasmézas C.I., Fournier J.G., Nouvel V., Boe H., Marcé D., Lamoury F., et al., Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications for human health, Proc. Natl. Acad. Sci. USA (2001) 98:4142–4147.

[71] Last J.M., A Dictionary of Epidemiology, 2nd ed., Oxford University Press, New York, USA, 1988.

[72] Lezmi S., Ronzon F., Bencsik A., Bedin A., Clavas D., Richard Y., et al., PrP^{d} accumulation in organs of ARQ/ARQ sheep experimentally infected with BSE by peripheral routes, Acta Biochim. Pol. (2006) 53:399–405.

[73] Ligios C., Sigurdson C.J., Santucciu C., Carcassola G., Manco G., Basagni M., et al., PrP^{Sc} in mammary glands of sheep affected by scrapie and mastitis, Nat. Med. (2005) 11:1137–1138.

[74] McIntyre M., Gubbins S., Sivam S.K., Baylis M., Flock-level risk factors for scrapie in Great Britain: analysis of a 2002 anonymous postal survey, BMC Vet. Res. (2006) 2:25.

[75] McLean A.R., Hoek A., Hoinville L.J., Gravenor M.B., Scrapie transmission in Britain: a recipe for a mathematical model, Proc. Biol. Sci. (1999) 266:2531–2538.

[76] Office Internationale Epizootie, Bovine spongiform encephalopathy, in: OIE manual of diagnostic

tests and vaccines for terrestrial animals, 5th ed., 2004, pp. 549–569.

[77] O'Rourke K.I., Baszler T.V., Besser T.E., Miller J.M., Cutlip R.C., Wells G.A., et al., Preclinical diagnosis of scrapie by immunohistochemistry of third eyelid lymphoid tissue, J. Clin. Microbiol. (2000) 38:3254–3259.

[78] Pattison I.H., Hoare M.N., Jebbett J.N., Watson W.A., Spread of scrapie to sheep and goats by oral dosing with foetal membranes from scrapie-affected sheep, Vet. Rec. (1972) 90:465–468.

[79] Pattison I.H., Hoare M.N., Jebbett J.N., Watson W.A., Further observations on the production of scrapie in sheep by oral dosing with foetal membranes from scrapie-affected sheep, Br. Vet. J. (1974) 130:65–67.

[80] Philippe S., Ducrot C., Roy P., Remontet L., Jarrige N., Calavas D., Sheep feed and scrapie, France, Emerging Infect. Dis. (2006) 11:1274–1279.

[81] Race R., Jenny A., Sutton D., Scrapie infectivity and proteinase K-resistant prion protein in sheep placenta, brain, spleen, and lymph node: implications for transmission and antemortem diagnosis, J. Infect. Dis. (1998) 178: 949–953.

[82] Robinson M.M., Hadlow W.J., Knowles D.P., Huff T.P., Lacy P.A., Marsh R.F., Gorham J.R., Experimental infection of cattle with the agents of transmissible mink encephalopathy and scrapie, J. Comp. Pathol. (1995) 113:241–251.

[83] Ronzon F., Bencsik A., Lezmi S., Vulin J., Kodjo A., Baron T., BSE inoculation to prion diseases-resistant sheep reveals tricky silent carriers, Biochem. Biophys. Res. Commun. (2006) 350:872–877.

[84] Ryder S., Dexter G., Bellworthy S., Tongue S., Demonstration of lateral transmission of scrapie between sheep kept under natural conditions using lymphoid tissue biopsy, Res. Vet. Sci. (2004) 76:211–217.

[85] Sharpe A.H., Hunter J.J., Chassler P., Jaenisch R., Role of abortive retroviral infection of neurons in spongiform CNS degeneration, Nature (1990) 346:181–183.

[86] Schoon H.A., Brunckhorst D., Pohlenz J., Spongiforme encephalopathie beim Rothalsstrauss (*Struthio camelus*) Eim Kasuistischer Beitrag, Teirartztl. Prax. (1991) 19:263–265.

[87] Schoon H.A., Brunckhorst D., Pohlenz J., Beitrag zur neuropathology beim Rothalsstrauss (*Struthio camelus*) – Spongiforme Encephalopathie, Verh. Ber. Erkrg. Zootiere (Akademischer Verlag) (1991) 33: 309.

[88] Schätzl H.M., The phylogeny of mammalian and nonmammalian prion proteins, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 119–121.

