Transmission of CWD from White-tailed Deer (*Odocoileus virginianus*), to Elk Transgenic Mice Results in Modifications to the Infectious Prion

by

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Abstract

Prion diseases are a class of fatal, neurodegenerative disorders resulting from a protein-misfolding event. Of particular concern is chronic wasting disease (CWD), a naturally occurring prion disease affecting cervids; this disease is prevalent in North America and is expanding its host range and geographic distribution. The full extent of CWD strains and the basis behind emergence of new strain properties is incompletely understood. Previously believed to be a single entity, recent evidence supports the idea that CWD can be attributed to several prion strains, which can be distinguished through passage between hosts of differing *PRNP* genotypes. This study explores interspecies CWD transmission between white-tailed deer (WTD) and elk. I hypothesize that passage of WTD CWD into elk results in a modified infectious prion. To explore this, mice expressing elk *PRNP* were challenged with CWD prions from elk, or from deer with four different PrP alleles (Wt/Wt, Wt/S96, Wt/H95, H95/S96). 100% penetrance in the mouse model was observed with all CWD isolates examined. Incubation periods varied depending on source material, with Wt/H95, Wt/S96 and H95/S96 showing extensions compared to those of elk and Wt/Wt CWD. Lesion profile analysis showed widespread vacuolation with elk CWD, however, regional differences were observed with WTD CWD. Accumulation of disease-associated PrP (PrP^d) appears extensive and severe in TgElk challenged with elk CWD. The WTD isolates resulted in accumulation of PrP^d of various densities, however, staining was localized to neuroanatomical regions that differed between CWD isolates. In the cervid prion cell assay (CPCA), Wt/H95 CWD isolate had higher spot counts than the other WTD isolates, comparable to elk CWD. This *in vitro* infectivity analysis demonstrated differences in susceptibility of cells to CWD agent from

one host species, demonstrating variability in virulence between the isolates, lending support to the presence of a strain mixture. In characterizing H95/S96 isolate properties; three distinct patterns in neuropathology and PrP^d deposition were identified. These passage lines were individually analyzed and differences were also observed in CPCA spot count.

Taken together, these data demonstrate that the infectious agent known as CWD is not a single agent, but can be attributed to many different CWD strains, and that through interspecies transmission novel properties can be attributed to the replicated agent.

Preface

This thesis represents an original work by Jeffrey Narayan. Dr. Jacques van der Merwe, Hristina Gapeshina and Camilo Duque Velásquez contributed to the experimental setup and data collection. The experiments were performed in accordance with the animal ethics outlined in AUP914, as part of the research project “Etiology and Pathogenesis of Prion Diseases”. Supervisory authors Drs. Debbie McKenzie and Judd Aiken contributed to concept development and the writing of the manuscript.

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Abbreviations

BSE bovine spongiform encephalopathy

CB Cerebellum

CC Cerebral cortex

CJD Creutzfeldt-Jakob disease

CKY Cheeky, hamster adapted CWD strain

CN cerebral nuclei

CNS Central Nervous system

CPCA Cervid Prion Cell Assay

CWD Chronic Wasting Disease

CWD1 CWD Strain

CWD2 CWD Strain

DNA deoxyribonucleic acid

DPI days post inoculation

DY Drowsy, hamster adapted TME strain

FFI fatal familial insomnia

FTIR Fourier-transform infrared spectroscopy

GPS global positioning system

GSS Gerstmann–Sträussler–Scheinker disease

H&E Hematoxylin and eosin staining

HC hippocampus

HT Hypothalamus

HY Hyper, hamster adapted TME strain

iCJD iatrogenic Creutzfeldt-Jakob disease

LpH proprietary phenolic disinfectant used against prions

MB Midbrain

MY Medulla

P Pons

PK proteinase K

PMCA protein misfolding cyclic amplification

PRNP Prion protein gene

PrP prion protein

PrP^{C} cellular prion protein

PrP^{CWD} pathogenic isoform of PrP specific to CWD

PrP^{d} disease-associated prion protein

PrP^{res} Proteinase k resistant prion protein

PrP^{Sc} pathogenic isoform of PrP^{C}

RT-QuIC real time quaking induced conversion, *in vitro* prion amplification assay

sCJD sporadic Creutzfeldt-Jakob disease

SDS sodium dodecyl sulfate

SSCA standard scrapie cell assay

T Thalamus

Tg33 transgenic mouse line expressing Wt white-tailed deer *PRNP*

TgElk elk transgenic mouse line

TME transmissible mink encephalopathy

TSE Transmissible Spongiform encephalopathy

UPR unfolded protein response

vCJD variant Creutzfeldt-Jakob disease

WST wasting hamster adapted CWD strain

Wt/Wt *PRNP* allele that is most prevalent; homozygous for glutamine at position 95 and glycine at position 96

Wt/H95 allele of *PRNP*, heterozygous for histidine substitution at position 95

Wt/S96 allele of *PRNP*, heterozygous for serine substitution at position 96

WTD White-tailed deer

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Introduction

1.1 Background of Prion Diseases and Chronic Wasting Disease Strains

Chronic wasting disease (CWD) is a prion disease affecting the animals in the family *Cervidae*. Since it was first described in Colorado in the 1960's, the disease has broadened its range in North America to 24 American states and two Canadian provinces[1,2]. In addition to geographic expansion, CWD has increased its host range in the wild, from mule deer (*Odocoileus hemionus*) to white-tailed deer (*Odocoileus virginianus*), moose (*Alces alces*), reindeer (*Rangifer tarandus tarandus*) and elk (*Cervus elaphus canadensis*)[3–5]. Though originating in the United States, CWD is no longer solely a disease of American cervids. CWD in captive deer and elk herds has facilitated its geographic spread to Canada, and outside of North America, to Korea, through importation of infected elk[6]. Of concern is the recent discovery of CWD in free ranging Reindeer and moose in Norway[7,8]. This marks not only its first appearance of CWD in Europe, but also an extension of the species naturally susceptible to the disease.

Etiology

Prion diseases, also known as transmissible spongiform encephalopathies (TSE) are a class of fatal, progressive neurodegenerative disorders. Prion diseases can be inherited, acquired or sporadic in nature. The causative agent is unique, as it is a protein-only molecule devoid of nucleic acids, resulting from a conformational change from the cellular form to the pathological form[9]. Prion diseases have been recognized anecdotally for hundreds of years in the case of sheep scrapie, however, the causative agent and its

properties have only become clear in the last few decades[9]. Hallmarks of TSEs include spongiform degeneration in the central nervous system (CNS), accumulation of protease-resistant material, and characteristically long incubation periods, which resulted in their early categorization as a "slow virus"[10,11]. Prions mainly affect the CNS, however, CWD prions have been detected in peripheral sites such as cardiac muscle, skeletal muscle[12,13]. Prions resist conventional approaches of decontamination such as exposure to ultraviolet light, formalin inactivation, denaturation in SDS and other detergents, and ionizing radiation[14–17]. Prion agents require stringent methods of inactivation, such as high concentrations of hypochlorite or sodium hydroxide, LpH (a mixture of phenolic compounds), or autoclaving at temperatures approaching 136°C[18–20].

A wide variety of mammalian species are susceptible to prion infection including humans, wild animals such as deer and elk, livestock such as cattle and sheep, laboratory species such as mice, hamsters, and rats. The gene encoding the prion protein is highly conserved across a broad range of taxa including mammals, birds, reptiles, amphibians, and fish[21,22]. This high level of conservation in the prion protein gene (*PRNP)* also facilitates interspecies transmission, including zoonotic.

Prion diseases are categorized into three classes: inherited, sporadic and acquired. Inherited prion diseases such as Gerstmann–Sträussler–Scheinker disease (GSS), and fatal familial insomnia (FFI), involve pathogenic mutation to the *PNRP* gene, resulting in altered PrP. Sporadic prion diseases arise from a stochastic conversion of PrP^{C} to PrP^{Sc}, though the exact mechanism is unclear. Sporadic Creutzfeldt-Jakob disease (sCJD) in humans is the prototypical example of sporadic prion disease. Acquired prion diseases occur through exposure to infectious prions, this can include intraspecies transmission

such as bovine spongiform encephalopathy (BSE), or interspecies such as variant CJD in humans[23,24].

Though they affect the same host species, human prion diseases exist in several forms with distinct etiologies and presentations[25]. CJD presents in several forms including familial CJD, sporadic CJD, and two forms of acquired disease, iatrogenic and variant CJD[23,26]. Iatrogenic CJD is acquired through exposure to prions during medical procedures such as receiving cadaveric derived growth hormone[26]. Variant CJD is transmitted to humans through the consumption of BSE-containing meat and through transfusion of contaminated blood products[23,27]. Kuru is a rare prion disease that affected the Fore peoples of Papua New Guinea. Kuru was transmitted orally in the communities through ritualistic cannibalism involved in funerary rights[28]. GSS is an inherited prion disease involving pathogenic mutations in *PRNP*, resulting in neurological symptoms such as altered gait and cognitive dysfunction[29,30]. FFI is an another inherited prion disease of humans characterized by progressive insomnia and dysautonomia[31,32]. This range in clinical presentation illustrates the diversity in the prion strains that can affect a single host.

Prion protein can adopt many isoforms, including the native folded cellular prion protein PrP^{C} and those belonging to the misfolded oligomer/amyloid conformers of PrP^{Sc}. PrP^{C} is a membrane-bound glycoprotein encoded by the *PRNP* gene and is highly expressed in the CNS of mammals[33].

PrP^{C} consists of a structured domain with two β-sheets, three α-helices, a C-terminal GPI anchor and an unstructured N-terminal domain containing metal ion coordinating octapeptide repeats[34–37]. PrP also contains two C-terminal sites of

glycosylation at residues 180 and 196 in murine numbering, which vary in glycan composition and result in three distinct bands in gel electrophoresis; diglycosylated PrP, monoglycosylated PrP and unglycosylated PrP[38,39].

Although highly expressed in the CNS, the function of PrP^{C} is incompletely understood[40,41]. It has, however, been shown to be neuroprotective and is implicated in a number of cellular processes such as metal ion coordination, cellular protection from apoptosis and oxidative stress, cell adhesion and cell signaling[42–46]. Ablation of *PRNP* is not lethal and does not result in any overt pathology, however, mild phenotypic abnormalities have been reported with some knock-out animal models, such as dysregulation of circadian rhythm, reduced copper levels in brain, increased seizure susceptibility and deficits in neuroprotection[44,47–50]. In 2012, a herd of goats was described that had a natural premature stop codon early in *PRNP* such that only seven amino acids of the mature protein remain[51]. These goats had no behavioural or biochemical abnormalities despite lacking functional PrP^{C}. Knockout animals have been generated to investigate the role of PrP^{C}, and to study the pathogenesis of PrP^{Sc} in both laboratory animals and natural hosts of disease[52–54].

The exact role that PrP^{C} plays in prion pathogenesis is not known. PrP^{C} is, however, essential for infection, as animals lacking PrP are not susceptible to disease[55]. In addition, PrP^{C} serves as a substrate for the misfolding events. Evidence shows a cellular response is initiated during prion infection that results in downregulation in PrP^{C} concentration and a change in the glycoform ratio as disease progresses[56]. This change in PrP^{C} serves as an additional variable in understanding the mechanisms involved in prion disease pathogenesis.

The misfolded PrP^{Sc} conformer forms oligomers and fibrils, which accumulate in CNS. Evidence shows oligomers to be more infectious than fibrillar PrP[57]. Although there is no consensus on the exact mechanism of neurotoxicity, several ideas have been put forward, such as PrP^{Sc} interacting with cell surface receptors and triggering neurotoxic signalling, or translational repression caused by unfolded protein response (UPR) to misfolded PrP[58,59].

The insolubility and aggregation properties of the misfolded PrP^{Sc} make it technically challenging to elucidate its structure. There are several models that fit the criteria outlined by experimental data, but no conclusive structure has been resolved. The structural data gathered using Fourier-transform infrared spectroscopy (FTIR) reveals that the α-helix rich PrP^{C} is converted into a conformer with a high β-sheet content, devoid of alpha helices[60]. X-ray fibre diffraction and electron microscopy analysis dictate a structure that is composed of beta sheets organized in a solenoid structure[61,62].

Prion Strains and the Conformational Basis

The concept of strains when applied to prions refers a set of distinct clinical signs and symptoms, pathology and biochemical properties that are consistent through transmission of a prion isolate[63–66]. Prion strains were first recognized in goats infected with scrapie isolates, where clinical signs observed with one isolate are retained upon serial infections, demonstrating that strains breed true[67].

As prions lack nucleotides, strain information must be encoded in a different manner. Prions accomplish this through conformation of the misfolded protein. This also allows for a multitude of prion strains to exist depending on the exact secondary structure adopted. This conformational change also gives rise to the pathogenicity of the infectious

prion. This change does not affect primary structure of the protein, nor does it confer any post-translational modifications to PrP^{Sc}[39,68].

Multiple prion strains can exist within a single host, and the interactions between these agents can affect the transmission properties[69]. In an interspecies transmission of transmissible mink encephalopathy (TME) into hamsters, two distinct clinical patterns are observed[70,71]. These two syndromes, termed hyper (HY) and drowsy (DY) for the resulting clinical signs, are the result of two distinct prion strains, which are separable through this interspecies transmission. As each hamster-adapted TME strain is successively transmitted through hamsters, the agents adapt to the new host, resulting in adjustments to the strain properties such as incubation periods[72]. This interspecies strain adaptation through serial passages is not specific to HY and DY, and occurs with many other prion strains, eventually resulting in a stable incubation in the host[70,72,73]. Co-infection of prion strains can alter the infection characteristics such that establishment of infection by one strain is precluded by the other. This is the case with the DY, when co-inoculated with the short-incubation strain HY, the interaction between these two agents extends the incubation period of HY[74]. In addition to interacting with HY, DY can interfere with the ability of hamster adapted-CWD and the 263K scrapie agent to establish infection in hamsters[75].

Impact of *PRNP* Polymorphisms

Previously explored in sheep, humans and mice, the sequence of *PRNP,* the gene encoding PrP, has been shown to play a role in the transmission properties of a prion agent to a host. Proteins are transcribed and translated from DNA, therefore, changes in the DNA sequence change the primary structure of the mature protein, which in turn

affects secondary and tertiary structural properties such as folding and aggregation[76]. In mice, there are two PrP alleles, with variability at codons 108L/F and 189T/V where PrP A encodes 108L and 189T, and PrP B encodes 108L and 189V[77]. Susceptibility to scrapie agents is affected by the allele of *PRNP* encoded by the host mouse[78–80]. This can be observed in ME7 infection of C57Bl or VM mice, where mean incubation period in the PrP A encoding C57Bl mice was 171 days, but was extended in the PrP B encoding VM mice to 328 days[77,81]. Mice with a single induced amino acid substitution at position 101 in *PRNP* have alteration in incubation periods in multiple interspecies transmission of prion strains[82]. This demonstrates that a single amino acid substitution is sufficient to affect changes in the transmission properties, and that interspecies transmission can be facilitated through this change.

