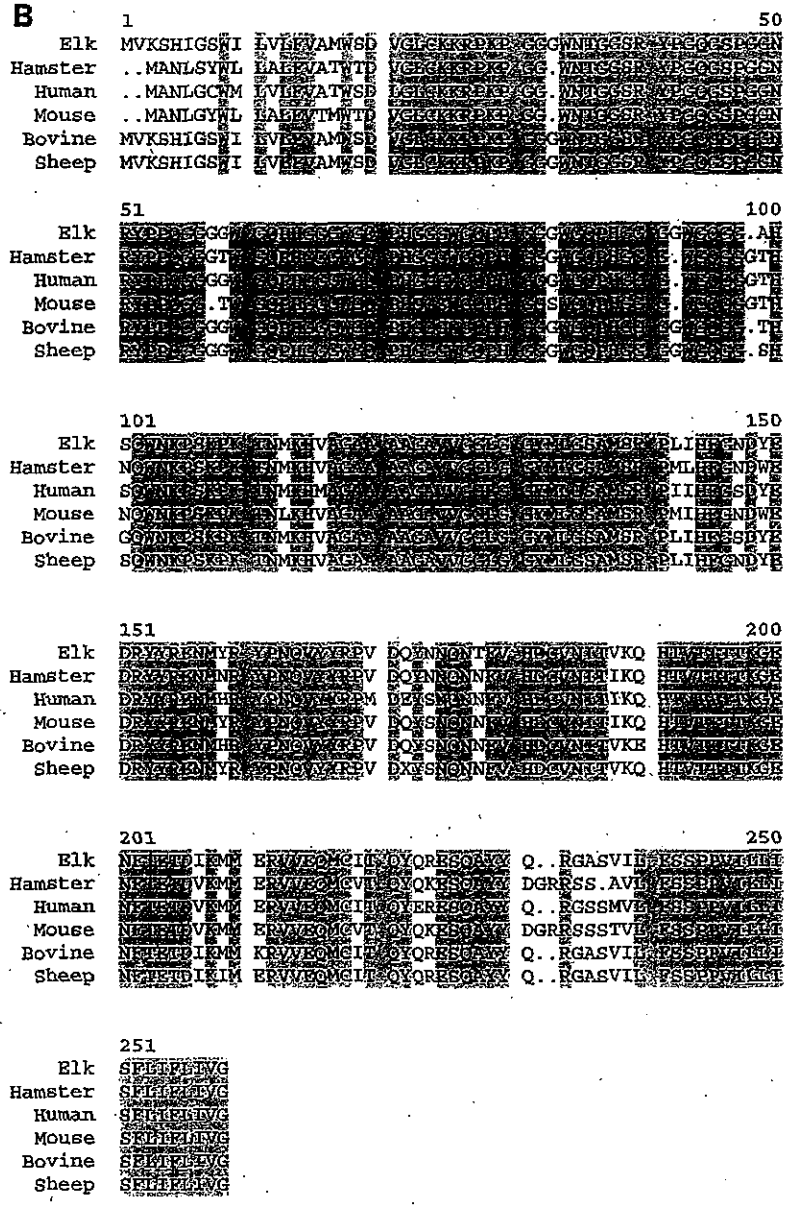

Fig. 1 (*continued*)

ated horizontal contact [5,9,18,19]. We aligned the amino acid sequences from species of cervid species which were used in the experiment: elk (*Cervus elaphus*; GenBank Accession No. CAA70902) reindeer/caribou, (*Rangifer tarandus*; GenBank Accession No. AAZ81477—reindeer is the European name for wild caribou), and moose (*Alces alces*; GenBank Accession No. AAZ81479) (Fig. 1A). The protein sequence of these three cervid species is highly conserved, with only one amino acid polymorphism reported in GenBank. We also aligned the amino acid sequences of elk with other species, such as hamster, human, mouse, bovine, and sheep, which reveals that the protein sequence of PrP^C is more than 90% conserved (Fig. 1B).