[89] Sigurdson C.J., A prion disease of cervids: chronic wasting disease, Vet. Res. (2008) 39:41.

[90] Sisó S., Gonzalez L., Jeffrey M., Martin S., Chianini F., Steele P., Prion protein in kidneys of scrapie-infected sheep, Vet. Rec. (2006) 159:327–328.

[91] Somerville R.A., Oberthur R.C., Havekost U., MacDonald F., Taylor D.M., Dickinson A.G., Characterization of thermodynamic diversity between transmissible spongiform encephalopathy agent strains and its theoretical implications, J. Biol. Chem. (2002) 277:11084–11089.

[92] Soto C., Prions: the new biology of proteins, CRC Press, Florida, USA, 2006.

[93] St Rose S.G., Hunter N., Matthews L., Foster J.D., Chase-Topping M.E., Kruuk L.E., et al., Comparative evidence for a link between Peyer's patch development and susceptibility to transmissible spongiform encephalopathies, BMC Infect. Dis. (2006) 6:5.

[94] Taylor D.M., Woodgate S.L., Atkinson M.J., Inactivation of the bovine spongiform encephalopathy agent by rendering procedures, Vet. Rec. (1995) 137:605–610.

[95] Taylor D.M., Woodgate S.L., Fleetwood A.J., Cawthorne R.J., The effect of rendering procedures on scrapie agent, Vet. Rec. (1997) 141:643–649.

[96] Taylor D.M., Woodgate S.L., Rendering practices and inactivation of transmissible spongiform encephalopathy agents, Rev. Sci. Tech. (2003) 22:297–310.

[97] Terry L.A., Marsh S., Ryder S.J., Hawkins S.A., Wells G.A., Spencer Y.I., Detection of disease-specific PrP in the distal ileum of cattle orally exposed to the agent of bovine spongiform encephalopathy, Vet. Rec. (2003) 152:387–392.

[98] Tongue S.C., Pfeiffer D.U., Warner R., Elliott H., del Rio Vilas V., Estimation of the relative risk of developing clinical scrapie: the role of prion protein (PrP) genotype and selection bias, Vet. Rec. (2006) 158:43–50.

[99] Touzeau S., Chase-Topping M.E., Matthews L., Lajous D., Eychenne F., Hunter N., et al., Modelling the spread of scrapie in a sheep flock: evidence for increased transmission during lambing seasons, Arch. Virol. (2006) 151:735–751.

[100] Tranulis M., Influence of the prion protein gene, *Prnp*, on scrapie susceptibility in sheep, APMIS (2002) 110:33–43.

[101] Tuo W., Zhuang D., Knowles D.P., Cheevers W.P., Sy M.S., O'Rourke K.I., PrP^{c} and PrP^{Sc} at the fetal-maternal interface, J. Biol. Chem. (2001) 276:18229–18234.

[102] Van den Ingh T.S., Mandigers P.J., van Nes J.J., A neuronal vacuolar disorder in young rottweiler dogs, Vet. Rec. (1998) 142:245–247.

[103] Van Keulen L.J., Schreuder B.E., Meloen R.H., Mooij-Harkes G., Vromans M.E., Langeveld J., Immunohistochemical detection of prion protein in lymphoid tissues of sheep with natural scrapie, J. Clin. Microbiol. (1996) 34:1228–1231.

[104] Vascellari M., Nonno R., Mutinelli F., Bigolaro M., Di Bari M.A., Melchiotti E., et al., PrP^{Sc} in salivary glands of scrapie-affected sheep. J. Virol. (2007) 81:4872–4876.

[105] Vilotte J.L., Soulier S., Essalmani R., Stinnakre M.G., Vaiman D., Lepourry L., et al., Markedly increased susceptibility to natural sheep scrapie of transgenic mice expressing ovine PrP. J. Virol. (2001) 75:5977–5984.

[106] Wadsworth J.D., Asante E.A., Desbruslais M., Linehan J.M., Joiner S., Gowland I., et al., Human prion protein with valine 129 prevents expression of variant CJD phenotype, Science (2004) 306:1793–1796.

[107] Wells G.A., Scott A.C., Johnson C.T., Gunning R.F., Hancock R.D., Jeffrey M., et al., A novel progressive spongiform encephalopathy in cattle, Vet. Rec. (1987) 121:419–420.

[108] Wells G.A., Wells M., Neuropil vacuolation in brain: a reproducible histological processing artefact, J. Comp. Pathol. (1989) 101:355–362.