In humans, variation at residue 129 in *PRNP* is associated with susceptibility to CJD. Human PrP encodes methionine or valine at position 129, the normal frequency is 51% M129/V129 heterozygous, 37% M129 homozygous, and 12% V129 homozygous[26]. Humans encoding 129VV are more susceptible to iatrogenic CJD, and 129VV or 128MM predisposes to sporadic CJD[26,83]. Variations in codon 129 combined with D178N substitution are linked to progression of either FFI or inherited CJD[84]. In a population of individuals encoding the D178 substitution, M129 expression correlates with the FFI phenotype and V129 with the familial CJD phenotype[32,84]. Codon 129 also affects variant CJD, with methionine homozygotes demonstrating increased susceptibility, while valine substitutions granted resistance[23,85]. Other polymorphic sites in human *PRNP* affect susceptibility to prion disease, such as the E200K substitution, which is can be found CJD populations in Europe, Asia and North America [86–90]. This polymorphism was

discovered in a population with high incidences of inherited CJD, and has been found to be solely sufficient to result in prion disease[91,92]. Other polymorphisms in human *PRNP* associated with prion disease include codons 198 and 217, which have been linked to GSS presenting with neurofibrillary tangles[30].

Ovine *PRNP* encodes four polymorphisms, 112M/T, 136A/V, 154A/H and 171Q/R/H, which affect susceptibility to scrapie[93,94]. In the Texel breed of sheep, allelic variants of *PRNP* were investigated for influence on scrapie infection, with V136 linked to increased susceptibility, and R171 linked to reduced susceptibility[94]. Together, the PrP^{VRQ}, positions 136, 154 and 171 respectively, is more susceptible to scrapie. In contrast, PrP^{ARQ} is linked to reduced susceptibility, and PrP^{ARQ} and PrP^{ARH} result in equal susceptibility and resistance[94]. Allelic composition also influences scrapie progression, with 171Q homozygous sheep over-represented in a scrapie-positive Suffolk flock[95].

PRNP genotype has been shown to affect the transmission of bovine spongiform encephalopathy (BSE). BSE exists in two forms, classical BSE, and atypical BSE, (further divided into H-type and L-type), based on electrophoretic mobility of PrP^{res}[96]. A E to K substitution at amino acid 221 in cattle *PRNP* has been linked to cases of H-type atypical BSE in 2006[97,98].

In elk *PNRP,* a polymorphism at codon 132 encodes either methionine or leucine[99]. This polymorphic region is analogous to codon 129 in humans and also influences susceptibility to prion disease in a similar manner. Elk homozygous for methionine at codon 132 were overrepresented in CWD positive populations, compared to heterozygotes or leucine homozygotes[99,100]. Studies on transgenic mice expressing elk

PrP with methionine or leucine at position 132 found that L132 animals were less susceptible to CWD infection than M132 mice[101].

Effect of 95/96 polymorphisms in CWD

The most prevalent sequence of white-tailed deer *PRNP* encodes glutamine (Q) at position 95 and glycine (G) at position 96, is termed the wild-type (Wt) allele. In CWD enzootic areas of Wisconsin, two amino substitution variants are observed in the white-tailed deer populations, a substitution of glutamine with histidine (H) at position 95, and s substitution of glycine with serine (S) at position 96[102]. Deer with polymorphisms in these two amino acids were underrepresented in CWD positive populations linking these substitutions to reduced CWD susceptibility[103]. Experimental infection studies have shown that white-tailed deer (WTD) heterozygous for these polymorphisms (Wt/Q95H, Wt/G96S) have extended incubation periods compared to Wt homozygotes when orally challenged with white-tailed deer Wt/Wt CWD[104]. This effect was investigated in mice transgenic for Wt (96G) WTD *PRNP,* using CWD material from deer with four genotypes (Wt/Wt, Wt/H95, Wt/S96, H95/S96)[105]. Transgenic mice expressing Wt/Wt allele were susceptible and infection characteristics indicated a common strain was isolated, though four different inocula were used. However, when the four WTD isolates were used to infect mice expressing the S96 allele (Tg60), the Wt/Wt and Wt/S96 isolates did not result in clinical disease, only the Wt/H95 and H95/S96 isolates resulted in infection. Serial passage of these isolates resulted in identification of a new CWD strain, ($H95^{+}$) resulting from the transmission between hosts of differing *PRNP* genotypes[105]. These data demonstrate the role of these polymorphisms not only in influencing CWD

progression, but also the role that *PRNP* polymorphisms have as a source for strain diversity.

CWD in North America

There are three main foci of CWD existing in North America, one in Colorado and Wyoming where CWD was first described, another in Wisconsin and Illinois, and the third in Canada, on the Alberta-Saskatchewan border. Interspecies CWD transmission is a cause for concern as populations of cervids differ at each focus. In Colorado/ Wyoming, there are mule deer, white-tailed deer and elk, whereas in Wisconsin/ Illinois, WTD only are present. The Alberta/ Saskatchewan enzootic area has populations of WTD, elk, and mule deer, with moose ranging throughout both provinces[106]. As the disease spreads across the prairies, the potential for transmission to elk and caribou increases. The influence that passage of CWD agents through multiple species has on agent properties is unclear, however, it has been demonstrated that transmission of CWD in white-tailed deer with differing *PRNP* genotypes results in emergence of new strains[105]. The potential for this emergence of strains increases as the disease progresses across North America, and encounters susceptible hosts of differing *PRNP* genotypes, such as would occur in an interspecies transmission. Another cause for concern is the potential for transmission of CWD into Caribou (*Rangifer tarandus)* populations in Canada. Previously only demonstrated in lab conditions, CWD has now been detected in free ranging reindeer in Norway[7,107]. This puts the Caribou population in Canada is at an risk of developing CWD, and has the potential to increase the geographic range of CWD in Canada as the caribou cross provincial borders on their migratory route[108,109].

CWD Globally

In 2002, the first reported case of CWD outside North America occurred in South Korea[6]. While the natural movement of animals had previously limited the spread of CWD to North America, the shipment of infected animals has facilitated the spread of CWD to other regions of the world. Asian and European nations have implemented surveillance programs in attempt to assess the spread of CWD. To date, surveillance programs in Belgium, Japan and Switzerland have not detected any CWD positive animals[110–113].

The Republic of Korea reported importation of CWD through infected farmed elk from Saskatchewan[6]. Further cases of CWD were documented through 2004-2006, but were isolated to imported Canadian elk. The CWD positive animals were later determined to be infected with the same CWD strain[114].

In early 2016, the Norwegian Veterinary Institute announced that a free-ranging reindeer was determined to be positive for CWD. The female reindeer was discovered during a GPS collaring operation where it died and subsequently, was determined positive for CWD. This marks the first case of CWD in Europe and a marked extension to the range of disease[7,115]. Following the identification of CWD in reindeer, the Norwegian Veterinary Institute announced it had detected CWD prions in two moose, further demonstrating the presence of CWD in Norway[8].

CWD Transmission in Cervids

CWD is naturally transmitted in populations of mule deer, white-tailed deer, elk, moose and recently, in reindeer (*Rangifer tarandus)*. Reeves muntjac deer and fallow deer (*Dama dama)* have also been shown to be susceptible to CWD following

experimental infection[107,116]. The clinical signs observed in cervids affected with CWD include weakness, cachexia (weight loss progressing to wasting), sialorrhea, dysphagia and other neurological symptoms such as lack of coordination[5]. The mechanism of CWD transmission within cervid populations is not fully understood, what is known is that CWD can be acquired through direct contact of infected animal or through environmental contamination. CWD prions can be detected in body fluids from infected animals, including blood, saliva, feces and urine[117–121]. This may facilitate direct cervid-to-cervid transmission as the infected animals congregate for grooming, fighting and mating[122]. Vertical transmission has been demonstrated in an experimental setting between Reeves' Muntjac doe and their fawn, and has been documented in free-ranging Rocky Mountain elk[123,124].

In addition to blood, feces and urine, CWD prions can also be found in muscle, fat, and antler velvet from infected animals[13,125,126]. This allows for environmental contamination, through excreta or carcasses of infected animals. Initial studies done on persistence of sheep scrapie in the environment showed retained infectivity after three years in soil[127]. Studies with CWD have demonstrated persistence in the environment for years allowing naïve deer to become infected after exposure to contaminated paddocks[118,128]. Soil-bound prions have shown resistance to degradation by ruminant digestive conditions and retain the capability to infect naïve animals[129]. Furthermore, prions are able bind soil and remain infectious, this binding can enhance the infectivity, further increasing the potential of CWD transmission from environmental sources[130,131].

Management of CWD is difficult, with the only effective strategy being culls to remove infected animals and reduce naïve populations.[132] Modelling predicts that the

intervention strategy of removing of male elk and deer would be the most effective, as it removes population with the highest infected animals[133–135]. In addition to human intervention in host depletion, strategies using predation have been investigated; wolves (*Canis lupis)* predation could be used as a means to reduce numbers of CWD positive animals[136]. Unfortunately, due to the uncertainties involved in environmental persistence modelling analysis suggests that the CWD management strategy of culling may not be sustainable as the disease expands[137].

CWD transmission into humans

Although the transmission of CWD into humans has not been documented, concerns of zoonotic potential, as occurred with BSE resulting in cases of variant CJD, spur further investigation. Experimental infection of squirrel monkeys showed susceptibility to CWD via intracerebral route and further studies demonstrated oral susceptibility[138,139]. The failure of transmission of CWD into cynomolgus macaques (*Macaca fascicularis*), which are evolutionarily closer to humans suggest a robust species barrier[139].

Several studies have been performed using transgenic mice expressing human PrP as a model for CWD infection. Early work demonstrated resistance of two humanized mouse lines to CWD from elk[140]. A study using humanized mice overexpressing *PRNP,* and encoding variations at residue 129 that alter susceptibility to CJD, were not susceptible to CWD from mule deer[141]. CWD from white-tailed deer did not cause disease in a humanized mouse line with wild-type expression levels of PrP[142]. However, recent work by Qing *et al* demonstrates the zoonotic potential for CWD in second passage through humanized mice[143].

This robust species barrier preventing zoonotic CWD transmission may be a result of sequence diversity between humans and cervid species. An investigation into the sequence diversity's role in the species barrier found that humanized mice were resistant to elk CWD, however, substituting four residues in the β2-α2 loop of human PrP^C to those of elk PrP^C allowed transmission[144].

CWD transmission into other species

It is possible that CWD could enter the human food supply through transmission to livestock. Cattle have been shown to be susceptible to experimental infection with white-tailed deer and mule deer CWD [145,146], CWD from mule deer is also transmissible to sheep [147].

In vitro methods such as prion misfolding cyclic amplification (PMCA) have been employed to assess transmissibility of CWD to non-cervid species. PMCA uses successive rounds of sonication to convert a substrate containing PrP^C and a prion seed into PK resistant material. Studies employing PMCA have previously been used to determine infectivity of tissues such as blood and antler velvet, as well as saliva and excreta, have shown rabbits to be capable of amplifying CWD prions[117,120,121,148]. Additionally, PMCA has shown bighorn sheep (*Ovis canadensis),* a species not yet shown to be susceptible to prion diseases but whose range is sympatric with species in which CWD occurs, capable of amplifying CWD prions with a low species barrier[149].

Bank voles (*Myodes glareolus*) have been termed a universal acceptor for prions due to their ability to replicate a large number of prion strains. PMCA studies have demonstrated that bank vole PrP is capable of amplifying CWD and retaining

infectivity[150]. *In vivo* studies demonstrate efficient CWD transmission to bank voles and transgenic mice containing bank vole *PRNP*[150,151].

Due to the overlap in habitats of red-backed voles (*Myodes gapperi*), meadow voles (*Microtus pennsylvanicus*), deer mice (*Peromyscus maniculatus*), and white-footed mice (*Peromyscus leucopus*), with the CWD enzootics in North America, transmissibility to these rodent species has been investigated. The study found that CWD was transmitted efficiently to all four species, with the vole species displaying incubation periods comparable to those of transgenic mice[152].

Many species visit carcasses and gut-piles from CWD infected cervids, often as scavengers, which can facilitate CWD dissemination and transmission[153]. Laboratory infected raccoons (*Procyon lotor*) failed to show clinical signs of CWD[154]. In a survey of scavengers from enzootic regions of Wisconsin, signs of CWD infection could not be detected in raccoons, badgers (*Taxidea taxus*), domestic cats (*Felis catus)* Red fox (*Vulpes vulpes*), or Virginia opossums (*Didelphis virginiana*)[155]. Interestingly, American mink, (*Mustela vison*), striped skunks (*Mephitis mephitis*), raccoons and cats did not demonstrate CWD infection, although in a laboratory setting they are capable of supporting prion infections[156–159]. Though this study was not conclusive, it does illustrate the potential for CWD transmission into scavengers. Although coyotes (*Canis latrans*) have not demonstrated CWD susceptibility, they have been implicated in the dissemination of infectious material from the carcasses of infected deer[155,160].

Models of CWD

Due to the difficulties in studying the native CWD hosts in an experimental setting, two types of animal models have been employed to investigate the disease;

adaptation of the agent to an existing model or creating a transgenic model. CWD has been experimentally transmitted into the existing animal models of ferrets, mice and Syrian golden hamsters (*Mesocricetus auratus*)[161–164]. These models allow study of CWD without the cost and time scale involved with cervid hosts.

However, transmitting CWD to laboratory animals does present challenges, primarily, the transmission barrier that exists between animals encoding different *PRNP* genotypes. One method of limiting the effect of this transmission barrier is to use transgenic animals that lack the *PRNP* of their species and instead encode the sequence of another species as a transgene. Transgenic models have been used to study zoonotic potential of animal prion diseases[140,144].

TgElk as a model for CWD

Mice expressing *PRNP* from cervids have proved to be an invaluable tool for studying CWD transmission. The TgElk mouse is one such line, developed by the Rubenstein lab in 2006[165]. Created from the PrP null on a FVB/N background, these mice express Rocky Mountain elk (*Cervus elaphus nelsoni)* PrP under the Syrian hamster PrP promoter. These transgenic mice express PrP at 2.5-fold higher level than wild type mice making the line highly suitable for transmission studies. The elk sequence differs from the wild-type WTD sequence at one amino acid, at position 226, where white-tailed deer express glutamine and elk express glutamic acid. The TgElk mice are susceptible to CWD from elk and mule deer, on first passage with 100% attack rates[165]. The TgElk mouse line has been further characterized for pathology resulting from infection of CWD from elk of the Korean outbreak [166].

