Health. The views expressed in this publication are those of the authors and not necessarily those of the Department of Health.

APPENDIX A

A.1. Leucodepletion

As described in §3.3, the optimistic scenario is taken to be a 60% effective ban, corresponding to a 40% reduction in the transmission coefficient. Let $t^{\mathrm{L}}=16$ be the time at which leucodepletion was first introduced, and substitute all occurrences of β_2 in the model above by $\beta_2(t)=(0.6)^{i_{\mathrm{L}}}(t)\beta_2$, where $i_{\mathrm{L}}(t)=1$ if $t\geq t^{\mathrm{L}}$ or 0 otherwise.

A.2. Ban on blood donations

The ban excludes those who received blood at any time since 1980 from donating blood after 2004, i.e. $t\geq 24$. We assume (optimistically) this ban is 90% effective. We do not explicitly exclude those who receive blood but are not themselves infected because we assume any shortfall are offset by eligible donors from the susceptible population; nor do we explicitly exclude those transfusion recipients who were infected by the primary route because the number of such people is small. Let $t^{\mathrm{B}}=24$ be the time at which the ban on blood donations from transfusion recipients was introduced, then equation (3.4) can be rewritten as

$$U'(t)=\sum_c X_c(t)\rho(a_{ct})P(a_{ct})^{i_{\mathrm{B}}(t)} - \sum_c N_c(t)\phi(t,a_{ct})U(t)/V,$$

$$C'(t)=\sum_{c,f}\left(Y_{c,f}(t)P(a_{ct})^{i_{\mathrm{B}}(t)}+(1-i_{\mathrm{B}}(t)Z_{c,f}(t)\right)\rho(a_{ct}) - \sum_c N_c(t)\phi(t,a_{ct})C(t)/V,$$

where $P(a_{ct})$ is the proportion of those age a_{ct}, who have not received any blood since 1980, and $i_{\mathrm{B}}(t)=0.9$ if $t\geq t^{\mathrm{B}}$ or 0 otherwise. In practice, we assume that $P(a)\approx 1$ and so $Y_{c,f}(t)P(a_{ct})\approx Y_{c,f}(t)$ and $X_c(t)P(a_{ct})\approx X_c(t)$. The exclusion from March 2004 onwards of those who have received blood transfusions after 1980 from donating blood will mean the effective reproduction number (i.e. the basic reproduction number immediately following intervention) is close to 0.

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一般的名称			研究報告の公表状況	Transmission of Elk and Deer Prions to Transgenic Mice. Tamguney, G. et al., J. Virol., 80; 9104-9114 (2006)	公表国 米国	
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研究報告の概要	使用上の注意記載状況・その他参考事項等
本論文はエルクとシカの脳から単離した慢性消耗病(CWD)プリオンのトランスジェニック(Tg)マウスへの感染成功例を示している。この目的のために，シカまたはエルクのプリオンタンパク質(ElkPrP，DePrpPp)のどちらかを発現する Tg マウスが作成された。これらの Tg マウスでは，マウス PrP は発現されず，シカ科の PrP のみ発現していた。いずれの系統の Tg マウスも自然発生の神経疾患は発症しなかった。シカ脳組織の調製液から，疾病に関与する PrP のレベルがシカ間で大きく異なることが示唆された。エルクとシカの CWD を接種した Tg マウス(ElkPrP または DePrP)では感染率が 100%であった(接種した全マウスで発症)。プリオン病の診断は，免疫組織化学と組み合わせた組織学的分析で行い，罹患したマウスの脳内に多数のプリオン・アミロイド斑が示された。Tg マウス(ElkPrP)では 180-200 日の間に，Tg マウス(DePrP)では 300-400 日の間にプリオン病が発症した。平均潜伏期間は神経病理学的損傷の変化をもたない次世代マウスでは短くなったが，導入 PrP の発現レベルとは反比例した。野生型マウス PrP を過剰発現した Tg マウス(MoPrP)にシカの脳組織を接種したところ，8 匹中 1 匹だけが感染した。この症例では，神経解剖学上の脳病変の特徴は，Tg マウス(ElkPrP 及び DePrP)で観察されるものとは異なっていた。対照的に，ウシ，ヒツジ，またはヒト PrP を過剰発現した Tg マウスへ CWD プリオンを接種したところ，500 日以上経ってもいかなる症状も発現しなかった。これらの結果から，エルクとシカの CWD プリオンはシカ科間で容易に感染することが確認された。	BYL-2007-0247

報告企業の意見	今後の対応
本文献ではエルクとシカの CWD プリオンはシカ科間で容易に感染することが確認された。CWD のウシまたはヒトへの感染を示す証拠は見つかっておらず，現時点では感染リスクは極めて低いと考える。	これまでに，種の壁を越えたという報告例はないため，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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Transmission of Elk and Deer Prions to Transgenic Mice†

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Chronic wasting disease (CWD) is a fatal prion disease in deer and elk. Unique among the prion diseases, it is transmitted among captive and free-ranging animals. To facilitate studies of the biology of CWD prions, we generated five lines of transgenic (Tg) mice expressing prion protein (PrP) from Rocky Mountain elk (*Cervus elaphus nelsoni*), denoted Tg(ElkPrP), and two lines of Tg mice expressing PrP common to white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*), denoted Tg(DePrP). None of the Tg(ElkPrP) or Tg(DePrP) mice exhibited spontaneous neurologic dysfunction at more than 600 days of age. Brain samples from CWD-positive elk, white-tailed deer, and mule deer produced disease in Tg(ElkPrP) mice between 180 and 200 days after inoculation and in Tg(DePrP) mice between 300 and 400 days. One of eight cervid brain inocula transmitted disease to Tg(MoPrP)4053 mice overexpressing wild-type mouse PrP-A in ~540 days. Neuropathologic analysis revealed abundant PrP amyloid plaques in the brains of ill mice. Brain homogenates from symptomatic Tg(ElkPrP) mice produced disease in 120 to 190 days in Tg(ElkPrP) mice. In contrast to the Tg(ElkPrP) and Tg(DePrP) mice, Tg mice overexpressing human, bovine, or ovine PrP did not develop prion disease after inoculation with CWD prions from among nine different isolates after >500 days. These findings suggest that CWD prions from elk, mule deer, and white-tailed deer can be readily transmitted among these three cervid species.