[109] Wells G.A., Dawson M., Hawkins S.A., Green R.B., Dexter I., Francis M.E., et al., Infectivity in the ileum of cattle challenged orally with bovine spongiform encephalopathy, Vet. Rec. (1994) 135:40–41.

[110] Wells G.A., Simmons M.M., The essential lesion profile of bovine spongiform encephalopathy (BSE) in cattle is unaffected by breed or route of infection, Neuropathol. Appl. Neurobiol. (1996) 22:453.

[111] Wells G.A., Hawkins S.A., Green R.B., Austin A.R., Dexter I., Spencer Y.I., et al., Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update, Vet. Rec. (1998) 142:103–106.

[112] Wells G.A., Hawkins S.A., Green R.B., Spencer Y.I., Dexter I., Dawson M., Limited detection of sternal bone marrow infectivity in the clinical phase of experimental bovine spongiform encephalopathy (BSE), Vet. Rec. (1999) 144:292–294.

[113] Wells G.A., Spiropoulos J., Hawkins S.A., Ryder S.J., Pathogenesis of experimental bovine spongiform encephalopathy (BSE): preclinical infectivity in tonsil and observations on the distribution of lingual tonsil in slaughtered cattle, Vet. Rec. (2005) 156:401–407.

[114] Wells G.A., Hawkins S.A., Austin A.R., Ryder S.J., Done S.H., Green R.B., et al., Studies of the transmissibility of the agent of bovine spongiform encephalopathy to pigs, J. Gen. Virol. (2003) 84:1021–1031.

[115] Wells G.A., Ryder S.J., Hadlow W.J., The pathology of prion diseases in animals, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 315–323.

[116] Wells G.A., Pohlenz J., Hawkins S.A., Matthews D., Portrait of a spongiform encephalopathy in birds and the transmissibility of mammalian prion diseases to birds, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 279–282.

[117] Wells G.A., Konold T., Arnold M.E., Austin A.R., Hawkins S.A., Stack M., et al., Bovine spongiform encephalopathy: the effect of oral exposure dose on attack rate and incubation period in cattle, J. Gen. Virol. (2007) 88:1363–1373.

[118] Wilesmith J.W., Wells G.A., Cranwell M.P., Ryan J.B., Bovine spongiform encephalopathy: epidemiological studies, Vet. Rec. (1988) 123:638–644.

[119] Wilesmith J.W., Ryan J.B., Atkinson M.J., Bovine spongiform encephalopathy: epidemiological studies on the origin, Vet. Rec. (1991) 128:199–203.

[120] Wilesmith J.W., Ryan J.B., Hueston W.D., Bovine spongiform encephalopathy: case-control studies of calf feeding practices and meat and bonemeal inclusion in proprietary concentrates, Res. Vet. Sci. (1992) 52:325–331.

[121] Wilesmith J.W., Ryan J.B., Bovine spongiform encephalopathy: observations on the incidence during 1992, Vet. Rec. (1993) 132:300–301.

[122] Wilesmith J.W., Wells G.A., Ryan J.B., Gavier-Widen D., Simmons M.M., A cohort study to examine maternally-associated risk factors for bovine spongiform encephalopathy, Vet. Rec. (1997) 141:239–243.

[123] Williams E.S., Miller M.W., Portrait of chronic wasting disease in deer species, in: Hörnlimann B., Riesner D., Kretzschmar H. (Eds.), Prions in humans and animals, de Gruyter, Berlin, Germany, 2007, pp. 257–262.

[124] Wrathall A.E., Brown K.F., Sayers A.R., Wells G.A., Simmons M.M., Farrelly S., et al., Studies of embryo transfer from cattle clinically affected by bovine spongiform encephalopathy (BSE), Vet. Rec. (2002) 150:365–378.

[125] Wyatt J.M., Pearson G.R., Smerdon T.N., Gruffydd-Jones T.J., Wells G.A., Wilesmith J.W., Naturally occurring scrapie-like spongiform encephalopathy in five domestic cats, Vet. Rec. (1991) 129:233–236.

[126] Zlotnik I., Rennie J.C., A comparative study of the incidence of vacuolated neurons in the medulla from apparently healthy sheep of various breeds, J. Comp. Pathol. (1958) 68:411–415.