Cervid Prion Cell Assay (CPCA)

The cervid prion cell assay is an *in vitro* method for characterizing prion isolates in a high throughput manner. This technique is based on the standard scrapie cell assay (SSCA), developed by the Weissmann lab[167]. In the SSCA, cell cultures are exposed to the prion strain or isolate, and if the cells are permissive to infection by the agent, PrP^{Sc} is generated. The PrP^{Sc} positive cells are then quantified in terms of proteinase K resistant spots, which are representative of the source inoculum infectivity and titre. Using this method prion infectivity can be characterized, and compared between strains and isolates.

The CPCA is a modified version of the SSCA, it utilizes rabbit kidney cells expressing elk *PRNP* transfected to express HIV-1 GAG precursor protein[168]. These cells, termed Elk21 cells, can then infected with CWD, and the infectious agents further characterized.

CWD Strains

Prion agents can be classified into strains, based on infection characteristics such incubation period, clinical signs, and neuropathology, as well as biochemical characteristics such as proteinase K resistance, glycoform pattern, electrophoretic mobility[64,66,70,169]. First identified in goats, strains have been extensively studied in mouse-adapted scrapie and hamster-adapted TME[67,70,170].

The idea of CWD strains is still expanding, with the prevalence and number of strains not yet defined. Transmission of CWD prions from elk, mule deer and white-tailed deer to mice expressing cervid PrP (Tg(CerPrP)) revealed elk isolates produce one of two distinct patterns in a stable manner, while deer isolates can produce a mixture.

Characterization of incubation periods and neuropathology revealed the presence of two main strains in the CWD population, CWD1 and CWD2[171].

Evidence for interspecies transmission resulting in emergent strains was observed upon adaption of CWD into ferrets (*Mustela putorius furo*). Mule deer CWD passaged through ferrets causes clinical disease in Syrian hamsters (*Mesocricetus auratus),* while the initial mule deer CWD inocula did not[172]. This expansion of host range demonstrates that transmission properties are altered on passage into new hosts.

CWD has also been adapted to Syrian hamsters, a model that has been utilized to separate strains of transmissible mink encephalopathy[70,173]. CWD transmission into transgenic mice overexpressing hamster PrP, followed by transmission into hamsters resulted in the isolation of two distinct phenotypes of incubation periods and clinical signs[173]. These two observed phenotypes, termed CKY and WST, were further characterized and determined to be two distinct strains, demonstrating that cross-species CWD transmission can result in new strains[173].

Complementing the work done with ferret and hamster models of CWD, transgenic approaches have also supported interspecies transmission as a means of studying CWD strains. Recent work done on transgenic mice expressing white-tailed deer *PNRP* has been used to resolve a novel strain of CWD. CWD from deer with different allotypes was used to infect mice expressing Wt/Wt WTD PrP. These mice replicated one strain that was present in all inocula tested. Transgenic mice expressing the 96S amino acid variant were susceptible only to the H95/S96 and Wt/H95 inoculum[105]. This transmission across *PRNP* genotype resulted in generation of a novel prion strain,

supporting the idea that cross-species transmission would result in modification to the amplified agent.

I hypothesize that the transmission of CWD between different cervid species and within the same species with *PRNP* polymorphisms will result in emergence of new strains. To test this hypothesis, I transmitted CWD from white-tailed deer to a transgenic mouse model expressing elk *PRNP* to determine the effect of this transmission on strain properties. As primary sequence of PrP influences transmission properties of CWD and other prion diseases, transgenic elk mice were challenged with CWD agent from deer expressing common *PRNP* allele variants (Wt/Wt, Wt/H95, Wt/S96, H95/S96), to determine the effect of *PRNP* polymorphisms.

Materials and Methods

Inocula Preparation and Transmission into TgElk mice

Brains from white-tailed deer experimentally infected with CWD in a previous study were homogenized in phosphate buffered saline to 10% (w/v). The white-tailed deer expressed four different *PRNP* alleles; the wild type allele Q95/G96, substitutions at positions 95 and 96 (Wt/95H and Wt/96S), as well as a double polymorphism Q95H/G96S[104].

Mice expressing *PRNP* from elk (*Cervus elaphus nelsoni*), previously used to model elk and mule deer CWD transmission, were obtained from Drs. Richard Rubenstein and Robert Rohwer[165]. TgElk mice weanlings were intracranially inoculated with 30µL of 2% w/v brain homogenates from uninfected white-tailed deer, elk CWD, or one of four white-tailed deer CWD isolates. Both the TgElk mice and the elk CWD inocula encoded the wild type *PRNP* allele, i.e., methionine at position 132 and glutamic acid at position 226.

Control groups consisted of uninfected white-tailed deer brain homogenate as a negative control, and CWD isolated from elk was used as a positive control.

Animals were monitored daily for onset of disease and sacrificed upon presentation of clinical signs. Brains were then collected and cut in the sagittal plane, with one hemisphere fixed in formalin for histological analysis and the other stored at -80°C for biochemical analysis.

Based on time interval between inoculation and onset of clinical signs, incubation periods in days post-inoculation (dpi) were calculated and statistical analysis performed

on the mean incubation periods for each inoculum using the Kruskal-Wallis test with Dunn's multiple comparison post-test in GraphPad Prism.

Second Passage Transmission

Brain homogenates from first passage of CWD isolated into the TgElk mouse line were used as inocula for second passage studies. TgElk mice weanlings were intracranially inoculated with 30µL 1% w/v brain homogenates from mice clinically affected with CWD isolates from WTD or elk. Animals were monitored daily for onset of disease and sacrificed upon presentation of clinical signs. Brains were then collected and cut in the sagittal plane, with one hemisphere fixed in formalin for histological analysis and the other stored at -80°C for biochemical analysis.

Initial examinations of neuropathology and distribution of disease-associated PrP revealed that after passage in the TgElk line the H95/S96 inocula produced three distinct profiles resulting from three individual mice. These mice, termed passage lines L1, L2 and L3, were isolated from the H95/S96 cohort and were examined separately, and transmitted as a second passage independently.

Proteinase K Digestion and Immunobloting

Brains of CWD-infected TgElk mice were homogenized in sterile water using a bead mill homogenizer (Omni Bead Ruptor) cooled to -10°C, to 10% (w/v). Protein concentrations were determined using MicroBCA assay kit (Life Technologies). Each brain homogenate (50µg) was digested with proteinase K (Roche Diagnostics) at a final concentration of 50µg/mL at 37°C for 45 minutes. These PK digestion conditions result in a protein to enzyme ratio of 1:1. PK-digested samples were denatured in 2.5X Laemmli buffer (25% glycerol, 12.5% β- mercaptoethanol, SDS 5% w/v, 0.5%

bromophenol blue and 150 mM Tris-Cl pH 6.8) at 100°C for 10 minutes. Samples were then stored at -80°C until western blot analysis.

Samples were heated at 100°C for 10 minutes before loading (1µg/lane) and electrophoresis at 110 volts on precast 12% NuPAGE Bis-tris gels (Life Technologies) in 1X MOPS running buffer (50mM MOPS, 50mM Tris, 0.1% SDS, 1 mM EDTA) for 3 hours. Proteins were then transferred in protein transfer buffer (190 mM glycine, 24.5 mM Tris, 10% methanol) to PVDF immobilon-P membranes (Millipore) at 35 volts for 2 hours. Membranes were blocked with 5% w/v skim milk in TBS-T (Tris-buffered saline, 0.1% v/v Tween-20) solution for 1 hour at room temperature, prior to probing with BAR224 anti-PrP monoclonal primary antibody (1:10,000 dilution in blocking solution). After 8 hours incubation with primary antibody at 4°C and subsequent washings with TBS-T, goat anti-mouse HRP-conjugated secondary antibody (1:20,000 in blocking solution) was applied and incubated at room temperature for 2 hours. Enhanced chemiluminesence (Thermo Scientific) and X- ray film (Super Rx Fujifilm™) were used to visualize protein bands.

Histopathological Analysis

Hematoxylin and Eosin (H&E) staining and immunostaining for PrP of sagittal brain sections of TgElk mice, were used to characterize lesion and disease-associated PrP deposition patterns associated with each CWD isolate. H&E staining and IHC were performed by Hristina Gapeshina, from The Centre For Prions And Protein Folding Diseases histology core. Pathology caused by prions is characteristic of the infecting strain, therefore, novel patterns would reveal presence of a modified agent[66]. Brains of clinically infected TgElk mice were collected, formalin fixed and paraffin embedded

prior to sectioning. Slides were deparaffinised in xylene and washed in ethanol and water before staining with Harris hematoxylin for 5 minutes. Slides were then washed in water and 1% acid alcohol (1% Hydrochloric acid in ethanol), and then stained with Eosin Y for 3 minutes. Slides were washed with water, ethanol and xylene prior to mounting.

Slides were digitized using NanoZoomer 2.0-series (Hamamatsu Photonics) and analyzed using NanoZoomer Digital Pathology (NDP.view) software (Hamamatsu Photonics). Mean scores of vacuolar degeneration and density were analyzed using GraphPad Prism.

Nine neuroanatomical regions from rostral to caudal, were designated in brain slices[174], cerebral cortex (CC), cerebral nuclei (CN), hippocampus (HC), thalamus (T), hypothalamus (HT), midbrain (MB), pons (P), medulla (MY) and cerebellum (CB). Slides were visually assessed for extent of vacuolation in these 9 regions. Three independent observers graded vacuolation based on a 0-4 scale modified from use in sheep scrapie, where 0 represents vacuolation comparable to uninfected control mice, 4 representing the severest vacuolation achieved[175].

Immunohistochemistry was performed to assess the level of disease associated PrP (PrP^d) deposition. Formalin fixed sagittal brain sections of TgElk mice were autoclaved at 2.1 bars (210 KPa) for 30 minutes at 121ºC. Citric acid pH 6.0 (10 mM) bathing was done for antigen retrieval. Slides are treated with 98% Formic acid treatment for 30 minutes and guanidine thiocyanate (4M) at room temperature for 2 hours. Sections are then immunostained using the anti-PrP primary antibody (1:2000) BAR224 (bertinpharma). Density of disease-associated PrP deposits was assessed visually by three independent observers in a manner modified from lesion profiling[175]. Briefly, a 0 score

was assigned to PrP^d accumulation comparable to that of the uninfected control, i.e. free of deposits, the largest and most densely clustered aggregates of PrP were assigned a 4. Intermediate distributions of deposits were scored 1-3 depending on their severity.

Cervid Prion Cell Assay (CPCA) Analysis

The CPCA is a modified form of the standard scrapie cell assay (SSCA) developed for use with cervid samples[167,168,176]. CPCA analysis was performed by Dr. Jacques van der Merwe. The modified assay utilizes Elk21 cells, RK13 cells expressing *PRNP* from elk, in 96-well plates, exposed to 0.1% and 0.01% w/v brain homogenates from TgElk mice with or without CWD infection for 5 days. Cells (20,000) are collected after three passages, and transferred to $Multiscreen_{HTS}$ IP 96-well, 0.45-µM filter plates (Millipore).

Samples were digested with proteinase K (5µg/mL) for 90 minutes at 37ºC, followed by protease inhibition with 2 mM PMSF and denaturation in 3M guanidine thiocyanate. The anti-PrP antibody BAR224, followed by goat anti-mouse AP conjugated secondary antibody is used to detect PK resistant PrP in the Elispot reaction. The plates were visualized using BCIP/NBT and data collection and analysis was done using an Autoimmun Diagnostika GmbH Elispot plate reader.

Scoring scale used to visually assess level of spongiform degeneration in sagittal brain sections of CWD infected TgElk mice. Adapted from lesion profiling of sheep scrapie by Ligios *et al* in 2002[175].