Prions are transmissible pathogens that accumulate in the central nervous system (CNS) and cause fatal neurodegeneration (34). Prions are composed of an alternatively folded isoform of the prion protein (PrP), denoted PrP^{Sc}. The precursor of PrP^{Sc} is a cellular protein designated PrP^{C} that is encoded by a chromosomal gene. Prion diseases afflict humans as well as livestock, such as cattle, goats, and sheep; additionally, prions cause CNS disease in captive and wild populations of deer and elk (51, 53). In contrast to scrapie of sheep and goats, bovine spongiform encephalopathy (BSE) in cattle has been transmitted to humans and has killed more than 170 teenagers and young adults as variant Creutzfeldt-Jakob disease (vCJD) (44, 49, 51, 52). BSE prions have been experimentally transmitted to sheep and appear to be transmitted naturally among sheep (6). Recently, BSE prions were found in goats (14).

The transmission of BSE prions to humans has elevated concern about the possibility of the zoonotic transmission of chronic wasting disease (CWD) from deer and elk to humans (5, 56). Hunters and other consumers of venison are potentially at risk to acquire prion disease from infected deer and elk. CWD was first observed in 1967 in cervids and was recognized as a prion disease a decade later (54). CWD has been reported in 14 U.S. states and 2 Canadian provinces.

The epidemiology of CWD is unclear. In contrast to BSE and scrapie, CWD is highly transmissible among cervids. In some captive mule deer (*Odocoileus hemionus*) herds, 90% of the animals have been reported to be infected with CWD prions (28). The prevalence of CWD cases in free-ranging deer populations can be up to ~30% (53). The number of cases of CWD that arises spontaneously and then spreads horizontally, however, is unknown. Commercial farming and trade with cervids may foster horizontal transmission of CWD. It seems likely that, as surveillance improves, the known geographic distribution of CWD will increase. Cases of CWD have been reported in South Korea where elk had been imported from Canada (21). Furthermore, free-ranging elk and deer occasionally share the same pastures with cattle and sheep. It is therefore of concern whether CWD prions can be transmitted to livestock and on to humans. The passage of prions into a new host species can alter the host range: hamsters are resistant to CWD prions from deer and elk but are susceptible to CWD prions previously passaged in ferrets (4).

While our work was in progress, two reports appeared describing the transmission of CWD prions to transgenic (Tg) mice expressing cervid PrP (9, 23). The first study showed transmission of CWD prions to Tg(CerPrP) mice expressing the S2 PrP allele (GenBank accession no. AF009180) of mule deer, and the second study reported transmission of CWD prions to Tg12 mice expressing the eGMSE PrP allele (GenBank accession no. AF156183) of Rocky Mountain elk (*Cervus elaphus nelsoni*). The respective alleles are expressed exclusively in deer or elk and give rise to PrP molecules that differ only at residue 226, which is glutamine in deer PrP and glutamate in elk PrP. Transmission of a CWD brain sample from Rocky Mountain elk to Tg(CerPrP) mice resulted in incubation times of ~240 days. Transmission times of several CWD brain samples from mule deer were between 230 and 260 days. On second passage, the incubation time was 160 days in mice

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homozygous for the transgene (9). The second study showed transmission of CWD prions from elk to Tg12 mice within 120 to 140 days; no change in incubation time was observed on second passage (23).

In the findings reported here, we describe studies with Tg mice expressing either elk PrP [Tg(ElkPrP)] or deer PrP [Tg(DePrP)]. The Tg(ElkPrP) mice used in this study express the same PrP as Tg12 mice (23), and the Tg(DePrP) mice express the same PrP as Tg(CerPrP) mice (9). We generated four lines of Tg(ElkPrP) mice, one of which was bred to homozygosity, and two lines of Tg(DePrP) mice. We inoculated all lines with CWD prions from elk ($n = 1$). Additionally, we inoculated Tg(ElkPrP) and Tg(DePrP) lines with CWD prions from mule deer ($n = 2$) and white-tailed deer (*Odocoileus virginianus*; $n = 2$). Tg(ElkPrP) mice succumbed to prion disease within 180 to 200 days and Tg(DePrP) mice, with slightly lower DePrP transgene expression levels, within 300 to 400 days. Neuropathologic analysis showed spongiform degeneration, florid PrP amyloid plaques, and astrocytic gliosis in ill mice.

Based on the data reported here and those of others cited above, CWD prions from elk, mule deer, or white-tailed deer seem to be equally transmissible among these three cervid species. In addition, CWD prions do not readily transmit disease to Tg mice expressing human, bovine, or ovine PrP. Whether CWD prions cause disease in humans or livestock remains uncertain.

MATERIALS AND METHODS

Source of transgenic mice. Unless otherwise specified, all Tg mice originated from Zrch.*Prnp*$^{0/0}$ mice, which do not express endogenous PrP from mouse (MoPrP) (10). Tg(BoPrP)4092 and Tg(OvPrP,VRQ)338 mice are homozygous for the respective transgene; all other lines used in this study are hemizygous. Tg(HuPrP)440, Tg(BoPrP)4092, Tg(BoPrP)4125, and Tg(MoPrP)4053 mice have been described previously (39, 42, 46, 47). Transgenic lines were maintained by breeding with FVB/*Prnp*$^{0/0}$ mice, except for Tg(MoPrP)4053, in which the endogenous *Prnp* gene was maintained by breeding with FVB mice (Charles River Laboratories, Wilmington, MA). Tg(OvPrP,VRQ)338 mice were a generous gift from H. Laude (26).

