Potential Risk of Variant Creutzfeldt-Jakob Disease (vCJD)

From Plasma-Derived Products

In recent years, questions have been raised concerning the potential risk of variant Creutzfeldt-Jakob disease (vCJD - a rare but fatal brain infection) for recipients of plasma- derived clotting factors, including United States (US) licensed Factor Eight (pdFVIII), Factor Nine (pdFIX), and other plasma-derived products such as immune globulins and albumin. In response to these questions, FDA conducted a risk assessment. Based on the risk assessment, the US Public Health Service believes that the risk of vCJD to patients who receive US licensed pdFVIII products is most likely to be extremely small, although we do not know the risk with certainty. vCJD risk from other plasma derived products, including Factor IX, is likely to be as small or smaller.

This web page provides FDA's risk assessment for US licensed pdFVIII and risk communication materials for this product and other plasma derivatives. Included are Key Points, and Questions and Answers. Additional links are provided to FDA's current guidance documents on deferral of blood and plasma donors who may be at increased risk of vCJD, and to other sources of information regarding vCJD.

Documents Regarding US Licensed pdFVIII, and Other US Licensed Plasma Derivatives Including pdFIX

- Potential vCJD Risk From US Licensed Plasma-Derived Factor VIII (pdFVIII, Antihemophilic Factor) Products: Summary Information, Key Points
- Risk Assessment (PDF, 582 KB)
- Risk Assessment Appendix (PDF, 623 KB)
- Questions and Answers on vCJD and pdFVIII
- Questions and Answers on vCJD and Plasma Derivatives Other than pdFVIII

Guidance on Donor Deferral Related to CJD and vCJD

- Draft Guidance for Industry: Amendment (Donor Deferral for Transfusion in France Since 1980) to "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products" - 8/2006
- Questions and Answers on FDA Guidance: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob (CJD) Disease and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products - 1/22/2004
- Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products - 1/2002

Other Sources of Information

- Transmissible Spongiform Encephalopathies Advisory Committee
- Blood Products Advisory Committee Meeting – Summary of Recent TSEAC Meeting and Statement about FXI from the UK, on October 21, 2004
- Information on vCJD: Centers for Disease Control and Prevention
- Information on Bovine Spongiform Encephalopathy ("Mad Cow Disease"): US Department of Agriculture

Patient Organizations:

- Committee of Ten Thousand
- Hemophilia Federation of America
- National Hemophilia Foundation and/or HANDI
- World Federation of Hemophilia

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研究報告の概要

本文献では米国で牛海綿状脳症（BSE）関連の診断を受けた 2 症例（2003 年の報告例：症例 1，2004 年の報告例、症例 2 の特徴について報告する。両症例の脳切片について免疫組織化学検査及びウェスタンブロット法を実施したところ，微妙な違いが明らかになった。症例 1 では脳幹の閂領域に海綿状変性及び広範な異常プリオン蛋白（PrP^{Sc}）の異常沈着がみられた一方，症例 2 では空胞状の変化はみられず PrP^{Sc} はそれほど多くなかった。ウェスタンブロット法によって，症例 1 及び 2 の PrP^{Sc} は主として 2 つの特異的なモノクローナル抗体に反応したことが示された。また，症例 2 の PrP^{Sc} は高分子量に位置する異常な電気泳動パターンを示した。両症例のプリオン蛋白遺伝子の配列を決定したところ，ウシについて過去に報告された種々の配列に当てはまることが示されたため，生殖細胞突然変異によって当該疾患が誘発される可能性は低い。症例 2 に認められた異常な特性は「高分子量 BSE」又は H 型 BSE に一致し，高分子量の非グリコシル化 PrP^{Sc} を示す。現在のところ，H 型 BSE が報告されているのはフランス及びドイツのみである。

使用上の注意記載状況・その他参考事項等

BYL-2007-0284

報告企業の意見	今後の対応
米国で報告された BSE の最初の 2 例の分子特性は BSE 誘発物質の 2 つの異なる株により引き起こされやすく，実質的に「散発性」BSE の仮説が除外されることを示唆するものである。興味深いことに，1 例では H 型 BSE である可能性が高く，欧州以外では過去に報告がなかったものである。以前に報告された米国農務省当局によるリスク評価では，米国の BSE 発症のリスクが増大することはほとんどないと結論付けられた。	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。引き続き関連情報の収集に努める。