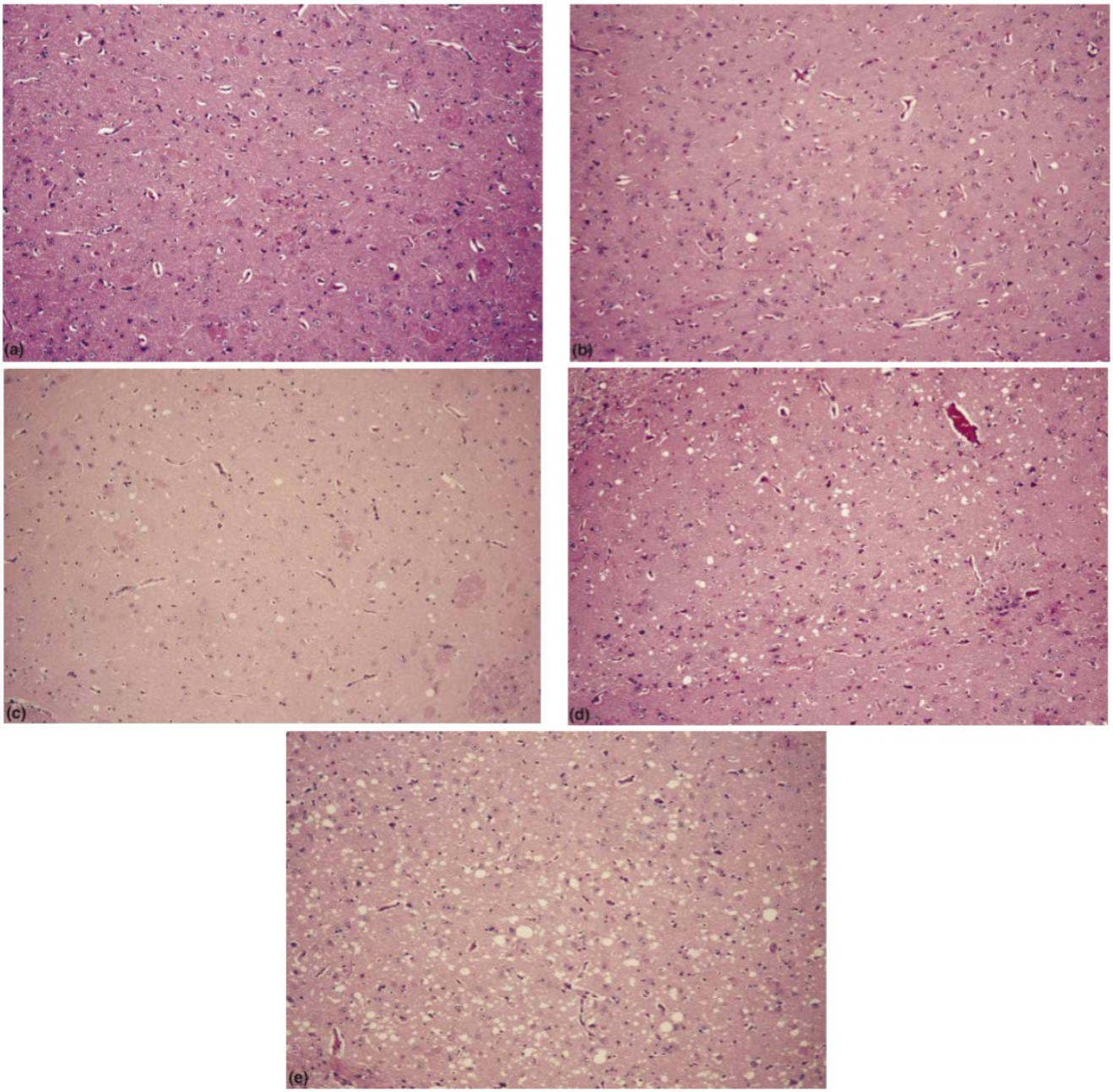
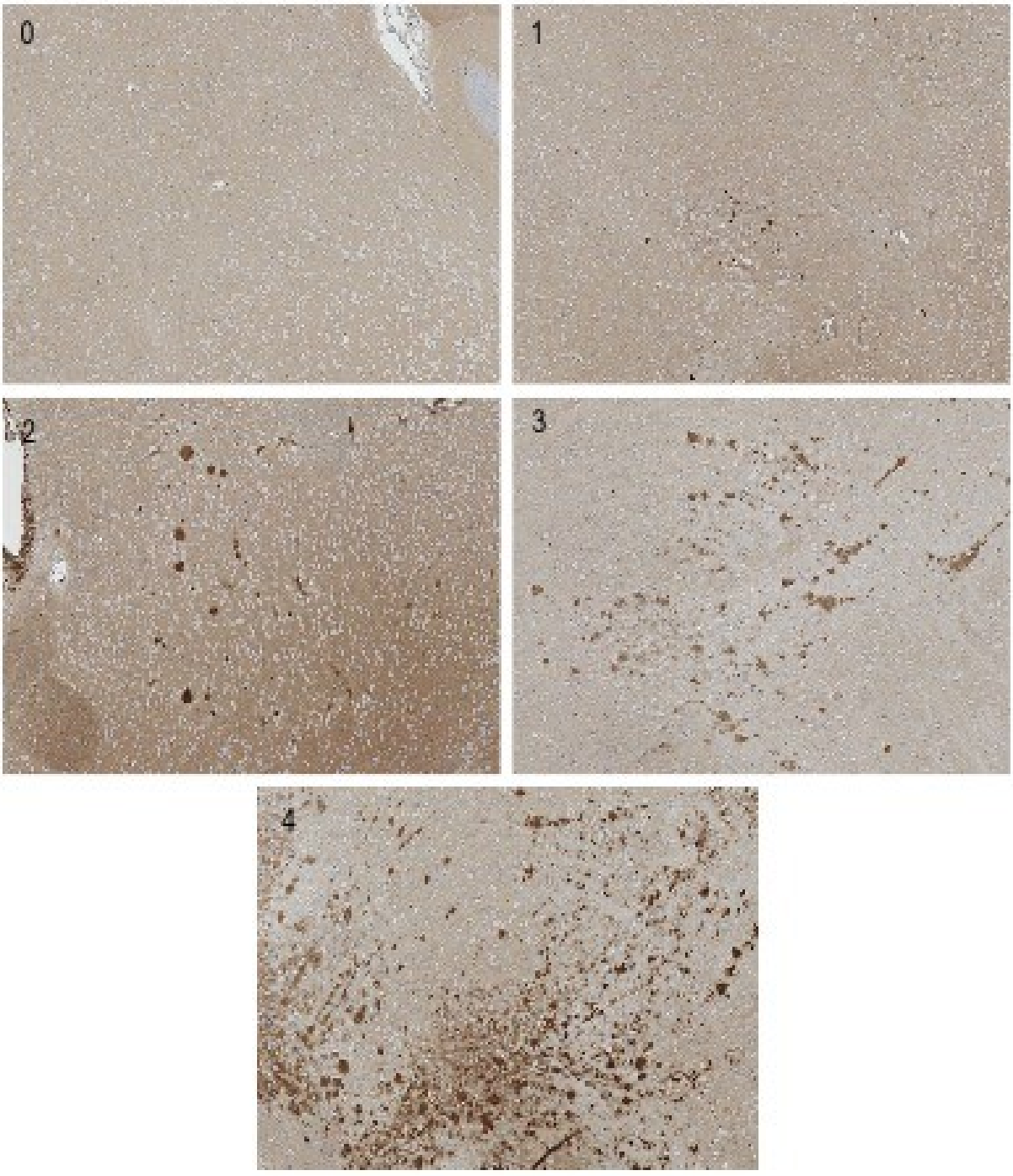


Fig. 2a–e. An example of typical 0–4 scoring, illustrated here in the nucleus accumbens. (a) Score 0—no lesions. (b) Score 1—minimum 3 vacuoles in half a field. (c) Score 2—several vacuoles evenly scattered. (d) Score 3—many vacuoles distributed in at least half a field. (e) Score 4—numerous vacuoles often coalescing, which must be observed in at least half a field. HE. Viewed with ×10 objective.

Scoring scale used to visually assess density of disease-associated PrP deposition in sagittal brain sections of CWD infected TgElk mice. Based on lesion profiling of sheep scrapie by Ligios *et al* in 2002[175].


0
1
2
3
4

Results

Transmission of elk and white-tailed deer CWD into TgElk Mice

To determine the effect of interspecies CWD transmission on agent properties, TgElk mice were intracerebrally challenged with CWD from white-tailed deer encoding one of four *PRNP* genotypes, or elk CWD[104]. Differences in transmission properties such as mean and maximum incubation period, and clinical signs would be indicative of modification of infectious agent. All CWD sources examined, elk and deer (Wt/Wt, Wt/H95, Wt/S96, H95/S96), resulted in clinically positive TgElk. Clinical signs included decline in grooming quality, kyphosis, and ataxia, eventually progressing to reduction of hind limb coordination. There were no observable differences between clinical signs of mice inoculated with WTD or elk CWD. Mice inoculated with uninfected WTD brain homogenate showed no signs of clinical disease. Elk CWD infection resulted in a mean survival of 106 days post-inoculation (dpi) grouped closely around the mean, with a standard deviation of 2 days (Figure 3A). WTD isolates have greater standard deviation, ranging from 101 days for Wt/Wt to 174 days with H95/S96. WTD isolates also showed higher variability within, and between each inoculum, as the standard deviations range from 5 days for Wt/95 to 12.6 days with H95/S96. The means of elk CWD and Wt/Wt CWD are not significantly different, however, the Wt/H95, Wt/S96 and H95/S96 inocula significantly differed from both the elk CWD and Wt/Wt ($P<0.05$).

Second passage of CWD into TgElk Mice

Brain homogenates collected from first passage of CWD isolates into TgElk mice and used to initiate a second passage. Figure 3B shows survival of second passage TgElk

mice. As observed with first passage transmission, there were no observable differences in clinical signs between inocula groups, however, three animals with incubation periods greater than 120 dpi did demonstrate agitation and increased movement. This increased agitation was observed in animals of the Wt/H95 and H95/S96 L1 groups and was not observed in any animal with an incubation periods less than 120 dpi. TgElk-elk CWD resulted in a mean survival of 97 dpi, a small reduction from first passage, however, the standard deviation was higher in second passage. The mean survival of the TgElk-WTD isolate infected mice were reduced compared to first passage transmission, most notably the H95/S96, where first passage resulted in a mean of 161 dpi and transmission of passage lines L1, L2 and L3 resulted in means of 109, 103 and 102, respectively. Comparing the mean incubation periods of all isolates resulted in no statistical significance. The Wt/H95 isolate produced a distribution of incubation periods that was markedly different from the remaining isolates, resulting in three groups of survival times (Figure 3B). Comparing means of H95/S96 passage lines resulted in no statistical significance.

Analysis of PrP

Western blot analysis was performed on 10% (w/v) brain homogenates from TgElk infected with CWD from elk or WTD, and levels of total PrP as well as proteinase K resistant PrP were examined. After passage in TgElk, elk CWD resulted in a weak signal from diglycosylated proteinase K resistant PrP and a strong monoglycosylated band. Passage of Wt/Wt, Wt/H95 and Wt/S96 white-tailed deer CWD produced robust signals from both di- and monoglycosylated PrP (Figure 4). Three individual TgElk mice of the H95/S96 cohort (L1, L2, L3) were assessed for total and PK resistant PrP, and

H95/S96 L1 resulted in a pattern that varied from L2 and L3, with a stronger monoglycosylated band.

Sensitivity to PK digestion varied between elk and WTD isolates. Elk, Wt/Wt, Wt/H95 and Wt/S96 were more protease resistant than the H95/S96 inoculum in TgElk mice. Differences can be observed within the deer-CWD infected mice, as Wt/Wt and Wt/H95 display strong signal in first and an increase after second passage, whereas H95/S96 shows high sensitivity to PK after both passages. Interestingly, Wt/S96 shows a robust signal after first passage in TgElk mice, but after second passage in TgElk mice, the signal decreases. Second passage of elk CWD in TgElk mice results in an increase in the signal from diglycosylated PrP (Figure 4).

Neuropathology of TgElk infected with CWD

To ascertain whether the regions affected by spongiform degeneration are influenced by the CWD source, sagittal brain sections of TgElk infected with CWD were assessed for neuropathology following hematoxylin and eosin staining (Figure 5). Differences in regional vacuolation, dependent on CWD source, would be indicative of modifications to the strain properties. Brain sections from mice challenged with elk CWD show widespread and severe vacuolation, which is clearly demonstrated in the hippocampus, (HC), cerebral cortex (CC), medulla (MY) and hypothalamus (HT) (Figure 5A). In contrast to the widespread spongiform degeneration observed with elk CWD, the pattern observed in TgElk infected with WTD CWD isolates appears localized to neuroanatomical structures (Figure 5 B-E). This regionalization varies depending on the source genotype of inocula. To better characterize these patterns, lesion profiling was performed (Figure 6A) on 9 brain regions, cerebral cortex (CC), cerebral nuclei (CN),

hippocampus (HC), thalamus (T), hypothalamus (HT), midbrain (MB), pons (P), medulla (MY) and cerebellum (CB). The elk CWD profile appeared high across 8/9 surveyed regions (Figure 6A). The HC region shows the regional nature of vacuolation as elk CWD, Wt/Wt, and Wt/S96 present high grades of degeneration whereas Wt/H95 has statistically lower values. The H95/S96 WTD isolate generated two distinct patterns of neuropathology. Figure 6B shows three animals from the H95/S96 inoculum group, later used as passage lines 1, 2 and 3 (L1, L2, L3) in the second passage. The H95/S96 L3 shows a lesion a profile that parallels the Wt/Wt inoculum, however, the H95/S96 L1 and L2 show a distinct and contrasting pattern, similar to each other but different from L1 and Wt/Wt.

Accumulation of PrP^d

Areas affected by disease-associated PrP in the brain are representative of the infecting prion strain; therefore differences in PrP^d deposition represent modification of the replicated prion. To determine if passage of white-tailed deer prions into TgElk affects PrP^d deposition, immunohistochemistry was performed to evaluate the levels of, and regions affected by, disease-associated PrP (Figure 7). Sagittal brain sections TgElk infected with elk CWD show widespread accumulation of disease-associated PrP^d across most surveyed regions with high densities of distribution (Figure 7). The trend of regionalization noted with vacuolation in WTD isolates is also observed with density of PrP^d distribution (Figure 7B-E). To discern differences in neuroanatomical PrP^d accumulation, profiling was done on 9 brain regions and density of deposition was assessed (Figure 8). Elk CWD produced extensive PrP^d accumulation across 8/9 surveyed regions, whereas density of distribution varied with each WTD isolate (Figure 8A). The

hippocampal (HC) region displays a wide range in PrP^d distribution with elk CWD, Wt/Wt, and Wt/S96 having high levels, while Wt/H95 has low levels of accumulation (Figure 8A). Similar to the vacuolation, the H95/S95 inoculum produced multiple differing profiles that correlated with passage lines (Figure 8B).

Cervid Prion Cell Assay (CPCA)

Brain homogenates from TgElk mice infected with elk or white-tailed deer CWD was subjected to the cervid prion cell assay (CPCA) performed by Dr. Jacques van der Merwe. CPCA is an *in vitro* infectivity assay where cells expressing cervid *PRNP* cells are challenged with prion inocula and spot counts are reflective of level of protease resistant PrP generated. Elk CWD generated a robust response of 3,000 spots/20,000 cells. In contrast first passage material from TgElk infected with WTD CWD produced lower spot counts of 1500-2000 spots/20,000 cells, with the notable exception of Wt/H95 which resulted in spot counts comparable to those generated with elk CWD.

Material from second passage in the TgElk mice was also analyzed via the CPCA, with H95/S96 passage lines analyzed individually. All second passage isolates resulted in increased spot counts compared to first passage material. The resultant spot counts were comparable between all second passage isolates analyzed, and no difference was observed between second passage WTD and elk CWD isolates.

Discussion

This study involves transmission of CWD isolates from white-tailed deer to Rocky Mountain elk to investigate the properties of interspecies transmission and to determine if this transmission modifies the infectious prion. We modeled this interspecies transmission using mice transgenic for elk (*Cervus elaphus nelsoni*) *PRNP* and CWD prions from four white-tailed deer (*Odocoileus virginianus*) each expressing a different *PRNP* genotype. Based on previous data form Duque Velasquez *et al,* illustrating that transmission of CWD between hosts of differing *PRNP* genotypes allowing emergence of novel CWD strains, we aimed to determine to what extent this effect occurs between animals expressing *PRNP* from different species[105]. The decision to use CWD isolates from WTD with amino acid substitutions at positions 95 and 96 in *PNRP* was based on data demonstrating reduced CWD susceptibility and disease progression in animals with these alleles[103,104]. My hypothesis is that cross-species transmission of CWD from deer to elk would result in a modified infectious prion.

Incubation periods

Our investigation modeled interspecies CWD transmission by utilizing TgElk mice which express elk *PNRP*[165]. After exposing mice to CWD isolates, we observed the mice for clinical signs of prion infection and determined the incubation periods of each source inocula (Figure 3). For TgElk mice infected with the elk CWD isolate, the mean survival of 106 days was consistent with the ranges previously reported[164–166]. We observed no difference in incubation times between elk CWD and the Wt/Wt isolate. The Wt/H95 and Wt/S96 isolates resulted in statistically increased incubation periods compared to the elk CWD; there was, however, no difference relative to the Wt/Wt

isolate. The equivalence in mean incubation periods of Wt/Wt, Wt/H95 and Wt/S96 indicates that one copy of the WTD wild type allele is sufficient to cause disease with efficiency comparable to wild type homozygotes. The H95/S96 inocula had a mean incubation of 174 dpi, significantly longer than with elk or Wt/Wt CWD. This increase in incubation time with the H95/S96 can be explained by the absence of Wt PrP in the inocula, and the less efficient conversion by the polymorphism-containing proteins. The distribution of incubation periods is greater with the deer isolates than with elk CWD, and this effect is most evident with the H95/S96 isolate. These effects may be explained by the difference in efficiency of conversion between elk and H95/S95 CWD. Another explanation may be that the elk isolate contains only one CWD strain, and the H95/S96 inocula represents a mixture of competing strains. It has been previously shown that co-infection with two prion strains will affect progression of disease as a result of interference between the strains, therefore, it is possible that competing strains exist within the H95/S96 isolate resulting in incubation extension and increase in variance compared to the single strain present in elk CWD[74,177]. This conclusion is supported by work done on characterizing elk and deer CWD in mice expressing cervid PrP. When elk CWD was used to infect Tg(CerPrP) mice, the transmission properties indicated one of two patterns would be produced from each isolate, indicating one strain present in any single elk CWD isolate. However, when CWD isolated from mule deer was used, the results showed a mixed response, indicating that a mixture of strains present in deer CWD samples[171].