The PrP open reading frame (ORF) for ElkPrP was PCR amplified from elk tissue using the sequence-specific primer pair 5′-GTCTGTCGACGATG GTGAAAAGCCACATAGGC-3′ and 5′-GTCCTCGAGCTATCCTACTAT GAGAAAAATGAG-3′. DePrP was obtained by introducing a E226Q mutation into ElkPrP by site-directed mutagenesis using a QuikChange Multi Site-Directed mutagenesis kit (Stratagene, La Jolla, CA) and the mutagenesis primer 5′-CACCCAGTACCAGAGAGAATCCCAGGCTTATTACCAAAG AGGGG-3′. Complete sequences of ORF constructs were determined and archived by using Vector NTI Advance software (Invitrogen Corp., Carlsbad, CA). Tg(ElkPrP)12577, Tg(ElkPrP)12580, Tg(ElkPrP)3934, Tg(ElkPrP)12584, Tg(DePrP)10945, and Tg(DePrP)10969 mice were generated using the cosSHa.Tet cosmid vector for transgenic expression as described previously (41). Only one line, Tg(ElkPrP$^{+/+}$)3934, was made homozygous for the transgene by intercrossing Tg(ElkPrP)3934 mice. Expression levels of PrP^{C} in the brains of Tg(ElkPrP) and Tg(DePrP) mice were determined by dot blotting using serial dilutions of homogenate and were compared to that of wild-type (WT) FVB mice (40). PrP was detected with the humanized recombinant fragment antibody (recFab) HuM-P (39) and developed with an enhanced chemiluminescent detection system (Amersham Biosciences, Piscataway, NJ) (44). The recFab HuM-P was expressed and fermented in *Escherichia coli* 33B6 competent cells and purified as described previously (33).

Prion isolates and transmission studies. The RML prion strain was derived from the Chandler isolate (12) passaged in CD-1 mice. The BSE isolate PG31/90 was obtained from John Wilesmith at the Central Veterinary Laboratory, Weybridge, United Kingdom. The sporadic CJD (sCJD) MM129 prion isolate HU00178 was obtained from a patient who was diagnosed with sCJD and whose PrP ORF revealed no mutations and methionine homozygosity at residue 129. The sheep scrapie isolate no. 027, derived from a Suffolk sheep with the ARQ genotype, was obtained from USDA. CWD elk isolates 03-12609 (Elk1), 03-01495 (Elk2), and 03-01483 (Elk3); mule deer (MD) isolates 03-12776 (MD1), 03-11714 (MD2), and 03-12812 (MD3); and white-tailed deer (WTD) isolates 03-12473 (WTD1), 8527 (WTD2), and 11993 (WTD3) were collected at the Wildlife Research Center in Fort Collins, Colorado. CWD elk isolate no. 99RA146 (Elk4) was obtained from Allen Jenny, formerly at the National Veterinary Services Laboratory, Ames, IA. Elizabeth Williams, formerly at the Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, provided the following CWD isolates: nos. 99W4049 (Elk5), 99W2864 (Elk6), 99W4050 (Elk7), 98W770 (MD4), 98W1243 (MD5), 98W6679 (WTD4), 94W3471 (WTD5), and 95W9717 (WTD6).

Transmissions of prion isolates to Tg mice were performed as previously described (43). For samples Elk1, MD1, MD3, WTD1, and WTD2, 15% (wt/vol) brain homogenates were prepared in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS; pH 7.4) by three 75-s cycles in a reciprocal homogenizer (Mini-BeadBeater-8; BioSpec Products, Inc., Bartlesville, OK) as described previously (38, 39). The resulting homogenate was diluted to a final concentration of 1% (wt/vol) using PBS containing 5% (wt/vol) bovine albumin Fraction V (ICN) and 0.5 U/ml penicillin (Sigma, St. Louis, MO) and with 0.5 μg/ml streptomycin (Sigma). For all other inocula, 10% (wt/vol) brain homogenates in PBS were obtained by 10 repeated extrusions through syringe needles of successively smaller sizes, from 22 to 18 gauge. For inoculation, brain homogenates were further diluted in 5% (wt/vol) bovine albumin Fraction V and PBS to obtain a final 1% (wt/vol) brain homogenate. Mice were inoculated in the right parietal lobe with 30 μl of the 1% (wt/vol) brain homogenate using a 27-gauge, disposable hypodermic syringe. The clinical status of the mice was monitored daily, while the neurologic status was assessed three times per week. Animals were euthanized following evidence of progressive neurologic dysfunction (11, 40).

Preparation of brain homogenates. For Western blotting analysis, 10% (wt/vol) brain homogenates were prepared in PBS and 4% (wt/vol) Sarkosyl by three 75-s cycles in a reciprocal homogenizer (Mini-BeadBeater-8). The resulting homogenate was diluted to a final 5% (wt/vol) using PBS containing 4% (wt/vol) Sarkosyl. Homogenates were clarified by centrifugation at 500 × *g* for 5 min on a tabletop centrifuge. Samples of 5% brain homogenates were incubated with 20 μg/ml of proteinase K (PK) (Invitrogen) for 1 h at 37°C. The reaction was stopped with 2 mM phenylmethylsulfonyl fluoride (PMSF). Samples were centrifuged at 100,000 × *g* for 1 h at 4°C. Pellets were resuspended in 10 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 0.5% (wt/vol) NP-40, and 0.5% (wt/vol) sodium deoxycholate. An equal volume of 2× sodium dodecyl sulfate sample buffer was added (25), and the mixture was boiled for 5 min prior to electrophoresis. Sodium dodecyl sulfate gel electrophoresis and Western blotting were performed as previously described (44). PrP was detected with the recFab HuM-P and developed with an enhanced chemiluminescent detection system (Amersham Biosciences). To determine the glycoform ratios of Western blot signals on exposed and developed film, we scanned the film using Kodak image station 440 CF (Kodak) and then quantified the signals using Kodak molecular imaging software (v. 4.0.3).