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Identification and characterization of two bovine spongiform encephalopathy cases diagnosed in the United States

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Abstract. Bovine spongiform encephalopathy (BSE) is a transmissible spongiform encephalopathy of cattle, first detected in 1986 in the United Kingdom and subsequently in other countries. It is the most likely cause of variant Creutzfeldt-Jakob disease (vCJD) in humans, but the origin of BSE has not been elucidated so far. This report describes the identification and characterization of two cases of BSE diagnosed in the United States. Case 1 (December 2003) exhibited spongiform changes in the obex area of the brainstem and the presence of the abnormal form of the prion protein, PrP^{Sc}, in the same brain area, by immunohistochemistry (IHC) and Western blot analysis. Initial suspect diagnosis of BSE for case 2 (November 2004) was made by a rapid ELISA-based BSE test. Case 2 did not exhibit unambiguous spongiform changes in the obex area, but PrP^{Sc} was detected by IHC and enrichment Western blot analysis in the obex. Using Western blot analysis, PrP^{Sc} from case 1 showed molecular features similar to typical BSE isolates, whereas PrP^{Sc} from case 2 revealed an unusual molecular PrP^{Sc} pattern: molecular mass of the unglycosylated and monoglycosylated isoform was higher than that of typical BSE isolates and case 2 was strongly labeled with antibody P4, which is consistent with a higher molecular mass. Sequencing of the prion protein gene of both BSE-positive animals revealed that the sequences of both animals were?within the range of the prion protein gene sequence diversity previously reported for cattle.

Key words: Bovine spongiform encephalopathy; cattle; immunohistochemistry; *Prnp* gene; Western blot.

Introduction

Transmissible spongiform encephalopathy (TSE) agents or prions induce fatal neurodegenerative diseases in humans and in other mammals. They are transmissible among their species of origin, but they can also cross some species barriers and induce infection with or without disease in other species. Human TSEs include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome, Kuru, and fatal familial insomnia.[38] In animals, 4 distinct TSE diseases are recognized: scrapie in sheep and goats, transmissible mink encephalopathy (TME) in mink, chronic wasting disease (CWD) in cervids, and bovine spongiform encephalopathy (BSE) in cattle. BSE is transmissible via BSE-contaminated feed to cats (feline spongiform encephalopathy, FSE) and exotic ungulates (exotic ungulate encephalopathy, EUE).[50,51]

Prions are proteinaceous infectious particles and are the causative agents of TSEs. They are host-coded proteins that have undergone conformational changes and have biological and physicochemical characteristics that differ significantly from those of other infectious agents. For example, they are resistant to inactivation processes that are effective against conventional viruses including those that alter nucleic acid structure or function. These include ionizing and UV radiation,[1] and inactivation by formalin.[21] In contrast, infectivity is highly susceptible to procedures that modify protein conformation.[41] In TSE disease, the normal cellular protein, PrP^{C}, is converted to abnormal prion protein, PrP^{Sc}. PrP^{Sc} exhibits increased beta sheet content, a change that may drive the additional changes in solubility and protease resistance.[40] Unlike normal cellular protein, PrP^{Sc} is relatively insoluble in detergents, is relatively resistant to proteases,[39] and is capable of causing a conformational change in additional molecules of PrP^{c}. The precise function of the normal PrP^{c} in healthy animals remains unknown. There is some evidence to show that PrP^{c} might play a role in sleep physiology, in

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resistance to oxidative stress, in signal transduction, and in self-renewal of hematopoietic stem cells.[17,31,33,55]

TSE disease involves the accumulation of PrP^{Sc} in the central nervous system (CNS) of the host, eventually leading to neurodegeneration and disease. In TSE-affected animals, PrP^{c} has a determinant role in the incubation time and species barrier.[8] Mice lacking prion protein gene (*Prnp*) expression are not susceptible to TSE agents or prion infection, demonstrating the key role of PrP^{c} in TSEs.[8] Susceptibility to prions thus depends upon the presence of PrP^{c} on the cell membrane of the host; prions do not propagate in brains that lack PrP^{c}.[6]