Second passage incubation

Previous studies have shown that subsequent passages of an isolate allow strain properties, such as neuropathology and incubation periods, to manifest more clearly[70,178]. Brain homogenate from TgElk infected with elk and white-tailed deer CWD isolates was passaged a second time in TgElk mice, to determine if changes to agent properties could be further resolved). There was no significant difference between mean incubation periods of mice inoculated with each CWD isolate (Figure 3B). The similarity in mean incubation periods can be explained by the source material composition of elk PrP, as generated in TgElk mice, rendering the transmission intraspecies rather than interspecies. Despite the neuropathological differences that separated animals of the H95/S96 inoculum, there was no statistical difference between mean incubation periods. It is, however, noteworthy that the incubation periods of TgElk infected with TgElk-Wt/H95 prions separated into three groups. This separation in incubation periods may be indicative of a strain mixture in the TgElk-Wt/H95 inocula, supporting the generation of new strains. Work remains to be done to distinguish the components of this mixture through dilutions of inocula, similar to the isolation of HY and DY from TME[70].

Clinical signs of disease

The TgElk mouse model has been characterized by other groups who have documented the clinical signs produced with CWD[114,165,166]. However, the clinical signs reported are not consistent with the observations from this study. This difference may be attributed to the different sources of CWD inocula. Previous studies with TgElk mice used CWD sourced from Wyoming mule deer, elk in Korea, or elk of unreported origin, whereas, this study utilized CWD agents derived from white-tailed deer expressing *PRNP*

alleles prevalent in Wisconsin where a focus of disease exists. This would support the idea that multiple strains of CWD exist naturally. In the second passage, a phenotype was observed in TgElk that differed from that of the first passage. This agitated phenotype was only observed in three animals, two of the Wt/H95 inocula and one of H95/S96 L1, all with incubation periods over 120 dpi. This may indicate emergence of new properties, which like HY and DY, may become separable in subsequent passages.

Analysis of PrP^{CWD} in TgElk mice

Differences in protein profiles including the migration pattern, glycoform profile, and sensitivity to proteinase K digestion of PrP^{CWD} can be associated with specific prion strains; therefore alterations in these patterns would demonstrate novel strain generation. Samples of TgElk infected with CWD were examined for total PrP as well as proteinase K resistant PrP^{CWD} (Figure 4). Elk CWD resulted in a strong monoglycosylated signal, and weak diglycosylated signal on first passage that increased after second passage. The changing pattern between first and second passage may be a result of the CWD agent adapting to the transgenic mouse model. Although the TgElk express *PRNP* from elk, the majority of expressed genes and physiology are murine, therefore, not a pure intraspecific transmission.

The proteinase K sensitivity of the Wt/S96 inocula after passage was higher than that of the Wt/Wt, Wt/H95 and H95/S96 inocula in the TgElk mice. This discrepancy in PK sensitivity could be attributed to a lower titer in the Wt/S96 inoculum, however, this does not appear to be the case, as the incubation period of Wt/H95 and Wt/S96 do not significantly differ. This similarity in incubation periods indicates that the increased PK sensitivity is a property of the agent in the TgElk line, not a concentration difference.

Furthermore, second passage of the Wt/S96 inoculum results in an increase in the PK resistant signal, indicating adaptation to the host and demonstrating that second passage of this WTD CWD isolated selected a prion conformer that is more PK resistant than the inoculum.

Histological analysis

Two histological analyses were performed on sagittal brain sections of the CWD infected TgElk mice to determine if differences exist between brain regions affected by spongiform degeneration or accumulation of disease-associated PrP. Regional differences would be indicative of generation of novel CWD strains.

Neuropathological analysis

The elk CWD isolate resulted in high levels of vacuolation throughout most brain regions surveyed, this is consistent with intra-species, syngeneic transmission, as agent derived in elk is transmitted into *PRNP* expressing hosts. The WTD isolates, however, produced variable results with grade of vacuolar degeneration with the hippocampus (HC) being most revealing region. The elk, Wt/Wt, and Wt/S96 inocula all produce high levels of vacuolation in the HC region. Conversely, the Wt/H95 isolate results in noticeably lower levels of vacuolation in the same region. The difference in severity of affected regions supports the idea that passage of white-tailed deer CWD into elk results in alterations to the replicated prion.

Disease-associated PrP accumulation

Widespread and dense PrP^{d} deposits result from the elk CWD, which again is explained by the syngeneic nature of this transmission. Patterns from white-tailed deer CWD

isolates varied between each other and between to the elk CWD. This is supportive of the interspecies transmission affecting a change to the infectious prion. The hippocampal region displays the variability that can occur in the accumulation, with elk, Wt/Wt and Wt/S96 CWD resulting in high densities of distribution, and Wt/H95 with significantly lower score. This finding is consistent with the pattern observed with the lesion profiling (Figure 6).

Cervid Prion Cell Assay (CPCA) analysis

CPCA is a method of investigating infectivity *in vitro.* Cultured cells expressing elk *PRNP* are exposed to CWD, and levels of infectivity can be assessed through counts of proteinase K resistant spots[168,179]. CWD from elk assayed by CPCA demonstrated a robust spot count. This robust spot count is reflected when brain material generated in TgElk mice infected with elk CWD is evaluated with CPCA, which again agrees with intraspecific transmission as both the TgElk and the elk21 cells express elk PrP.

White-tailed deer CWD isolates resulted in variable spot-count responses, isolates from Wt/Wt, and Wt/S96 infected TgElk analyzed by CPCA resulted in spot counts significantly lower that that observed with elk CWD. However, brain homogenate from a Wt/H95 isolate infected TgElk showed spot count significantly higher than those of the remaining deer CWD isolates, and comparable to what is observed with elk CWD. This indicates that passage of Wt/H95 through the TgElk mice selected a prion with higher infectivity in CPCA, than those of the remaining isolates.

H95/S96 Passage Lines

Upon characterization of first passage TgElk mice infected with the H95/S96 WTD CWD isolate, three patterns of disease-associated PrP distribution were observed. These animals, termed passage lines L1, L2 and L3, were individually examined and used to create inocula for second passage studies. Variances in location and grade were noted when assessing vacuolation, such that two patterns are visible in the lesion profile (Figure 6B). Passage line L3 resembles Wt/Wt WTD inocula, with high vacuolation in CN, HC and T, and low HT, MB and M degeneration. This is markedly different from the overlapping pattern observed with passage lines L1 and L2, which show lower CN, HC, T vacuolation and higher HT, MB and M scores.

Differences are observed in the pattern of PrP^{d} accumulation in which served as the basis for the delineation each passage line. The distribution of disease-associated PrP observed with the L3 passage line is similar to that of the Wt/Wt inocula. These data show that infected animals can manifest different neuropathology and deposition of disease-associated PrP, despite being exposed to the same CWD isolate. These changes in affected brain regions support the conclusion of a modified prion.

In addition to histopathological differences in passage lines infected with H95/S96 CWD, we observed changes in the spot counts observed with CPCA. When passage lines are examined individually, the three passage lines significantly differ from one another. A 0.01% concentration of L2 brain homogenate was used to infect cells, resulting in the highest spot counts, comparable to elk CWD. At the same concentration, L3 results in the lowest spot count followed by L1 with an intermediate value. This demonstrates different levels of CPCA infectivity in individual TgElk mice inoculated with the same white-

tailed CWD inocula. Another possible interpretation of these data is that the passage lines isolated from the TgElk mice have different cell tropism. The cells used in CPCA are the rabbit kidney epithelial cell (RK13) line, expressing elk *PRNP*^{179}. The low spot counts generated from L1 and L3 could result from a lowered tropism towards epithelial cells. Conversely, L2 generates a strong spot count in the same cell line, which would suggest an increased tropism towards epithelia compared to L1 and L3. The possible differences in tropism would still support the conclusion of a modified prion, as all three passage lines originated from the same deer CWD isolate.

The incubation periods of the passage lines supports the conclusion that interspecies transmission serves as a means of strain generation. On second passage, L1 and L2 have the same incubation periods, whereas L3 results in an extension. This is in agreement with the lesion profiling that demonstrates overlapping patterns for L1 and L2 and a dissimilar pattern formed by the L3. When considering all data incubation periods, neuropathology and CPCA, the picture becomes more complex. Passage in the TgElk mice gives equivalent incubation periods, however, two different patterns are observed in the lesion profiling and CPCA spot counts, and three distinct patterns in the PrP^{d} deposition profiling.

CWD Strains

This study investigates CWD transmission between white-tailed deer and elk to determine whether agent properties are affected as a result of interspecies transmission. The CWD agent has, until recently, been believed to be a single entity, however current research supports the idea of a multitude of strains existing. One potential method for generation of new strains is interspecies transmission of CWD. Passage of CWD between

hosts of the same species, but expressing different *PRNP* alleles result in generation of new strains[105]. This study provides evidence that novel strain properties are generated when CWD isolates from white-tailed deer are transmitted to elk transgenic mice.

Interspecies Prion Transmission as a Means of Novel Strain Generation

The idea of prion transmission between hosts with differing *PNRP* genotypes generating new strains is not a new concept. Previously explored in adapting scrapie strains in mice, adapting BSE strains into ovine transgenic mice, and more recently applied to CWD in deer, these date have demonstrated this principle holds within CWD transmission between elk and deer [105,178,180].

This study observed differences in incubation periods, neuropathology, PrP^{d} deposition and spot counts generated with CPCA upon passage of H95/S96 WTD CWD through TgElk mice. Further supporting the idea that transmission of CWD between species contributes to strain diversity. This first passage observation is supported by results from second passage transmission, as shown by the Wt/H95 and H95/S96 inocula, demonstrating that novel clinical signs result from extended incubation periods.

One explanation for this emergence of new strain properties following interspecies transmission is selection of prion conformers. Though prions do not possess nucleic acids, they are still capable of encoding information; this is done through the protein conformation. There is mounting evidence for a multitude of prion protein conformations in a given prion isolate. These conformers would exist as a simultaneously as a cloud and depending on the substrate PrP, a prion conformer would be selected to replicate and become the dominant agent. The first passage of WTD CWD into TgElk

mice selected different prion conformers depending on the inoculum, which were able to replicate and which resulted in different strain properties observable after transmission.

Future Directions

This study has expanded on the idea that CWD is not a single infectious entity but exists as a multitude of species, and that interspecies transmission may serve as a means to generate new strains. To further this conclusion several experiments can be carried out.

This study investigates CWD transmission between white-tailed deer and elk at on first passage, however owing to the sympatric nature of these two species and the mostly horizontal spread of CWD, transmission of elk generated WTD-CWD back into white-tailed deer warrants investigation. This transmission study can be initiated using the Tg33 mouse line, which expresses Wt white-tailed deer *PRNP,* allowing insight into processes occurring in natural transmission.

Characterization of Second Passage

One characteristic of prion strains is that they breed true, in that the traits associated with a given isolate are reiterated in subsequent passages of that isolate[67]. Second passage of Wt/H95 CWD into TgElk mice produced incubation periods that can be grouped into three cohorts. In addition to the variation in incubation periods, clinical signs differed between animals with short incubation periods versus those with extensions. The isolates can be characterized first by resistance to proteinase K, with positive isolates further subjected to stability in guanidine, diglycosylation by PNGase to determine protein size. Should any isolates result in PK negativity, digestion conditions could be altered, or thermolysin digestion could be carried out to determine if infectious material exists that is PK sensitive. Should low levels of PK resistant material result from this transmission, *in*

vitro amplification assays such as PMCA or QuIC will aid in detection. Comparison of strain properties between these groups, and between the inocula will allow a profile of strain properties to be created and allow conclusions as to whether generation of new prion strain or strains has occurred. Previous studies involving transmission of TME to hamsters have shown that strain properties may not resolve until subsequent passages[70,72]. As a result, third passage studies may be necessary to fully grasp the strain properties generated in this interspecies transmission. Further, this establishment of novel infection characteristics would cement these as strain properties, as further transmissions are required to determine if they "breed true" as prion strains do.

Strain Identity

Previous publications have noted the existence of several strains of CWD based on transmission studies in other models[105,161,172,173]. From these observations, work can be initiated to characterize the agents developed in this study and determine if they belong to the previously identified species, or if they represent novel strains. Transmission of these isolates into the Tg(CerPrP) and techniques such as conformation dependent immunoassay would allow comparison of the strain properties. This would help to determine the full extent of CWD strains existing.

Transmission Characteristics of CWD with New Strain Properties

This work and those of previous authors have highlighted the effect that CWD transmission between hosts of differing *PRNP* genotypes has on generation of new strain properties. The characteristics that distinguish CWD from other TSE's: natural transmission through free-ranging hosts, affecting five host species, and being attributed to a multitude of strains, coupled with the mutability observed in interspecies

transmission facilitates the divergence of prion conformers with different infection characteristics. This is concerning for CWD research for a number of reasons, first, this complicates analysis of properties attributed to the CWD agent. Second, and more concerningly, this source of strain diversity may result in modifications to the agents composing CWD to the extent that the host range in significantly extended.

Previous work has shown alteration in the host range of a prion strain occur after interspecies transmission[161]. Therefore, it follows that the infectious material generated after passage of white-tailed deer in elk transgenic mice may be capable of infecting hosts not currently susceptible to CWD. Currently, CWD has been reported in white-tailed deer, mule deer, moose, elk, and reindeer, as well as cats, cattle, pigs, mice, and hamsters experimentally. However as the interspecies transmissions occur between free ranging hosts, there exists the potential for an increase in the susceptible host species as a result of the generation of novel strain properties. One host of particular interest is humans, as there exists the potential for zoonotic transmission. Humans currently populate regions where CWD is enzootic, and may be exposed to CWD agents through environmental contamination or hunting practices such as field dressing of harvested deer or consumption of meat from infected animals. Zoonotic transmission can be explored using humanized mice, expressing human *PRNP*. This model has previously been used to determine the zoonotic potential of CWD, and can be applied to examine material from first and second passages of white-tailed deer CWD into TgElk mice[140,141].