In vitro conversion of various species with CWD prion template

Normal brain homogenates from elk, reindeer, moose, caribou, human, hamster, mouse, bovine, and sheep, which were incubated with CWD-affected elk brain "seeds", were tested for conversion to a protease-resistant PrP isoform (Fig. 2) as previously described for human CJD *in vitro* conversion [17]. As a negative control, *Prnp* null mouse brain showed no signal corresponding to PK-resistant PrP^{Sc} (Fig. 2, K/O mouse bar). Partial denaturation of normal brain homogenates induced by exposure to low pH and guanidine enhanced *in vitro* conversion to PK-resistant PrP^{Sc} (Fig. 2) has been previously reported for the human

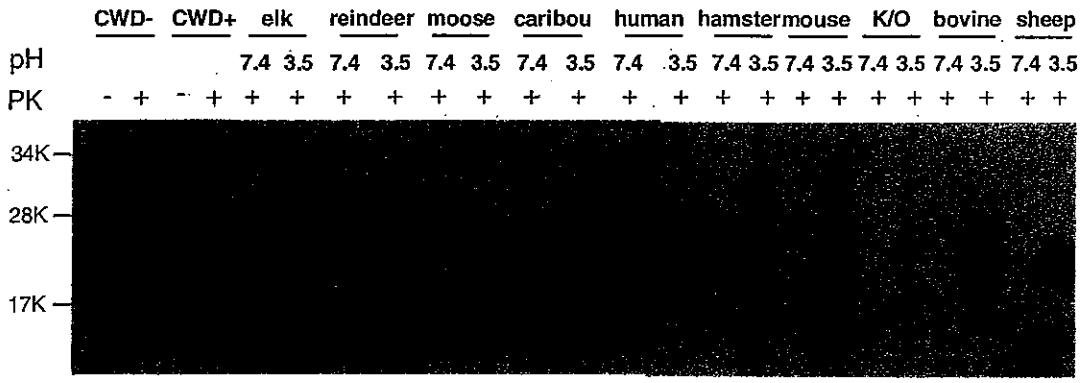

Fig. 2. *In vitro* conversion of treated PrP in the presence of PrP^{Sc} from CWD elk brain. Immunoblots of the PK-resistant PrP isoforms with 6H4 antibody. Samples were treated with GdnHCl and incubated in PBS (pH 7.4) with 0.05% SDS and 0.5% Triton X-100, at 37 °C for 12 h with shaking in the presence of trace amount of elk PrP^{Sc}. CWD−, normal elk brain homogenate as control; − and + indicates the PK treatment. CWD+, elk CWD brain homogenate as a control. The rests are the amplification of PrP^{Sc} in the different species, using elk CWD as seed, treated or untreated with acid (pH 7.4 or pH 3.5).

system [17]. All samples of normal brain contained PrP, which was sensitive to PK digestion (elk shown in Fig. 2, other species not shown). Five microliters of CWD brain homogenate was barely visible after PK digestion (Fig. 2), which was 25-fold greater than the dilution-adjusted CWD seed used in conversion system, excluding artifact from input PrP^{Sc}. Bands of the PK-resistant PrP^{Sc} form were present at ~21 kDa in all the species under acidic conditions (pH 3.5), except for the *Prnp* null mouse (Fig 2). However, PK-resistant PrP^{Sc} was poorly generated in some species in which the brain homogenates were treated under neutral conditions (pH 7.4), such as in human, hamster, mouse, bovine, and sheep. For homogenates treated at neutral pH (pH 7.4), the progression from most susceptible to least susceptible was: elk, reindeer > moose > caribou > hamster > human, bovine, sheep > mouse, with no detected conversion in *Prnp* null mouse brain.

PrP conversion efficiency enhancement by partial denaturation

Treatment of substrate brain with acidic pH (pH 3.5) enhanced PrP^{CWD}-induced conversion of all species, except *Prnp* null mice as expected (Fig. 3A). If the conversion of partially denatured PrP can be considered to be the maximum achievable conversion, the ratio of conversion of brain homogenates treated at pH 7.4 relative to pH 3.5 may provide a "conversion efficiency ratio" (CER) for that species. The comparative CER within different species is shown in Fig. 3B. Notably, some cervid species showed variability in crude conversion efficiency of native and denatured substrate, despite similar (or even identical) PrP amino acid sequences (e.g., caribou and reindeer). Although individual assays might vary for trivial reasons such as slightly differing concentration of brain homogenate, the adjusted CER seems to indicate all cervids display similar substrate conversion efficiency as expected from their evolutionary proximity. The CER analysis also