BSE first emerged in 1986 in the United Kingdom, where more than 180,000 cases have been diagnosed to date (August 2006). Widely referred to as "mad cow disease", BSE subsequently spread to many countries, predominantly in Western Europe. These outbreaks, caused by the consumption of infected meat and bone meal containing the malformed prion protein, have resulted in the destruction of thousands of cattle and have caused significant economic losses. BSE is a chronic degenerative disease affecting the central nervous system of cattle. Affected animals display changes in temperament, abnormal posture, incoordination and difficulty in rising, decreased milk production, and/or loss of body weight despite continued appetite.[42] The average incubation period is about 4–6 years and all affected animals succumb to the disease.[30] Following the onset of clinical signs, the animal's condition deteriorates until it either dies or is destroyed. This process usually takes from 2 weeks to 6 months. Most cases in the United Kingdom occurred in dairy cows between 3 and 6 years of age, with the highest susceptibility to infection being in the first 6 months of life; adult cattle appear to be at relatively low risk of infection.[3]

Epidemiological surveillance programs carried out in many European and non-European countries have discovered BSE-positive animals within the last decade.[18,36] In May 2003, Canada reported its first indigenous case of BSE, detected as part of the Canadian BSE surveillance program on a commercial cow-calf operation in Alberta, Canada [13,45]. Since then, Canada has reported 7 additional cases of BSE (two in 2005, five in 2006 – as of August 2006), all of them detected as part of the ongoing Canadian BSE surveillance and the majority of cases stemming from a distinct area in Alberta.

All currently validated diagnostic tests for BSE require brain tissue.[35,49] There is currently no validated *ante mortem* test for BSE. The original diagnostic test method was histopathology in which brain sections exhibiting the classical vacuoles and spongiform changes in specific areas are used for diagnosis.[35] In the mid-1990s, immunohistochemistry (IHC) and Western blotting were developed for the detection of PrP^{Sc} in tissues.[35] Both IHC and Western blot are considered confirmatory tests for BSE by the World Organization for Animal Health (OIE).[35] In the past decade, "rapid tests" have been introduced commercially for BSE surveillance.[35]

This report describes the identification and characterization of 2 BSE cases diagnosed in the United States. The first US BSE case (December 2003) was identified by using the BSE surveillance system that was in place in the United States from May 1990 until May 2004. Using this system, brainstem samples from field cases of cattle exhibiting signs of neurologic disease, cattle condemned at slaughter for neurologic reasons, rabies-negative cattle submitted to public health laboratories, neurologic cases submitted to veterinary diagnostic laboratories and veterinary teaching hospitals, and sampling of cattle that were nonambulatory (downer cattle/fallen stock) were sent to the National Veterinary Service Laboratory (NVSL) for confirmatory IHC testing. Before 1995, BSE testing at the NVSL was done by histological analysis of brain sections for the presence of BSE-typical spongiform lesions. After 1995, testing included the use of the newly established IHC procedures for the detection of PrP^{Sc} in brain sections of TSE-infected animals.[30] After the first case of BSE in the United States was diagnosed, an enhanced BSE surveillance program was established on June 1, 2004, based on the use of a rapid screening test[a], followed by confirmatory testing (IHC and/or Western blot, since June 2005 both methods are used in parallel) for any reactive sample, designated "inconclusive." As of August 6, 2006, more than 775,000 samples have been tested and 2 samples were found to be BSE positive. Currently, an "inconclusive" sample is defined as being positive by the initial one-well ELISA and again positive in at least 1 out of 2 wells in a repeat test of the original homogenate. USDA has designed its enhanced testing program to collect the majority of samples from high-risk animals in the following categories: nonambulatory cattle, cattle exhibiting signs of a central nervous system disorder, cattle exhibiting other signs that may be associated with BSE, such as emaciation or injury, and dead cattle. In addition, all cattle condemned on *ante mortem* inspection are sampled. In this report, detection of PrP^{Sc} by immunohistochemistry and Western blot analyses are presented. Brain material from both cases is compared with each other and with well-defined BSE isolates from other countries. In addition, sequences of the prion protein gene of both BSE-positive animals are discussed.