The insights gathered studying the unique characteristics of interspecies CWD transmission can be applied to the study of other interspecific prion diseases, particularly

zoonotic prion diseases, and help further evaluate potential for interspecies CWD transmission.

Figure 1: Cervid PrP amino acid alignment. Amino acid sequences for prion protein of Elk (*Cervus elaphus),* White-tailed deer (*Odocoileus virginianus)*, Mule deer (*Odocoileus hemionus)*, reindeer (*Rangifer tarandus),* and moose (*Alces alces*) were compared for sequence similarities, highlights indicate sequence differences and bolded indicate polymorphisms. Amino acid sequences obtained through https://www.ncbi.nlm.nih.gov/genbank/

Cervid Species PrP Amino Acid Alignment

1	MVKSHIGSWI	LVLFVAMWSD	VGLCKKRPKP	GGGWNTGGSR	YPGQGSPGGN	RYPPQGGGGW
1	MVKSHIGSWI	LVLFVAMWSD	VGLCKKRPKP	GGGWNTGGSR	YPGQGSPGGN	RYPPQGGGGW
1	MVKSHIGSWI	LVLFVAMWSD	VGLCKKRPKP	GGGWNTGGSR	YPGQGSPGGN	RYPPQGGGGW
1	MVKSHIGSWI	LVLFVAMWSD	VGLCKKRPKP	GGGWNTGGSR	YPGQGSPGGN	RYPPQGGGGW
1	MVKSHIGSWI	LVLFVAMWSD	VGLCKKRPKP	GGGWNTGGSR	YPGQGSPGGN	RYPPQGGGGW
61	GQPHGGGWGQ	PHGGGWGQPH	GGGWGQPHGG	GGWGQGGTHS	QWNKPSKPKT	NMKHVAGAAA
61	GQPHGGGWGQ	PHGGGWGQPH	GGGWGQPHGG	GGWG**QG**GTHS	QWNKPSKPKT	NMKHVAGAAA
61	GQPHGGGWGQ	PHGGGWGQPH	GGGWGQPHGG	GGWGQGGTHS	QWNKPSKPKT	NMKHVAGAAA
61	GQPHGGGWGQ	PHGGGWGQPH	GGGWGQPHGG	GGWGQGGTHS	QWNKPSKPKT	NMKHVAGAAA
61	GQPHGGGWGQ	PHGGGWGQPH	GGGWGQPHGG	GGWGQGGTHS	QWNKPSKPKT	NMKHVAGAAA
121	AGAVVGGLGG	Y**M**LGSAMSRP	LIHFGNDYED	RYYRENMYRY	PNQVYYRPVD	QYNNQNTFVH
121	AGAVVGGLGG	YMLGSAMSRP	LIHFGNDYED	RYYRENMYRY	PNQVYYRPVD	QYNNQNTFVH
121	AGAVVGGLGG	YMLGSAMSRP	LIHFGNDYED	RYYRENMYRY	PNQVYYRPVD	QYNNQNTFVH
121	AGAVVGGLGG	YMLGSAMSRP	LIHFGNDYED	RYYRENMYRY	PNQVYYRPVD	QYNNQNTFVH
121	AGAVVGGLGG	YMLGSAMSRP	LIHFGNDYED	RYYRENMYRY	PNQVYYRPVD	QYNNQNTFVH
181	DCVNITVKQH	TVTTTTKGEN	FTETDIKMME	RVVEQMCITQ	YQRESEAYYQ	RGASVILFSS
181	DCVNITVKQH	TVTTTTKGEN	FTETDIKMME	RVVEQMCITQ	YQRESQAYYQ	RGASVILFSS
181	DCVNITVKQH	TVTTTTKGEN	FTETDIKMME	RVVEQMCITQ	YQRE**S**QAYYQ	RGASVILFSS
181	DCVNITVKQH	TVTTTTKGEN	FTETDIKMME	RVVEQMCITQ	YQRESQAYYQ	RGASVILFSS
181	DCVNITVKQH	TVTTTTKGEN	FTETDIKMIE	RVVEQMCITQ	YQRESQAYYQ	RGASVILFSS
241	PPVILLISFL	IFLIVG	*Cervus elaphus*			GenBank: AAC12860.2
241	PPVILLISFL	IFLIVG	*Odocoileus virginianus*			GenBank: AAP37447.1
241	PPVILLISFL	IFLIVG	*Odocoileus hemionus*			GenBank: AAC33174.2
241	PPVILLISFL	IFLIVG	*Rangifer tarandus tarandus*			GenBank: AAT77253.1
241	PPVILLISFL	IFLIVG	*Alces alces shirasi*			GenBank: AAO67544.1

Figure 2: Distribution of CWD in North America. Obtained October 27 2016, from http://www.nwhc.usgs.gov/images/cwd/cwd_map.jpg

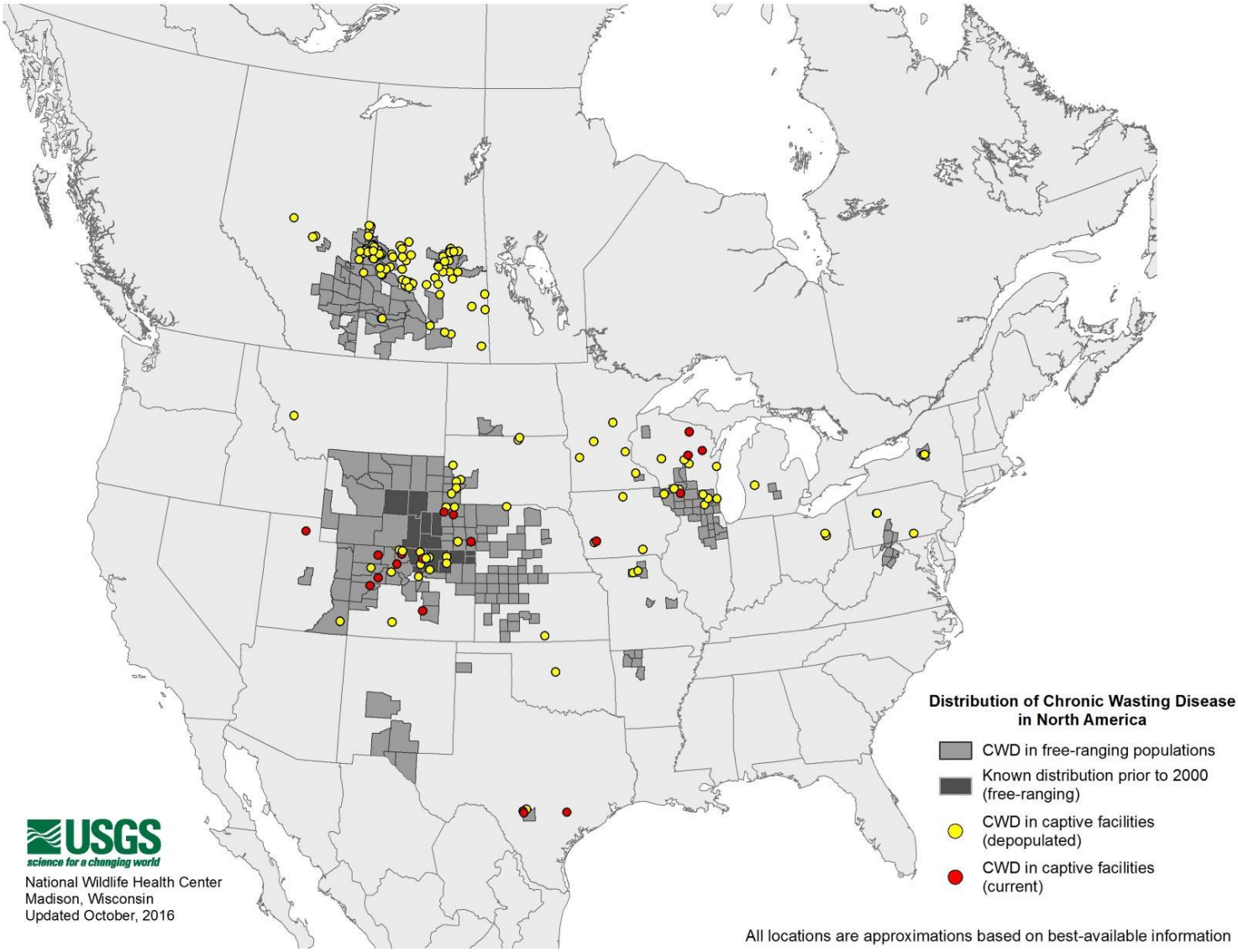
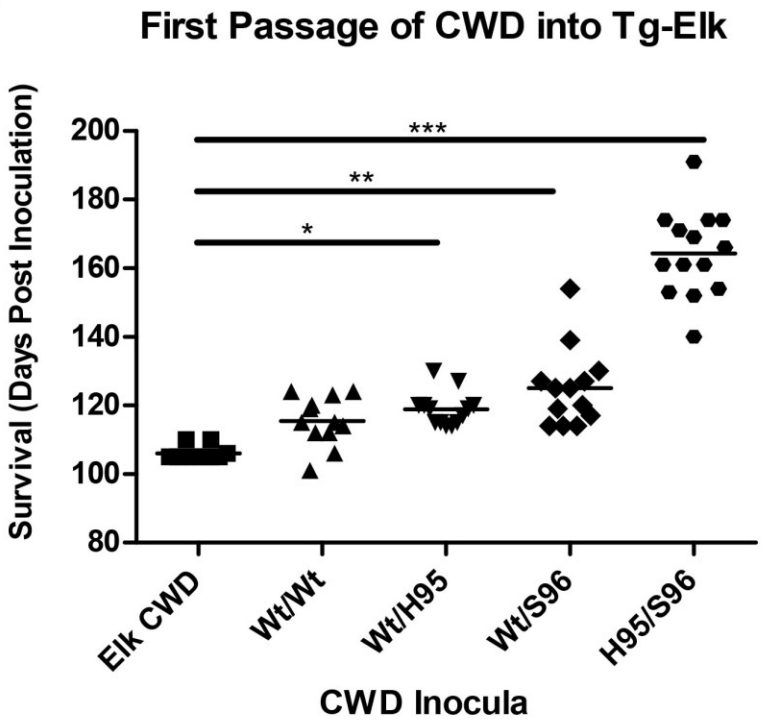

Distribution of Chronic Wasting Disease in North America
CWD in free-ranging populations
Known distribution prior to 2000 (free-ranging)
CWD in captive facilities (depopulated)
CWD in captive facilities (current)
USGS
science for a changing world
National Wildlife Health Center
Madison, Wisconsin
Updated October, 2016
All locations are approximations based on best-available information

Figure 3: Transmission of CWD into mice expressing elk PrP. Incubation periods of TgElk mice intracerebrally challenged with 2% w/v brain homogenates from elk, or CWD positive white-tailed deer expressing one of four *PNPR* genotypes (Wt/Wt, Wt/H95, Wt/S96, H95/S96) after A) first passage or B) second passage. *$P<0.05$ determined by Kruskal-Wallis test and Dunn's multiple comparisons post-test.

A)


First Passage of CWD into Tg-Elk
Survival (Days Post Inoculation)
200
180
160
140
120
100
80
***
**
*
Elk CWD
Wt/Wt
Wt/H95
Wt/S96
H95/S96
CWD Inocula

B)

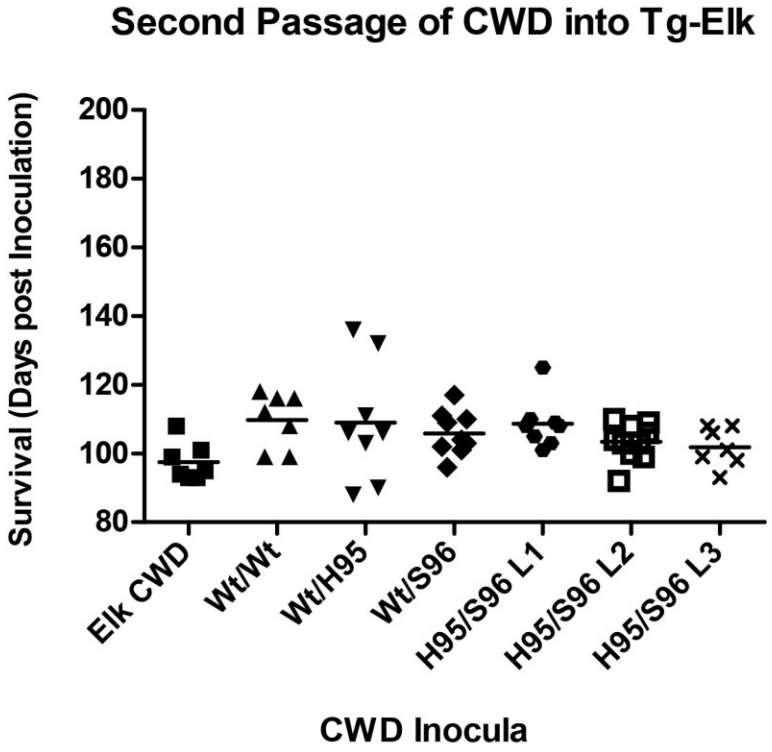
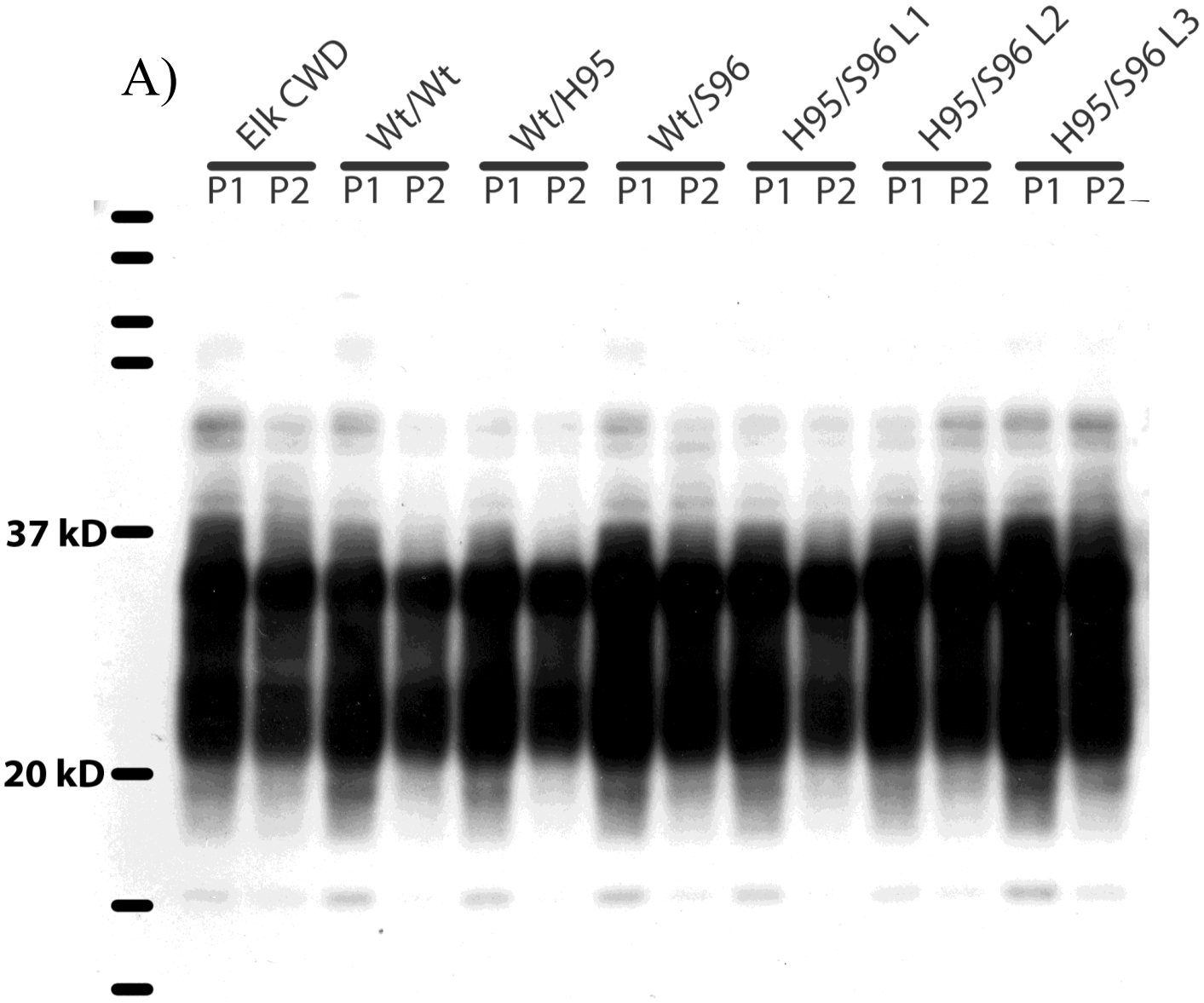