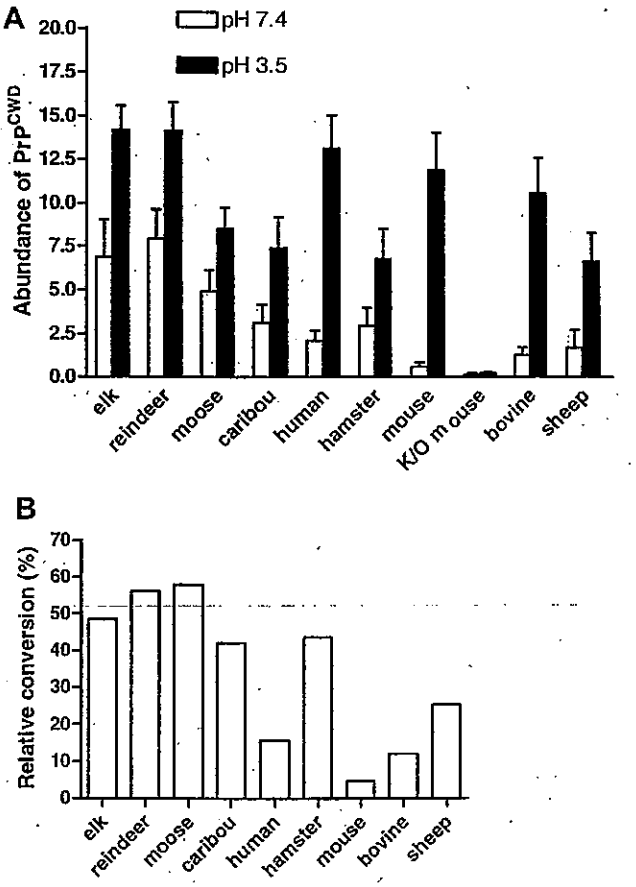

Fig. 3. (A) The immunoblots as in Fig. 2 were examined by densitometry to determine the ratio of neutral (pH 7.4) and acidic (pH 3.5) forms of PrP^{Sc} using Quantity One software (Bio-Rad). (B) Conservation efficiency ratio of native and denatured PrP substrate.

appears to show that hamster segregates with the cervids. Although Syrian hamsters were initially deemed resistant to CWD, a recent publication demonstrates that CWD can be transmitted and adapted to hamsters [20].

Measurement of species barriers by in vitro conversion assays

A number of studies have been published on the PrP^{Sc}-induced conversion of PrP^{C} [14,15,21–25]. However, in these assays require molecular cloning to obtain recombinant PrP of different species, derived from cells in culture that may not possess brain-specific PrP posttranslational modifications, and/or brain molecules which may facilitate PrP isoform conversion. Furthermore, it now appears that PMCA may trigger stochastic generation of PrP^{Sc} *de novo* [15], which may render this technique unsuitable for determining species barriers of prion infection.

Substrate denaturation and human health

We confirm with multiple species that acid/GdnHCl-treated brain PrP^{C} is a superior substrate for *in vitro* conversion than untreated PrP^{C}, possibly by overcoming conformational barriers in partial denaturation of substrate PrP^{C}. PrP conversion in scrapie-infected neuroblastoma cells is believed to occur in endosomes, a low-pH and reducing environment [26]. The non-ruminant stomach possesses a low pH lumen, and PrP^{C} is expressed in this organ [27]. Such acidic (denaturing) organ or cellular organellar environments might also promote CWD transmission to non-cervid species, including humans.

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研究報告の概要

ヒトおよび反芻動物における伝達性海綿状脳症(TSE)の診断は死後の脳組織中のプロテアーゼ抵抗性の宿主糖タンパク質 PrP の検出に依存している。この異常なアイソフォーム(PrP^{Sc})が組織中に存在することは TSE の感染性が存在することを示すものとされている。
本研究は、PrP^{Sc} のレベルが低いか、もしくは検出されない動物の TSE 疾患の臨床的および空胞化徴候を示す脳組織内に、高タイターの TSE 感染性が存在しうることを明確に示している。本研究は PrP^{Sc} のレベルと感染価との間の相関性に疑問を投げかけるものであり、プロテアーゼ K 抵抗性の PrP をほとんどもしくは全く含まない組織が感染源となりうること、および高タイターの TSE 感染性を有しうることを示すものである。
従って、プロテアーゼ抵抗性の PrP^{Sc} を感染性の唯一の尺度としてそれに依存することは、場合によっては診断しようとするサンプルの生物学的特性を著しく過小評価し、そのことによって TSE を防止し根絶しようとする努力を減弱させる可能性がある。

報告企業の意見	今後の対応
PrP^{Sc} のレベルが低いか、もしくは検出されない動物の TSE 疾患の臨床的および空胞化徴候を示す脳組織内に、高タイターの TSE 感染性が存在するとの報告である。 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

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
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。