Preparation of brain homogenates for the CDI. For biochemical analysis only, slices from cervid brainstems weighing 250 to 350 mg were homogenized to a final concentration of 15% (wt/vol) in PBS containing 4% (wt/vol) Sarkosyl by three 75-s cycles in a reciprocal homogenizer Mini-BeadBeater-8. The resulting homogenate was diluted to a final 5% (wt/vol) using PBS containing 4% (wt/vol) Sarkosyl. The diluted samples were digested with 10 μg/ml PK for 1 h at 37°C using the shaker. After a clarification spin at 500 × *g* for 5 min at room temperature (RT) in a drum rotor (Jouan, Milford, MA), the samples were mixed with stock solution containing 10% phosphotungstate and 85 mM $MgCl_2$, pH 7.4, to obtain a final concentration of 0.31% sodium phosphotungstate and 2.6 mM of $MgCl_2$. After a 1-h incubation at 37°C on a rocking platform, the samples were centrifuged at 14,000 × *g* in a Jouan MR23i centrifuge for 30 min at RT. The resulting pellets were resuspended in H_2O containing protease inhibitors (0.5 mM PMSF; aprotinin and leupeptin, 2 μg/ml each) and assayed by the conformation-dependent immunoassay (CDI).

Sandwich CDI for cervid PrP^{Sc}. The CDI data described in this paper were generated with recFab HuM-P labeled with Eu chelate of *N*-(*p*-isothiocyanatobenzyl)-diethylenetriamine-N^1,N^2,N^3,N^3-tetraacetic acid (DTTA) at pH 8.5 for 16 h at RT according to the manufacturer's protocols (Wallac, Inc., Turku, Finland). The final Eu/Fab molar ratio was 4.3.

The principle, development, calibration, and calculation of cervid PrP^{Sc} concentration from CDI data have been described previously (38, 39). Briefly, each sample was divided into two aliquots: (i) untreated (designated native [N]) and (ii) mixed to a final concentration of 4 M guanidine hydrochloride and heated for 5 min at 80°C (designated denatured [D]). Both samples were immediately diluted 20-fold with H_2O containing protease inhibitors (5 mM PMSF; aprotinin

and leupeptin, 4 μg/ml each), and aliquots were loaded on a 96-well, black polystyrene plate (Packard, Meriden, CT) that was coated overnight with 5 μg/ml of recFab HuM-D18 (33) in sodium phosphate buffer (pH 7.4) and blocked with Tris-buffered saline (pH 7.8) containing 0.25% (wt/vol) bovine serum albumin and 0.1% (wt/vol) Tween 20 for 1 h at RT. The plates containing native and denatured aliquots of each sample were then incubated for 2 h at RT. The plates were washed three times with Tris-buffered saline (pH 7.8) containing 0.05% (vol/vol) Tween 20, incubated with 0.25 μg/ml of Eu-labeled recFab HuM-P at RT for 2 h, and then developed after seven washing steps in the enhancement solution provided by the europium label supplier (Wallac, Inc.). The signal was counted on a Discovery dual-wavelength, time-resolved fluorometer (Packard). As a standard, we used denatured recombinant mouse-bovine PrP (MBo2M) (43). Each plate contained positive and negative controls prepared from pooled CWD-infected or uninfected brains. The results were expressed as (D − N), the difference of the time-resolved fluorescence (TRF) results for D and N aliquots, measured in counts per minute. The concentration of cervid PrP^{Sc} is directly proportional to the (D − N) value (37–39, 48).

Neuropathology. For neuropathologic analysis, brains were removed rapidly from animals and either immersion fixed in 10% buffered formalin or frozen on dry ice. Paraffin-embedded brain sections of 8 μm were stained with hematoxylin and eosin for evaluation of neurodegeneration. Immunohistochemical detection of PrP^{Sc} on formalin-fixed, paraffin-embedded tissue sections was performed by the hydrolytic autoclaving method and by using recFab HuM-P for PrP detection (30). Histoblotting was performed using 10-μm-thick frozen coronal sections that were blotted onto nitrocellulose membranes and processed for immunohistochemistry using recFab HuM-P directed against PrP (45). Reactive astrocytic gliosis was evaluated using peroxidase immunohistochemistry with a rabbit antiserum to glial fibrillary acidic protein (GFAP; Dako, Carpinteria, CA), as previously described (29).

RESULTS

Construction of Tg(ElkPrP) and Tg(DePrP) mice. In order to obtain mice that are susceptible to CWD prions, we used the cosSHa.Tet vector to generate Tg mice that express ElkPrP or DePrP on the *Prnp*$^{0/0}$ background (41). Tg(ElkPrP) mice express ElkPrP with methionine at codon 132 (GenPept accession number AAF80282), which is commonly associated with CWD in elk (31). Tg(DePrP) mice express a PrP common to mule and white-tailed deer (GenPept accession numbers AAC33174 and AAF80284, respectively) (8, 18, 20, 32). Thus, both Tg(ElkPrP) and Tg(DePrP) express PrP with D20, Q95, G96, A116, M132, and S225 and that differs only at codon 226, which encodes glutamate in ElkPrP and glutamine in DePrP.

As shown in Table 1, we observed five lines of Tg mice overexpressing ElkPrP and two lines overexpressing DePrP for spontaneous disease. One line of uninoculated Tg(DePrP) mice was observed for ~650 days and the other for >600 days; neither line showed signs of disease. None of the uninoculated Tg(ElkPrP) mice under observation exhibited signs of disease; four lines of Tg(ElkPrP) mice were observed for >600 days and one additional line for >480 days. These healthy control Tg mice appear to demonstrate that mice tolerate the expression of cervid PrP without measurable illness.