別紙様式第2-1
番号9

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2008年3月21日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	乾燥濃縮人アンチトロンビンIII	研究報告の公表状況	56th Annual Meeting of the American-Society-of-Tropical-Medicine-and-Hygiene 1044	公表国 アメリカ
販売名（企業名）	①ノイアート（ベネシス） ②ノイアート静注用1500単位（ベネシス）			

研究報告の概要

ヒト顆粒球アナプラズマ症［Human Granulocytic Anaplasmosis (HGA)］の発生率は、1999年以来2倍になった。原因病原体のAnaplasma phagocytophilumは、ニューイングランドの風土病であり、主にマダニ Ixodes scapularis の流行によってヒトに感染する。A. phagocytophilumによって引き起こされる疾患は、無症候なものから重篤なものまであり、一様ではない。A. phagocytophilumの輸血感染が1例報告されているが、現在HGAのスクリーニングは実施されていない。
この病原体によって引き起こされる血液の安全リスクを調査するため、我々はコネチカット州及びマサチューセッツ州の血液ドナーの陽性率を測定した。血液サンプルを春の後半から冬の初め（2001-2005年）及び2006年の初めから1年間、採取した。参加ドナーからの血清について、間接蛍光分析（IFA）を使ってA. phagocytophilumのヒトIgG抗体の試験を実施した。IFA力価が≧1:64のときに陽性とした。IFAによって検査した15,828名のドナー中、432名（2.7%）がA. phagocytophilum抗体陽性であった。力価の分布は以下の通りであった。1:64が256名（59%）、1:128が115名（27%）、1:256が42名（9.7%）、1:512が14名（3.2%）、≧1:1024が5名（1.2%）であった。マサチューセッツ州ドナーの陽性率は2.2%（30/1,346）、コネチカット州ドナーの陽性率は2.8%（402/14,482）であった。血清陽性率ピークは、次の月に生じた：2月（4.7%）、12月（3.7%）と9月（3.4%）。全体的に、年間陽性率は1.7%（2004年）から4.1%（2001年）まで変化が見られた。年間血清陽性率で観察された変動は、おそらくA. phagocytophilumの複雑なライフサイクルに影響する気候および環境因子よるものであろう。
比較的高い陽性率が持続していることから、A. phagocytophilumの血液安全性に及ぼす影響を調査する必要がある。

報告企業の意見	今後の対応
米国ニューイングランド地方の供血者中のA. phagocytophilumの血清陽性率が比較的高い値を持続しているとの報告である。 アナプラズマ属菌は、ウシ科、シカ科、ラクダ科動物の赤血球内に寄生する直径0.2～1μmのグラム陰性桿菌である。万一、原料血漿にアナプラズマ属菌が混入したとしても、除菌ろ過等の製造工程にて除去されるものと考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてノイアート（献血）の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人アンチトロンビンIIIを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びろ過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

study we assessed the field test version of the new WHO JE surveillance standards. We applied the clinical case definition of acute encephalitis syndrome (AES), laboratory diagnostic criteria and case classifications to patients with suspected central nervous system (CNS) infections in southern Vietnam. 380 patients (149 children) with suspected CNS infections were recruited and evaluable, of whom 296 (96 children) met the AES case definition. 54 children were infected with JE virus (JEV), of whom 35 (65%) had AES, giving a sensitivity of 65% (95%CI 56-73%), and specificity 39% (30-48%). 9 adults with JEV all presented with AES. The 19 JEV-infected children missed by the surveillance included 10 with acute flaccid paralysis, 2 with a flaccid hemiparesis, and 6 with meningism only. Altering the case definition to include limb paralysis and meningism improved the sensitivity to 89% (83-95), whilst reducing the specificity to 23% (15-30). An acute serum sample diagnosed 41(68%) of 60 JEV positive patients; an admission CSF diagnosed 33(72%) of 46 patients with this sample, including 7 that were serum negative. Examining a 2nd sample at day 10 diagnosed 61 of 62 patients. 5 patients with neurological manifestations of dengue infection had JEV antibodies in serum, and would have been misdiagnosed had we not tested for dengue antibodies in parallel. In conclusion, the case definitions detected about two thirds of the children infected with JEV, missing those presenting with acute flaccid paralysis. A modified case definition which included acute paralysis and meningism detected nearly 90% of children. An acute CSF sample is more sensitive and specific than an acute serum sample. This formal evaluation of surveillance standards during their development provides an evidence base to support their recommendation, and should be encouraged for future WHO standards.