Second Passage of CWD into Tg-Elk
Survival (Days post Inoculation)
200
180
160
140
120
100
80
Elk CWD
Wt/Wt
Wt/H95
Wt/S96
H95/S96 L1
H95/S96 L2
H95/S96 L3
CWD Inocula

Figure 4: Proteinase K resistant protein from western blot analysis of TgElk mice infected with elk or white-tailed deer CWD. Brain homogenates from TgElk mice challenged with elk or white-tailed deer CWD isolates in first passage (P1) or second passage (P2) were probed for A) total PrP or B) proteinase K resistant PrP. Brain homogenates were digested with 50 μg/ml of proteinase K, probed with the monoclonal anti-PrP antibody BAR224.


A)
Elk CWD
Wt/Wt
Wt/H95
Wt/S96
H95/S96 L1
H95/S96 L2
H95/S96 L3
P1 P2 P1 P2 P1 P2 P1 P2 P1 P2 P1 P2 P1 P2
37 kD
20 kD

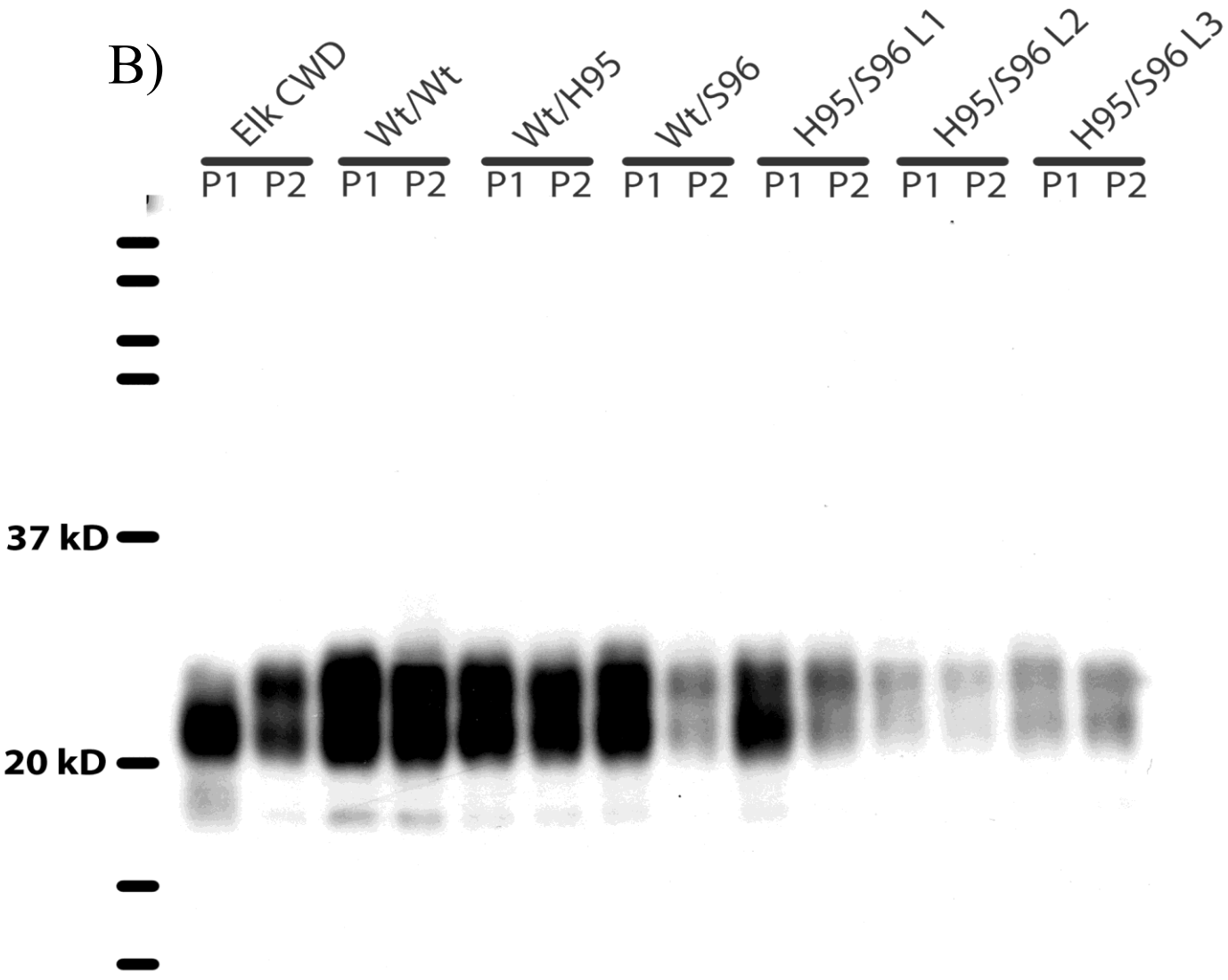

B)
Elk CWD
Wt/Wt
Wt/H95
Wt/S96
H95/S96 L1
H95/S96 L2
H95/S96 L3
P1 P2 P1 P2 P1 P2 P1 P2 P1 P2 P1 P2 P1 P2
37 kD
20 kD

Figure 5: Neuropathology of CWD in TgElk brains following first passage. Sagittal sections of brain from TgElk mice clinically affected with A) elk or B-E) white-tailed deer CWD were collected following first passage. Brain sections were stained with hematoxylin and eosin then surveyed for spongiform degeneration in hippocampus (HC), cerebral cortex (CC), medulla (MY) and hypothalamus (HT). H&E staining was performed by Hristina Gapeshina.

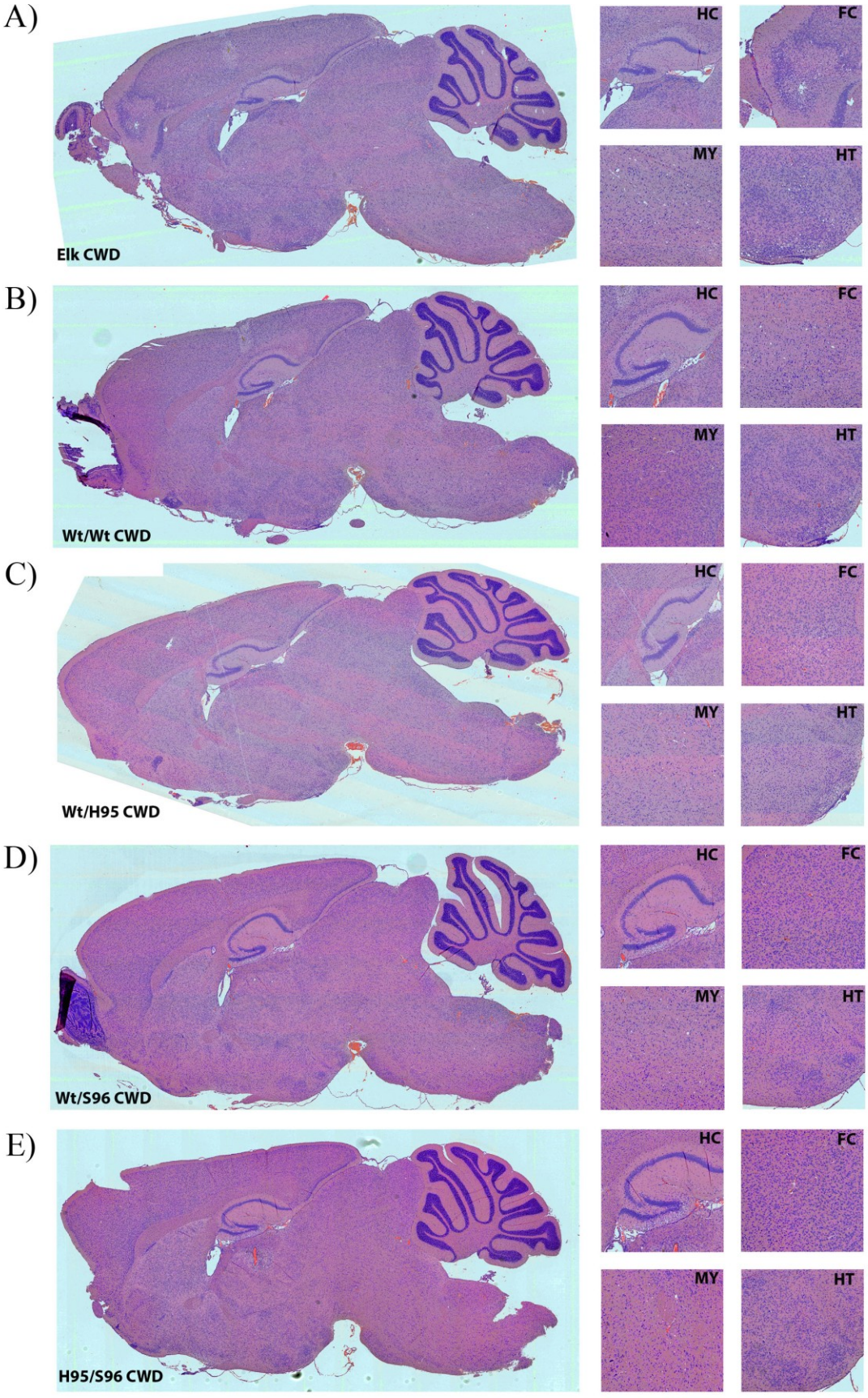
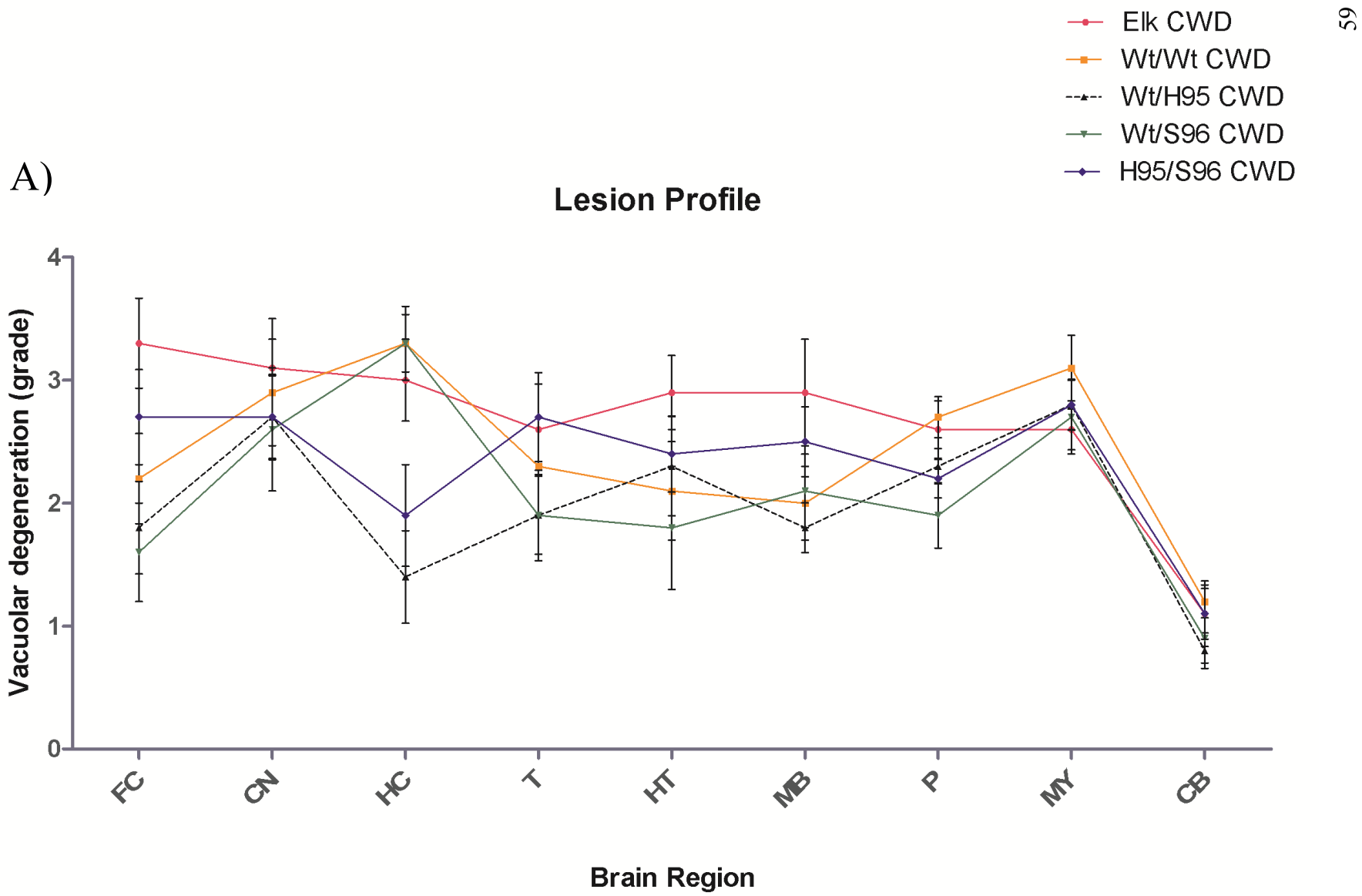

A)
Elk CWD
HC
FC
MY
HT
B)
Wt/Wt CWD
HC
FC
MY
HT
C)
Wt/H95 CWD
HC
FC
MY
HT
D)
Wt/S96 CWD
HC
FC
MY
HT
E)
H95/S96 CWD
HC
FC
MY
HT

Figure 6: Lesion profiles from TgElk infected with white-tailed deer or elk CWD. Hematoxylin and eosin stained sagittal brain sections of TgElk mice infected with A) elk or white-tailed deer CWD isolates were visually assessed for level of vacuolar degeneration by three observers on a 0-4 scale of intensity. Mean scores from 3-5 animals of each inoculum are displayed with SEM. B) Vacuolation of three animals from the H95/S96 inoculum, identified as passage lines 1, 2, 4, (L1, L2, L3) was analyzed separately. Mean scores for each passage line are displayed with SEM.