TABLE 1. Uninoculated Tg mice expressing cervid PrPs

Transgenic line	Cervid PrP expression (*n*-fold)	Age (days)[b]	No. of animals ill/no. of animals observed
Tg(ElkPrP)12577	2	>618	0/8
Tg(ElkPrP)12580	2	>597	0/5
Tg(ElkPrP)3934	1.5[a]	>603	0/6
Tg(ElkPrP$^{+/+}$)3934	3	>487	0/6
Tg(ElkPrP)12584	3	>606	0/5
Tg(DePrP)10945	1	>611	0/6
Tg(DePrP)10969	1	>642	0/8

[a] Half of the level of ElkPrP expression in Tg(ElkPrP$^{+/+}$)3934 mice.
[b] Age of the youngest animal when the experiment was terminated.

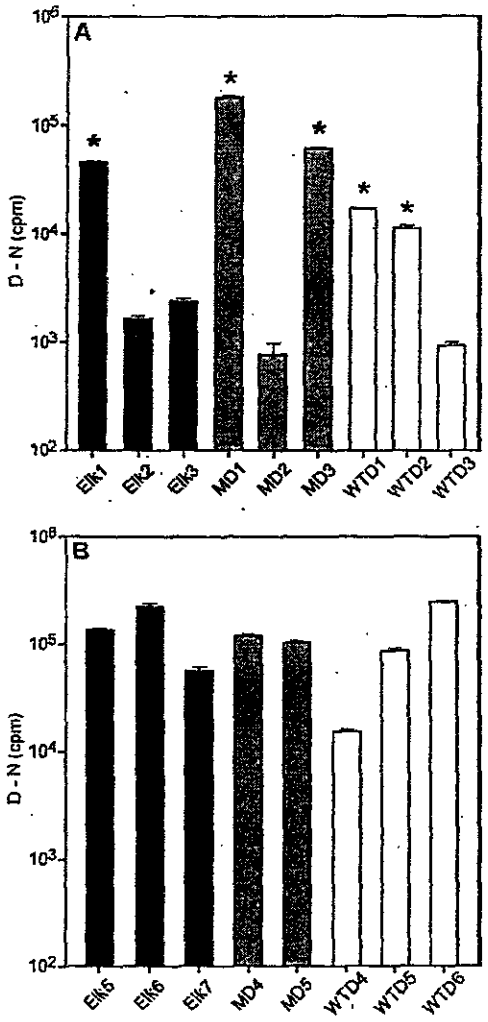

FIG. 1. Brainstem samples from 17 confirmed CWD cases in elk, mule deer, and white-tailed deer were assayed by CDI for relative PrP^{Sc} titers. The (D − N) value is directly proportional to the amount of infectious prions within each sample and is depicted in logarithmic scale (38, 39). Bars represent the averages ± standard deviations (SD) obtained from three independent measurements. The cutoff (D − N) value of 314 cpm was calculated by [median + 5(SD)] and determined from 40 brainstem samples of normal deer and elk tested by the sandwich CDI in duplicate (39). Individual CWD isolates differed 10- to 100-fold in their PrP^{Sc} content. (A) Only samples with relatively high prion titers [(D − N) > 10^4 cpm; marked with asterisks] were used to prepare inocula for transmission experiments with Tg mice expressing cervid PrP. (B) CWD samples used for transmission experiments with Tg mice expressing HuPrP, BoPrP, or OvPrP.

PrP^{Sc} levels in the brains of elk and deer with CWD. For transmission studies of CWD prions to Tg mice, we examined six brainstem samples from elk, five from mule deer, and six from white-tailed deer, all of which originated from confirmed CWD cases and contained PrP^{Sc}. Using the CDI, we determined that relative PrP^{Sc} levels varied 10- to 100-fold among the 17 samples (Fig. 1) (37, 39). In addition, DNA sequencing

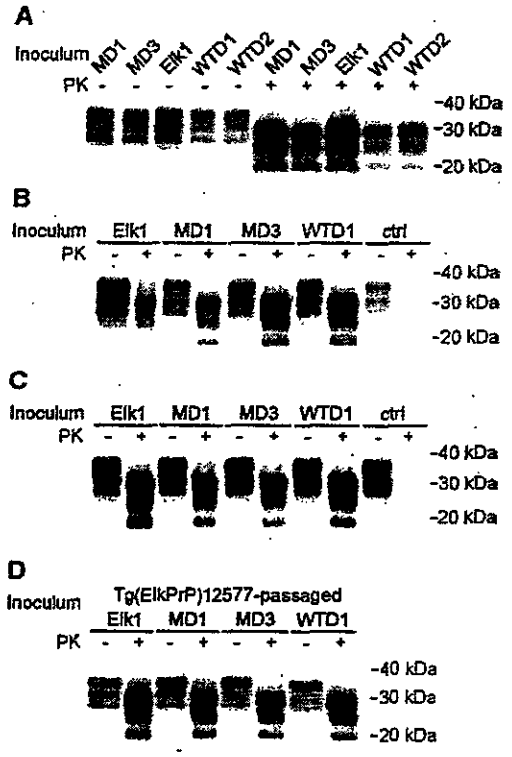

FIG. 2. Western blot analyses show that characteristics of CWD prions from diseased cervids remain similar after passage to Tg(DePrP) and Tg(ElkPrP) mice. (A) Brain homogenates of samples harboring high prion titers, as measured by the CDI and shown in Fig. 1. (B and C) Brain homogenates of diseased Tg(ElkPrP)12577 mice (B) and Tg(DePrP)10945 mice (C) infected with different CWD isolates after first passage, as indicated. ctrl, brain homogenate from an uninfected control mouse. (D) Brain homogenates of diseased Tg(ElkPrP)12577 upon second passage of different CWD isolates; first passage was in Tg(ElkPrP)12577 mice. In all panels, samples were either subjected to limited digestion with PK (+) or left undigested (−). Blots were probed using recFab HuM-P. Apparent molecular masses based on migration of protein standards are shown in kilodaltons.

of brain samples used to inoculate Tg mice expressing cervid PrP demonstrated that these samples were homozygous for *Prnp*. Among these, all elk samples had a PrP sequence identical to that expressed in Tg(ElkPrP) mice, and all mule deer and white-tailed deer samples expressed a PrP sequence identical to that expressed in Tg(DePrP) mice. Only CWD samples with high levels of PrP^{Sc} were used as inocula for transmission studies to Tg mice expressing cervid PrP; these were Elk1, MD1, MD3, WTD1, and WTD2 (Fig. 1A and 2A).