1043

EPIDEMIC CHIKUNGUNYA FEVER, INDIA AND INDIAN OCEAN, 2006: LABORATORY-BASED SURVEILLANCE FOR IMPORTED CASES, UNITED STATES

Eileen C. Farnon, Amanda J. Panella, Roselyn Hochbein, Olga L. Kosoy, Janeen J. Laveen, Robert S. Lanciotti, Grant L. Campbell

Centers for Disease Control and Prevention, Fort Collins, CO, United States

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus endemic to Africa and Asia. Chikungunya fever (CHIKF) is characterized by fever, rash, arthralgia, and sometimes arthritis; joint symptoms can be severe and prolonged. In 2005-2006, an unprecedented outbreak of CHIKF occurred on islands in the Indian Ocean and in India. Viremic travelers from epidemic areas could introduce CHIKV to the United States (U.S.) through infection of competent local mosquito species, including *Aedes aegypti* and *Aedes albopictus*, which are distributed throughout the southeastern U.S. and Hawaii. We investigated all cases of CHIKF among U.S.-bound travelers in 2006 that were confirmed at CDC. We searched the CDC Arboviral Diagnostic and Reference Laboratory's database for all patients with laboratory-confirmed CHIKF with onset in 2006, and abstracted demographic and travel information. Cases were confirmed using serology (IgM enzyme-linked immunosorbent assay and plaque reduction neutralization test), viral culture, and reverse transcriptase-PCR (RT-PCR). Thirty-eight people from 16 states and the District of Columbia had laboratory evidence of recent CHIKV infection. Their median age was 49 years (range, 22-78 years); 55% were female. India was the travel destination most frequently reported (87%), followed by Sri Lanka (11%), Réunion (3%) and Zimbabwe (3%). One person reported travel to both India and Sri Lanka. Evidence of recent infection was found by serology in 31 (82%), by viral culture and RT-PCR in 5 (13%), and by RT-PCR alone in 2 (5%). In contrast, only 3 cases of CHIKV infection among U.S.-bound travelers were diagnosed at CDC during the preceding 15-year period from 1991-2005. An unprecedented number of CHIKF cases were confirmed at CDC among travelers to the U.S. in 2006. The 5 culture-positive travelers, and others who might have had undetected viremia, posed a risk of introducing CHIKV into local mosquito populations. There was no evidence of local CHIKV transmission in the U.S. in 2006, but the potential for introducing CHIKV to the U.S. from areas with ongoing transmission still exists. Travelers to tropical areas of Asia and Africa should take precautions against mosquito bites. Travelers returning from epidemic or endemic areas with fever and joint symptoms should be tested for CHIKV infection, and positive cases reported promptly to local public health authorities.

BENE2008-005

O 1044

PERSISTENT SEROPREVALENCE OF *ANAPLASMA PHAGOCYTOPHILUM* IN NEW ENGLAND BLOOD DONORS

Melanie C. Proctor[1], David A. Leiby[1], Stephanie T. Johnson[2], Richard G. Cable[2]

[1]*American Red Cross Holland Laboratory, Rockville, MD, United States,* [2]*American Red Cross, Farmington, CT, United States*

The incidence of human granulocytic anaplasmosis (HGA) has doubled since 1999. The causative agent, *Anaplasma phagocytophilum*, is transmitted to humans primarily by the ixodid tick, *Ixodes scapularis*, endemic in New England. *A. phagocytophilum* causes an illness that ranges from asymptomatic to severe. There has been one reported case of transfusion-transmitted *A. phagocytophilum*, but blood donors are not currently screened for HGA. To determine the potential blood safety risk posed by this agent, we determined its seroprevalence in Connecticut (CT) and Massachusetts (MA) blood donors. Consenting CT and MA blood donors were enrolled in a comprehensive tick-borne disease study. Blood samples were collected during the late spring to early winter (2001-2005) and year round beginning in 2006. Serum collected from participating donors was tested for human IgG antibodies to *A. phagocytophilum* using an indirect immunofluorescent assay (IFA). A donor was considered positive if their IFA titer result was ≥ 1:64. Of 15, 828 donor sera tested by IFA, 432 (2.7%) were positive by IFA for *A. phagocytophilum* antibodies. The distribution of titers was as follows: 256 (59%) donors at 1:64, 115 (27%) at 1:128, 42 (9.7%) at 1:256, 14 (3.2%) at 1:512 and 5 (1.2%) at ≥1:1024. MA donors had a seroprevalence rate of 2.2% (30/1346), while the rate of CT donors was slightly higher, 2.8% (402/14,482). Seroprevalence peaks occurred in the following months: February (4.7%), December (3.7%) and September (3.4%). Overall, the seroprevalence data demonstrated variable yearly rates with a low of 1.7% in 2004 and a high of 4.1% in 2001. Observed fluctuations in yearly seroprevalence rates are likely the result of climactic and environmental factors that influence the complex lifecycle of *A. phagocytophilum*. The observed persistence of relatively high seroprevalence rates reinforces the need to examine the possible impact that *A. phagocytophilum* may have on blood safety. Limited transmission evidence to date may be attributable to the agent's short bacteremic phase, the effect of leukoreduction on this intra-granulocytic organism, or to transmission of primarily sub-clinical infection and resultant under-recognition.