Material and methods

Animals and tissues

Case 1 was an approximately 6.5-year-old, nonambulatory cow, slaughtered in December 2003 in Moses Lake, Washington State. The animal was imported from Canada in 2001. Case 2 was a downer cow, approximately 12 years old, and sent to a pet food plant in November 2004. The animal was born and raised in Texas. Brain samples were taken from both animals according to the USDA BSE surveillance plan and shipped to the National Veterinary Service Laboratories (NVSL) in Ames, Iowa. The obex sample from Case 2 was found to be reactive twice in a rapid BSE test performed by the Texas Veterinary Medical Diagnostic Laboratory at Texas A&M University, College Station, TX, before being shipped to NVSL. The rapid BSE test[a] used in the United States has been validated by the European Union, Canada, and the United States and has demonstrated both high sensitivity and specificity for detecting BSE prion protein in cattle. Frozen and formalin fixed samples from the medulla oblongata and the cerebellum were available for analysis. No portion of either cow's carcass entered the animal or human food chain.

Histopathology

Brain tissue was fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 5 micron thickness, and stained with hematoxylin and eosin (HE) for light microscopic examination.

Immunohistochemistry (IHC)

Brain tissue was placed in 10% buffered formalin and after a minimum of 4 days of fixation appropriate sections of obex were put in cassettes and kept in fresh formalin until they were processed for routine paraffin embedding. Using automated tissue processing, tissues were cut at 5 micron thickness and mounted onto charged glass slides[b]. Slides were set upright, drained and air-dried for a minimum of 3 hr, followed by 15 min at 80°C. The slides were then deparaffinized (xylene, ethyl alcohol gradient, distilled H_2O). Additionally, 5-micron sections of US BSE case 2 were treated with 95% formic acid for 5, 15, and 30 min at room temperature. Slides were incubated with a target retrieval solution[c], placed in a medical decloaking chamber[d], autoclaved for 30 (case 1) or 30 and 45 (case 2) min at 121°C, and then cooled for 25 min. Slides were soaked for a minimum of 5 min in Ventana APK Wash Solution[e]. The Ventana NexES carousel[e], filled with APK Wash Solution[e] and Liquid Coverslip bottles[e], was used with the PrP-specific antibody F99/97.6.1[f] at a concentration of 10 µg/ml. Slides were processed to completion using alkaline phosphatase red paraffin protocols as suggested by the manufacturer[e]. Then, slides were removed, placed in a rack and dipped thirty times in 250 ml of warm soapy tap water containing 2–3 drops liquid dishwashing detergent[g]. Afterwards, slides were rinsed in running tap water for 2 min and dehydrated in ethyl alcohol and xylene. Coverslips were added to slides and IHC results were interpreted as follows: 1) positive for PrP^{Sc}: pink to red and 2) background and negative for PrP^{Sc}: only blue background. It should be noted that the IHC procedure did not incorporate a proteinase K digestion step. For the purpose of simplicity of nomenclature, the term "PrP^{Sc}" is used to describe the abnormal prion protein. As positive controls, slides from the brainstem of a BSE-positive cow, obtained from the United Kingdom, and from the brainstem of a scrapie-positive sheep were used for Case 1; slides from brainstem of a BSE-positive cow obtained from the United Kingdom and from the US BSE Case 1 were used for Case 2. As negative controls, slides from brainstem material of BSE-negative cattle and scrapie-negative sheep were used.