Transmission of CWD prions to Tg mice. Inoculation of Tg(ElkPrP) and Tg(DePrP) mice resulted in transmission with all CWD isolates from elk, mule deer, and white-tailed deer (Table 2). In contrast, uninoculated Tg mice remained healthy for more than 600 days (Table 1). The Elk1 isolate caused disease in Tg(ElkPrP)12577 mice in 184 days (Table 2; Fig. 3A) and in Tg(DePrP)10945 mice in 329 days (Table 2; Fig. 4). First passage of isolates MD1 and MD3 into Tg(ElkPrP)12577 mice resulted in illness in 200 days (Table 2; Fig. 3B and C) and in Tg(DePrP)10945 mice in 339 and 292 days, respectively (Table 2; Fig. 4). For inoculation of isolates WTD1 (Fig. 3D) and WTD2, first passage in Tg(ElkPrP)12577 mice resulted in disease in 179 and 182 days, respectively (Table 2), and in Tg (DePrP)10945 mice in 408 and 399 days (Fig. 4), respectively. Western blot analysis showed identical PrP^{Sc}-banding patterns for all CWD inocula (Fig. 2A), which remained unchanged upon passage in Tg(ElkPrP)12577 mice (Fig. 2B) and Tg (DePrP)10945 mice (Fig. 2C). Brain homogenates prepared from CWD-inoculated Tg(ElkPrP)12577 and passaged into other Tg(ElkPrP)12577 mice also resulted in the same PrP^{Sc}-banding pattern (Fig. 2D). Additionally, quantification of the glycoforms in PK-resistant PrP^{Sc} of the natural and mouse-passaged CWD prions confirmed that all samples had similar glycosylation ratios (see Fig. S1 in the supplemental material).

Mean incubation times for Tg(ElkPrP) lines with higher PrP^{C} expression levels were shorter than for Tg(DePrP) lines (Table 2; Fig. 5). The level of ElkPrP and of DePrP was plotted as a function of the incubation time for the seven Tg cervid PrP lines inoculated with the Elk1 sample (Fig. 5). As shown, the length of the incubation time is inversely related to the level of expression of the cervid PrP transgene for the seven lines tested. This relationship is reminiscent of that found for Tg mice expressing Syrian hamster PrP (SHaPrP) (35).

Incubation periods in Tg mice decreased upon second passage. The second passage of Elk1, MD1, MD3, and WTD1 resulted in a shortening of the mean incubation periods by 10 to 70 days. For the second passage of each isolate, we prepared the inoculum from brain homogenates of individual Tg(ElkPrP)12577 mice that had been euthanized upon the onset of neurologic symptoms. Second passage of Elk1 prions reduced the mean incubation period from ~180 days to ~120 days and ~140 days (Table 3; Fig. 3A). The mean incubation period for the MD1 isolate decreased from 200 days to ~130 and ~140 days (Table 3; Fig. 3B), and that for the MD3 isolate decreased from 200 days to ~150 and ~190 days (Table 3; Fig. 3C). The second passage of the WTD1 isolate decreased the mean incubation period from ~180 days to ~150 and ~160 days (Table 3; Fig. 3D).

TABLE 2. Transmission of CWD prions to Tg mice expressing cervid PrP

Transgenic line	Cervid PrP expression (*n*-fold)	Inoculum	Incubation time ± SEM (days)	No. of animals ill/no. of animals inoculated
Tg(ElkPrP)12577	2	Elk1	184 ± 5.7	7/7
		MD1	200 ± 4.3	6/6
		MD3	200 ± 9.1	8/8
		WTD1	179 ± 9.1	7/7
		WTD2	182 ± 7.0	8/8
Tg(ElkPrP)12580	2	Elk1	204 ± 8.5	8/8
Tg(ElkPrP)3934	1.5[a]	Elk1	264 ± 15.8	8/8
Tg(ElkPrP$^{+/+}$)3934	3	Elk1	145 ± 9.0	7/7
Tg(ElkPrP)12584	3	Elk1	149 ± 8.3	7/7
Tg(DePrP)10945	1	Elk1	329 ± 22.2	8/8
		MD1	339 ± 25.9	8/8
		MD3	292 ± 17.6	8/8
		WTD1	408 ± 30.8	6/6
		WTD2	399 ± 21.6	5/5
Tg(DePrP)10969	1	Elk1	304 ± 21.0	6/7
		MD1	323 ± 16.1	8/8

[a] Half of the level of ElkPrP expression in Tg(ElkPrP$^{+/+}$)3934 mice.