(ACMCIP Abstract)

1045

FAILURE OF STANDARD BABESIOSIS THERAPY IN IMMUNOCOMPROMISED HOSTS

Peter J. Krause[1], Ben Gewurz[2], David Hill[3], Francisco Marty[2], Ivo Foppa[4], Edouard Vannier[5], Ellen Neuhaus[1], Gail Skowren[6], Shaili Gupta[7], Richard R. Furman[8], Carlo McCalla[9], Ed Pesanti[1], Mary Young[10], Donald F. Heiman[11], Jeffrey A. Gelfand[2], Gary Wormser[9], John Dickason[2], Samuel R. Telford[12], Barry Hartman[8], Frank Bia[7], Kenneth Dardick[1], Diane Christianson[1], Morton Coleman[13], Andrew Spielman[2]

[1]*University of Connecticut School of Medicine, Farmington, CT, United States,* [2]*Harvard University School of Medicine, Boston, MA, United States,* [3]*Hospital for Tropical Diseases, London, United Kingdom,* [4]*University of South Carolina School of Medicine, Charleston, SC, United States,* [5]*Tufts University School of Medicine, Boston, MA, United States,* [6]*Brown University School of Medicine, Providence, RI, United States,* [7]*Yale University School of Medicine, New Haven, CT, United States,* [8]*Cornell University School of Medicine, New York, NY, United States,* [9]*New York*

別紙様式第2-1

No. 3

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 1. 15	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	ABC Newsletter. 20008 Jan 11.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			米国

研究報告の概要

○血液安全パネルはHHSに病原体低減技術の開発を急ぐよう要請

血液安全・安定供給諮問委員会は、米国保健社会福祉省(HHS)事務局に対し、安全で効果的な輸血用血液製剤の病原体低減技術(不活化)の早急な開発を優先して進め、開発され次第実施するよう勧告した。

委員会はまた、HHSに低減技術の開発とバリデーションの障害を取り除くための手段を提供するよう要請した。現在、スクリーニング検査にかかる費用とその複雑さは、血液の安全性の革新を妨げている。これは、製造販売業者の血液安全技術への積極的な投資継続に見合うようなビジネスモデルがないためである。

2008年1月9日と10日の会合では、「病原体低減の効果と安全性を示すエビデンスの蓄積は、今後蔓延する可能性のある感染症に対し広く適応できるセーフガードとして、この技術の導入を保証する」という決議を採択した。こうした新しい技術の例は、血漿分画製剤では世界的に使用され、血液成分製剤ではヨーロッパで導入されている病原体低減システムである。

委員会はこの勧告の根拠として、受血者への既知の感染症の脅威をより低減する必要性を挙げた。また、感染性因子の特定後にドナーの検査を導入するという現行の方式では、新たな病原体が特定される前に感染が拡大する可能性があると指摘した。

病原体低減技術の費用は高額になると予想されるが、ガンマ線照射、白血球除去、細菌培養、マラリア予防のための渡航歴による供血制限など、導入後に不要となる現行の血液安全対策の削減によって相殺されると委員会は考えている。また、病原体低減技術の導入で、検査の偽陽性や精度の低い渡航歴による供血制限のためのドナー喪失が減るため、血液の供給量が増加すると推測している。

報告企業の意見	今後の対応
血液安全・安定供給諮問委員会は、米国保健社会福祉省事務局に対し、安全で効果的な輸血用血液製剤の病原体低減技術(不活化)の早急な開発を優先して進め、開発され次第実施するよう勧告したとの報告である。	日本赤十字社では8項目の安全対策の一つとして、不活化技術の導入について、各不活化技術の効果、血液成分への影響、製造作業への影響などについて評価検討を行っている。外国での不活化実施状況や効果、新たな技術、副作用等の情報収集も含め総合的に評価し、導入について関係機関と協議する予定である。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

(4-1)