Western blot analyses

Brain homogenates from US BSE case 1 were prepared from approximately 0.5 gram brainstem material and analyzed using the Prionics®-Check Western Kit[h] as suggested by the manufacturer with minor modifications regarding the detection system. It should be noted that the Prionics®-Check Western Kit[h] method is not a confirmatory assay for BSE. Samples were homogenized at room temperature with homogenization buffer[h] (10% w/v) using a PowerGen125 homogenizer[i] with a disposable probe[i] (5 times, 30 sec), and digested with proteinase K (PK)[h] for 40 min at 48°C. PK-digestion was stopped according to the manufacturer's protocol and 10–15 µl volume of sample was loaded onto pre-cast sodium dodecyl sulfate (SDS)-12% polyacrylamide gel electrophoresis (PAGE) gels[j]. SDS-PAGE was performed as described by the manufacturer and the proteins transferred from the gel to a polyvinylidene difluoride (PVDF)[k] membrane with transfer buffer[j]. The membranes were blocked with PVDF blocking buffer[j] and either incubated with antibody 6H4[h] (1:10,000 dilution or 0.1 µg/ml of a mouse IgG1 monoclonal antibody antiserum raised against human residues 144–152 [DYEDRYYRE] of the PrP peptide) or antibody P4[l] (1:10,000 dilution or 0.1 µg/ml of a mouse monoclonal antibody raised against synthetic ovine PrP residues 89–104 [GGGGWGQGG-SHSQWNK] of the PrP peptide) for 1 hr at 37°C or overnight at 4°C. After three washes in Tris-Buffered Saline Tween®-20 (TBST)[j], the membranes were incubated for 30 min at room temperature with a biotinylated sheep anti-mouse antibody[m] (1:10,000 dilution or 0.05 µg/ml in TBST). After 3 washes in TBST[j], a streptavidin-horseradish peroxidase conjugate[m] was added for 30 min. After another round of washes with TBST, bound antibodies were detected by using the ECL Plus[m] chemiluminescent substrate.

For US BSE case 2, the Prionics®-Check Western Kit[h] and the OIE-recommended Scrapie Associated Fibril (SAF)-Immunoblot method (http://www.oie.int/eng/normes/mmanual/ A_summry.htm) were used[35] with minor modifications. In contrast to the Prionics®-Check Western[h] method, the SAF Immunoblot method enriches brain samples for PrP^{Sc} by ultracentrifugation prior to loading them onto a SDS-PAGE gel. The SDS-PAGE electrophoresis conditions, subsequent transfer and immunodetection of PrP^{res} were carried out as described above. Therefore, only the enrichment method will be described in more detail. Material for analysis of case 2 was taken from the brainstem (2 g) and cerebellum (2 g) area and minced with

a new blade after removal of dura mater. A 10% (w/v) tissue homogenate in 10 mM Tris, pH 7.5, containing 5 mM $MgCl_2$ was prepared using a homogenizer[g] with a disposable probe[g] (5 times, 30 sec). The homogenate was mixed well and then again sonicated for 30 sec on ice bath (5–10 times). Benzonase®[n] was added to the mixture for a final concentration of 100 U/ml and incubated for 1 hr at 37°C while shaking. An equal volume of 20% (w/v) N-lauroylsarcosine[o] in 10 mM Tris, pH 7.5 and 1 mM DTT[o] was added to each homogenate, vortexed for 1 min every 10 min for a total of 30 min at room temperature. Homogenates were transferred to polyallomer tubes[p] and centrifuged at 20,000 × *g* for 25 min at 10°C. Supernatant was centrifuged again using polyallomer tubes[p] at 200,000 × *g* for 55 min at 10°C. The resultant supernatant was discarded, the pellet was resuspended in sterile, distilled H_2O (1 µl/mg tissue equivalent) and sonicated until suspended. Sample was split into 2 aliquots into micro-centrifuge tubes and one sample was treated with PK[q] (concentration 0.4 U/ml) by incubation at 37°C for 60 min with agitation while the control sample was not treated with PK. Phenylmethylsulphonyl fluoride[r] (PMSF) was added to a final concentration of 5 mM, incubated on ice for 15 min and transferred to a new 1.5-ml ultracentrifuge tube. Volume was brought up to 500 µl with H_2O and centrifuged at 200,000 × *g* for 1 hr at 10°C. Pellet was resuspended in SDS-PAGE sample buffer to at least 10 mg tissue equivalent per µl. Samples were sonicated on wet ice before loading on SDS-PAGE gel. For both Western blot techniques, detection was performed either on Biomax films[s] or scanned images were obtained with a Typhoon[m] imaging system. As positive control samples, BSE-positive brain material from Canadian and Swiss cattle, cattle-and sheep-passaged scrapie and CWD-positive elk and mule deer material were used for Case 1, brain material from Case 1 and sheep scrapie were used for Case 2. As negative controls, brain material from a BSE-negative cow was used.