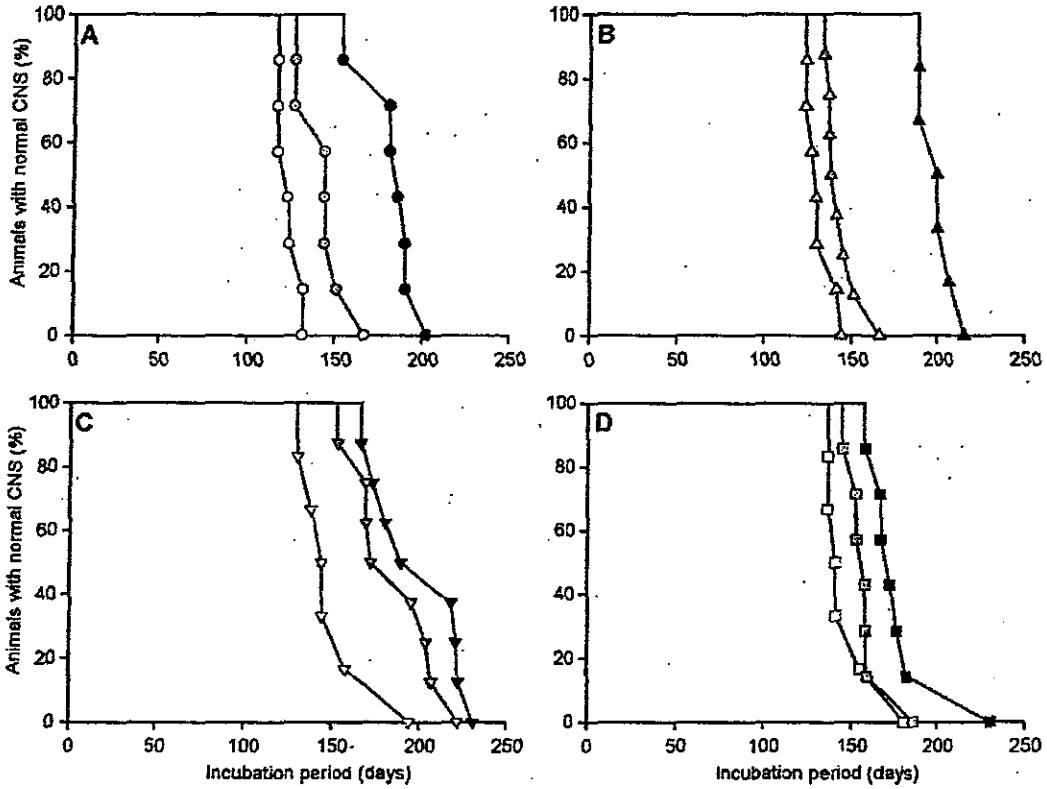

FIG. 3. Survival curves from transmissions of Elk1 (filled circles) (A), MD1 (filled triangles) (B), MD3 (filled inverted triangles) (C), and WTD1 (filled squares) (D) to Tg(ElkPrP)12577 mice. Serial transmission of 1% brain homogenates from Tg(ElkPrP)12577 mice that were diagnosed with clinical signs of prion disease after inoculation with the Elk1 isolate at 154 days (gray circles) and 181 days (open circles) (A), with the MD1 isolates at 189 days (two mice; gray and open triangles) (B), with the MD3 isolate at 166 days (gray triangles) and 173 days (open triangles) (C), and with the WTD1 isolate at 158 days (gray squares) and 167 days (open squares) (D).

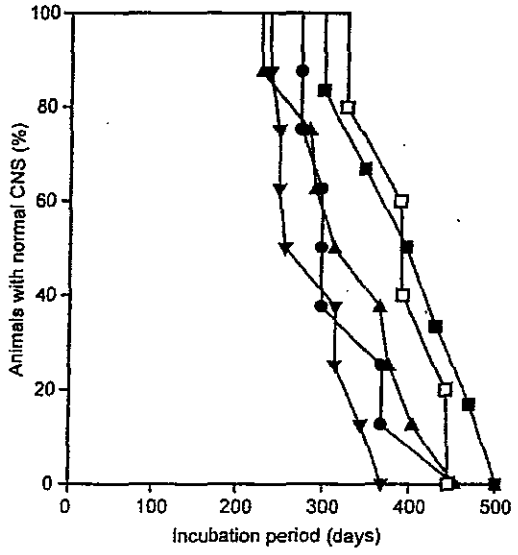

FIG. 4. Survival curves from transmissions of Elk1 (filled circles), MD1 (filled triangles), MD3 (filled inverted triangles), WTD1 (filled squares), and WTD2 (open squares) to Tg(DePrP)10945 mice.

Neuropathology in Tg mice. Histologic analysis showed that all CWD isolates caused similar neuropathologic changes in Tg(ElkPrP) and Tg(DePrP) mice, which did not vary upon second passage in Tg(ElkPrP)12577 mice. Immunohistochemistry with recFab HuM-P directed against PrP showed similar neuropathologic changes for all five isolates in the Tg(Elk

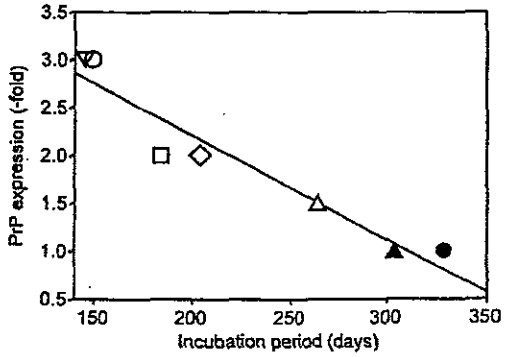

FIG. 5. Mean incubation periods in Tg mice expressing cervid PrP inoculated with Elk1 prions. The expression level of PrP inversely correlates with the incubation time (R = 0.96). Open inverted triangle, Tg(ElkPrP$^{+/+}$)3934; open circle, Tg(ElkPrP)12584; open square, Tg(ElkPrP)12577; open diamond, Tg(ElkPrP)12580; open triangle, Tg(ElkPrP)3934; filled triangle, Tg(DePrP)10969; filled circle, Tg(DePrP)10945.

TABLE 3. Serial passage of CWD prions in Tg(ElkPrP)12577 mice

Inoculum (day)[a]	Incubation time ± SEM (days)	No. of animals ill/no. of animals inoculated
Elk1 (181)	123 ± 2.5	7/7
Elk1 (154)	143 ± 5.2	7/7
MD1 (189)[b]	131 ± 3.0	7/7
MD1 (189)[b]	144 ± 3.7	8/8
MD3 (173)	151 ± 9.3	6/6
MD3 (166)	186 ± 8.5	8/8
WTD1 (167)	149 ± 6.9	6/6
WTD1 (158)	159 ± 4.9	7/7

[a] All inocula shown were serially passaged into Tg(ElkPrP)12577 mice. The day on which the animal was sacrificed due to the onset of prion disease is given for each inoculum.

[b] These inocula were prepared from two individual animals that were diagnosed with illness at 189 days postinoculation.

PrP)12577 (Fig. 6A to C) and Tg(DePrP)10945 (Fig. 6D to F) mice. Numerous PrP amyloid plaques, common in CWD (3, 15, 55), were seen in the brains of ill Tg(ElkPrP) and Tg(DePrP) mice. Most plaques were periventricular, but some were present away from the ventricle, particularly in the hippocampal neuropil (Fig. 6A to F). Florid plaques were abundant (Fig. 6G), and staining with Thioflavine S verified that many PrP-positive plaques were true amyloid (Fig. 6H). Hematoxylin and eosin staining showed widespread spongiform degeneration within the hippocampus, while staining with antibodies against GFAP revealed intensive astrocytic gliosis (data not shown). Histoblot analysis showed that CWD prions from elk, mule deer, and white-tailed deer resulted in similar patterns of PrP^{Sc} deposition in the brains of Tg(ElkPrP) (Fig. 7A to D) and Tg(DePrP) mice (Fig. 7E to H).

Inoculation of Tg(HuPrP), Tg(BoPrP), and Tg(OvPrP) mice with CWD prions. To test the susceptibility of humans, cattle, and sheep to CWD prions, we intracerebrally inoculated Tg mice that overexpress either human PrP [Tg(HuPrP)], bovine PrP [Tg(BoPrP)], sheep PrP [Tg(OvPrP)], or MoPrP [Tg(MoPrP)] (Table 4). The inocula used contained high PrP^{Sc} titers as confirmed by CDI (Fig. 1B). Tg(HuPrP)440 mice express HuPrP at a level twofold greater than that found in human brain; when inoculated with sCJD prions, these mice succumb to disease in ~150 days (47). In contrast, Tg(HuPrP)440 mice remained healthy for >500 days after infection with each of four different CWD isolates from elk (Elk4, Elk5, Elk6, and Elk7), two from mule deer (MD4 and MD5), and two from white-tailed deer (WTD4 and WTD5) (Table 4). Similar results were obtained with Tg(BoPrP) mice expressing PrP at levels 10-fold higher than

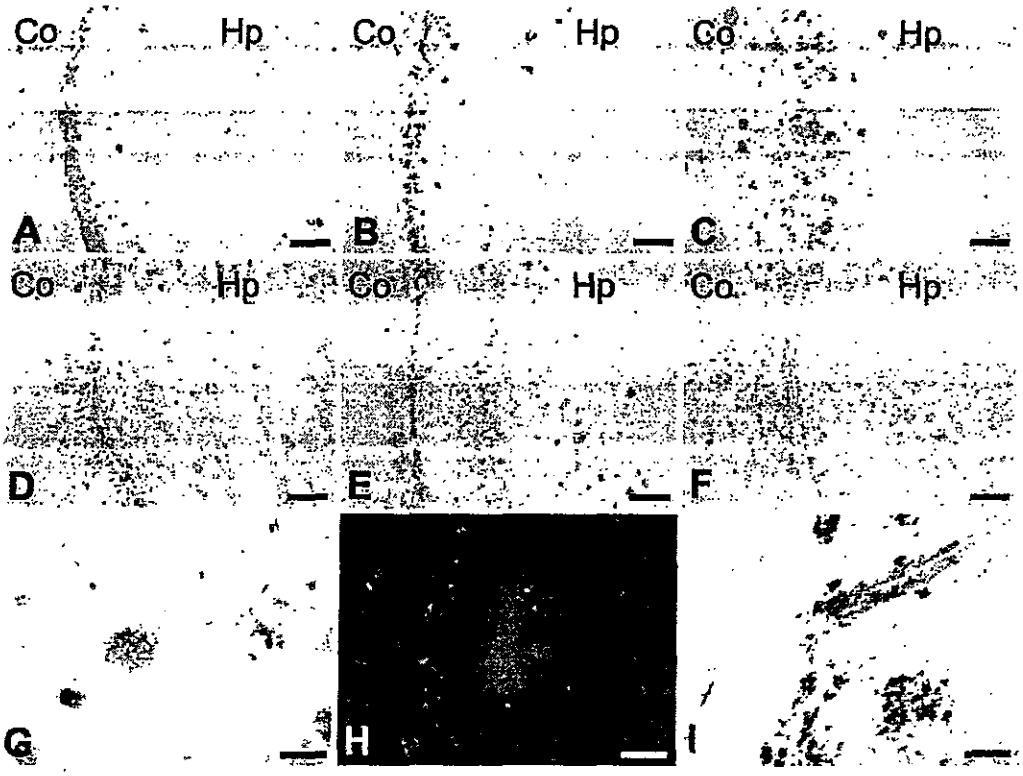

FIG. 6. Immunohistochemistry for PrP^{Sc} with recFab HuM-P shows PrP amyloid plaque deposition in brain sections from Tg(ElkPrP)12577 mice (A to C), Tg(DePrP)10945 mice (D to H), and Tg(MoPrP)4053 mice (I) inoculated intracerebrally with CWD prions from elk (A, D, G, H, and I), mule deer (B and E), and white-tailed deer (C and F). In Tg(ElkPrP)12577 mice, most of the PrP plaques were located in the periventricular region with each of the CWD inocula (A to C). In Tg(DePrP)10945 mice, large numbers of PrP plaques were located both in the periventricular region and in the brain parenchyma away from the ventricles (D to F). Many florid plaques, characterized by a single PrP^{Sc} deposit surrounded by vacuoles, were seen (G). Thioflavine S staining shows that many of the larger PrP plaque deposits are amyloid (H). Large deposits of PrP plaques were found in Tg(MoPrP)4053 mice following inoculation with elk CWD prions (I). Hp, Hippocampus; Co, Corpus callosum; bars in panels A to F, 150 μm; bars in panels G to I, 40 μm.