DNA isolation and PCR amplification

Genomic DNA was extracted from 200 µl of a 10% brain homogenate (cerebellum) using the DNeasy™ tissue kit[t] according to the manufacturer's instructions. PCR was performed in a 100 µl final reaction volume containing 0.2 pmole of forward primer (5'-CAT ATG ATG CTG ACA CCC TC -3'), 0.2 pmol of reverse primer (5'-AGA AGA TAA TGA AAA CAG GAA G -3') 1× Easy-A PCR buffer, 2.5 mM $MgCl_2$, 0.8 mM each deoxyribonucleotide triphosphate (dNTP Master Mix)[u], 2.5 U of Easy-A™ high-fidelity cloning Taq DNA polymerase[v], and 0.4 ug of total DNA. Amplification was performed with the following conditions: 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec (denaturation), 59°C for 30 sec (annealing), and 72°C for 1 min. A final extension step at 72°C was performed for 10 min. Amplified DNA product was purified using a GENECLEAN® spin kit[w] and sequencing was performed using the ABI 3700 DNA sequencer with the cycle sequencing kit[x]. The fragment was sequenced in duplicate using the original 2 primers and 2 internal primers 4142 and 9612 [4] for a total of 8 reactions. Databases were searched using standard nucleotide-nucleotide BLAST at the National Center for Biotechnology Information Web Site (http://www.ncbi.nlm.nih.gov). The database is a collection of sequences from several sources, including GenBank and Reference Sequence. The nucleotide sequences of cases 1 and 2 were aligned using both CLUSTAL V[26,27] and CLUSTAL W[48] with the following GENBANK accession numbers: AY335912 (bovine), AY367641 (bovine), AF016228 (elk), AY275712 (white-tailed deer), AF166334 (sheep), AJ567986 (sheep), and the Canadian BSE case using Lasergene version 5.07 software (DNASTAR-Madison WI).

Results

ELISA results

When brainstem samples from case 1 and case 2 were repeatedly tested for the presence of PrP^{Sc} using a validated ELISA test system, the mean optical density (OD) values were 1.86 for case 1 ($n = 2$) and 2.49 for case 2 ($n = 6$).

Histological and immunohistochemical examination

Brainstem samples at the level of obex were available from both cases and analyzed for the presence of spongiform changes and deposition of PrP^{Sc}. Lesions of spongiform encephalopathy diagnostic for BSE were detected in the obex region of case 1 (Fig. 1A, and were not present in the obex of case 2 (Fig. 1C). The tissue of case 2 had been frozen and therefore artifactual changes were present, but definitive lesions of BSE were not observed (Fig. 1C). In both cases the medulla at the level of the obex was examined. In case 1 there was neuropil vacuolation present in several areas and occasional scattered neuronal vacuolation. The neuropil vacuolation was most pronounced in the solitary nucleus and tract, the spinal nucleus and tract of the trigeminal nerve, the olivary nuclei, and less pronounced in the motor nucleus of the vagus. In case 2 there was a freezing artifact that precluded definitive histological interpretation of vacuolar changes, however overt TSE related vacuolar lesions were not observed. When IHC was performed, both cases were positive for the presence of PrP^{Sc}. Whereas significant amounts of PrP^{Sc} were detected in the obex area of case 1 (Fig. 1B), only weak staining for PrP^{Sc} was observed in the obex area of case 2 (Fig. 1D). Initially, the IHC of case 2 was negative, however, after formic acid treatment and extended antigen retrieval the case was positive by IHC (Fig. 1D). Extended antigen retrieval (up to 45 minutes) and the treatment of the slides with formic acid prior to immunostaining were necessary to obtain unambiguous PrP^{Sc} signals. The distribution of PrP^{Sc} in brainstem of case 2 was not as uniform nor as intense as seen with case 1 (Fig. 1B). Case 1 had intense widespread immunostaining