A)


Elk CWD
Wt/Wt CWD
Wt/H95 CWD
Wt/S96 CWD
H95/S96 CWD
Lesion Profile
Vacuolar degeneration (grade)
0
1
2
3
4
FC
CN
HC
T
HT
MB
P
MY
CB
Brain Region

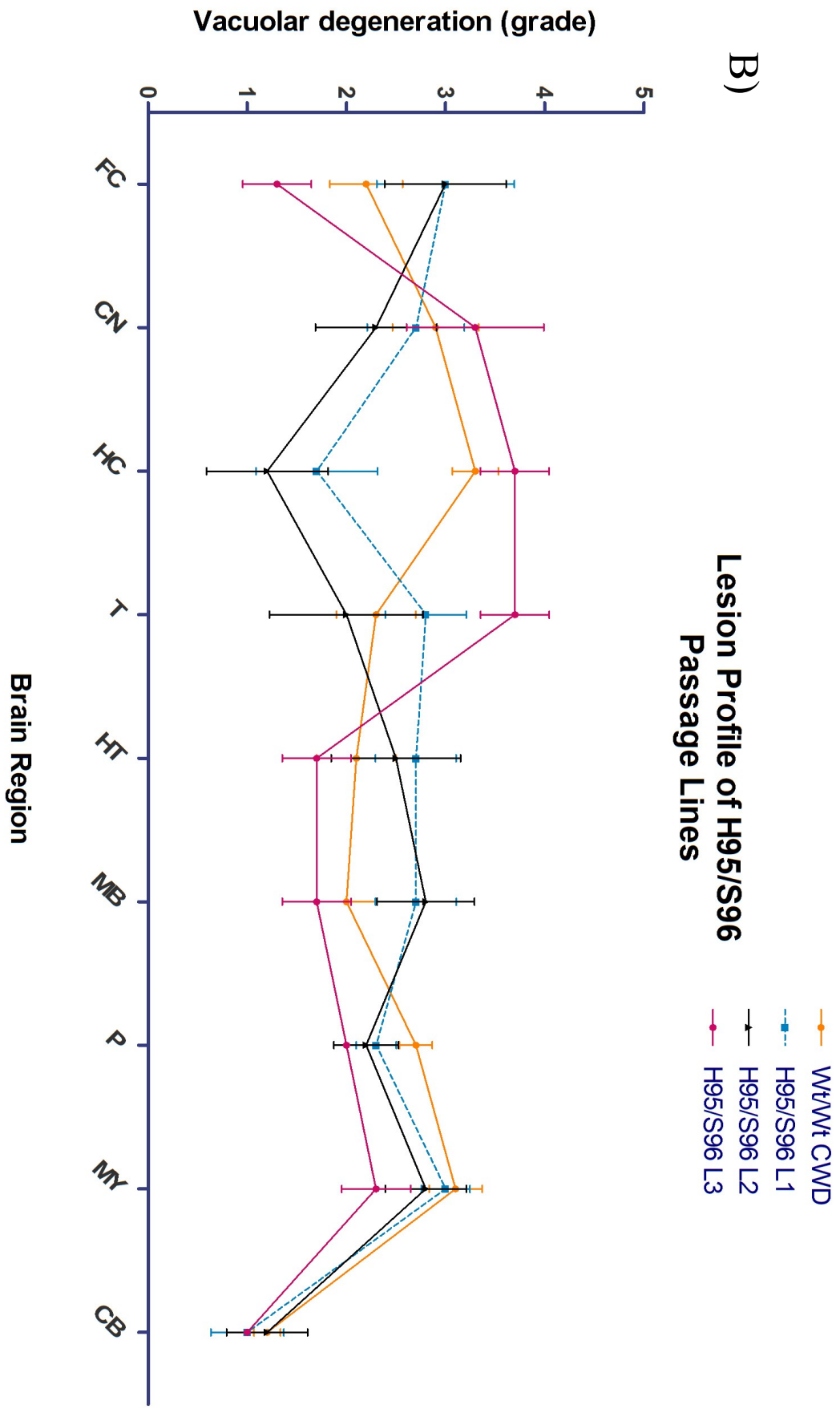

B)
Lesion Profile of H95/S96
Passage Lines
Wt/Wt CWD
H95/S96 L1
H95/S96 L2
H95/S96 L3
Vacuolar degeneration (grade)
0
1
2
3
4
5
FC
CN
HC
T
HT
MB
P
MY
CB
Brain Region

Figure 7: Distribution of PrP^d following first passage of CWD in TgElk mice. Sagittal sections of brain from TgElk mice clinically affected with A) elk or B-E) white-tailed deer CWD were collected following first passage. Disease-associated PrP was immunostained using the monoclonal anti-PrP antibody BAR224 (1: 2000), and density of PrP^d was surveyed in hippocampus (HC), frontal cortex (FC), medulla (MY) and hypothalamus (HT). Immunohistochemistry was performed by Hristina Gapeshina.

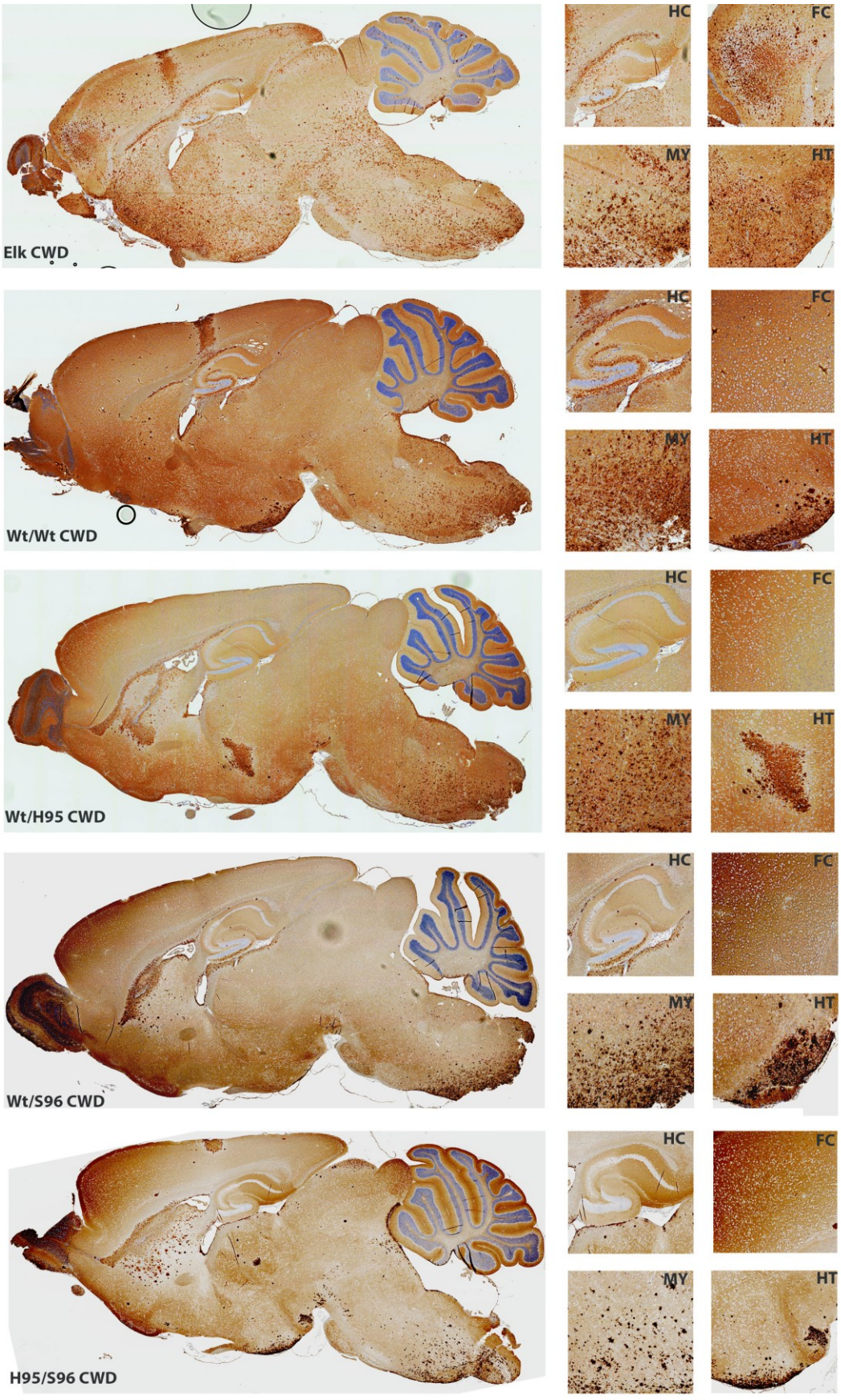
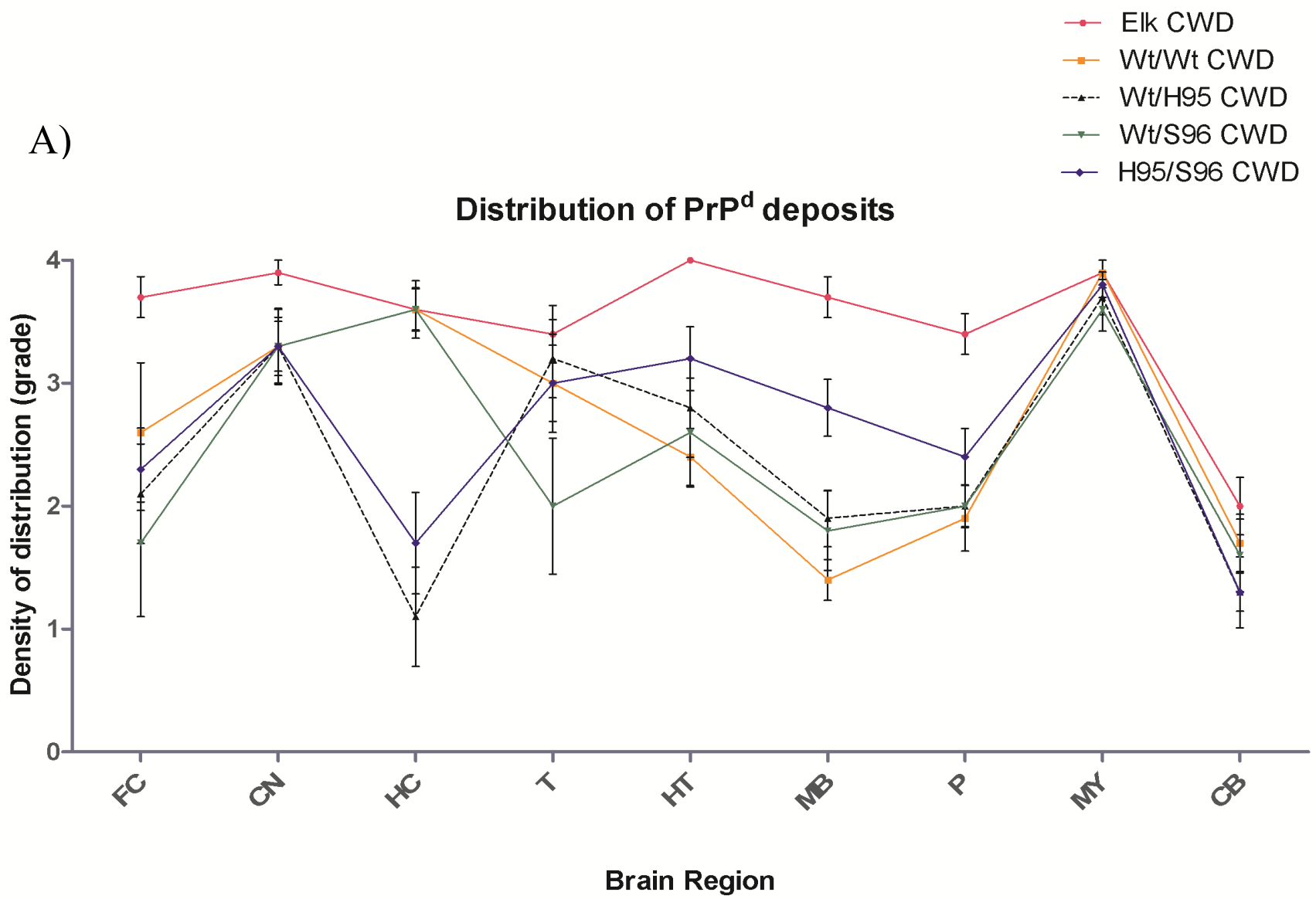

Elk CWD
HC
FC
MY
HT
Wt/Wt CWD
HC
FC
MY
HT
Wt/H95 CWD
HC
FC
MY
HT
Wt/S96 CWD
HC
FC
MY
HT
H95/S96 CWD
HC
FC
MY
HT

Figure 8: Profiles of PrP^d deposition in TgElk mice following transmission of CWD. Disease-associated PrP was immunostained using the anti-PrP antibody BAR224 in sagittal brain sections of CWD infected TgElk mice. Three independent observers on a 0-4 scale assessed the density of PrP^d deposition. A) Mean score for frontal cortex (FC), cerebral nuclei (CN), hippocampus (HC), thalamus (T), hypothalamus (HT), midbrain (MB), pons (P), medulla (MY) and cerebellum (CB) are displayed with SEM. B) Deposition of PrP^d of three animals from the H95/S96 inoculum, identified as passage lines 1, 2, 4, (L1, L2, L39 was analyzed separately. Mean scores for each passage line are displayed with SEM.

A)


Elk CWD
Wt/Wt CWD
Wt/H95 CWD
Wt/S96 CWD
H95/S96 CWD
Distribution of PrP^d deposits
Density of distribution (grade)
0
1
2
3
4
FC
CN
HC
T
HT
MB
P
MY
CB
Brain Region

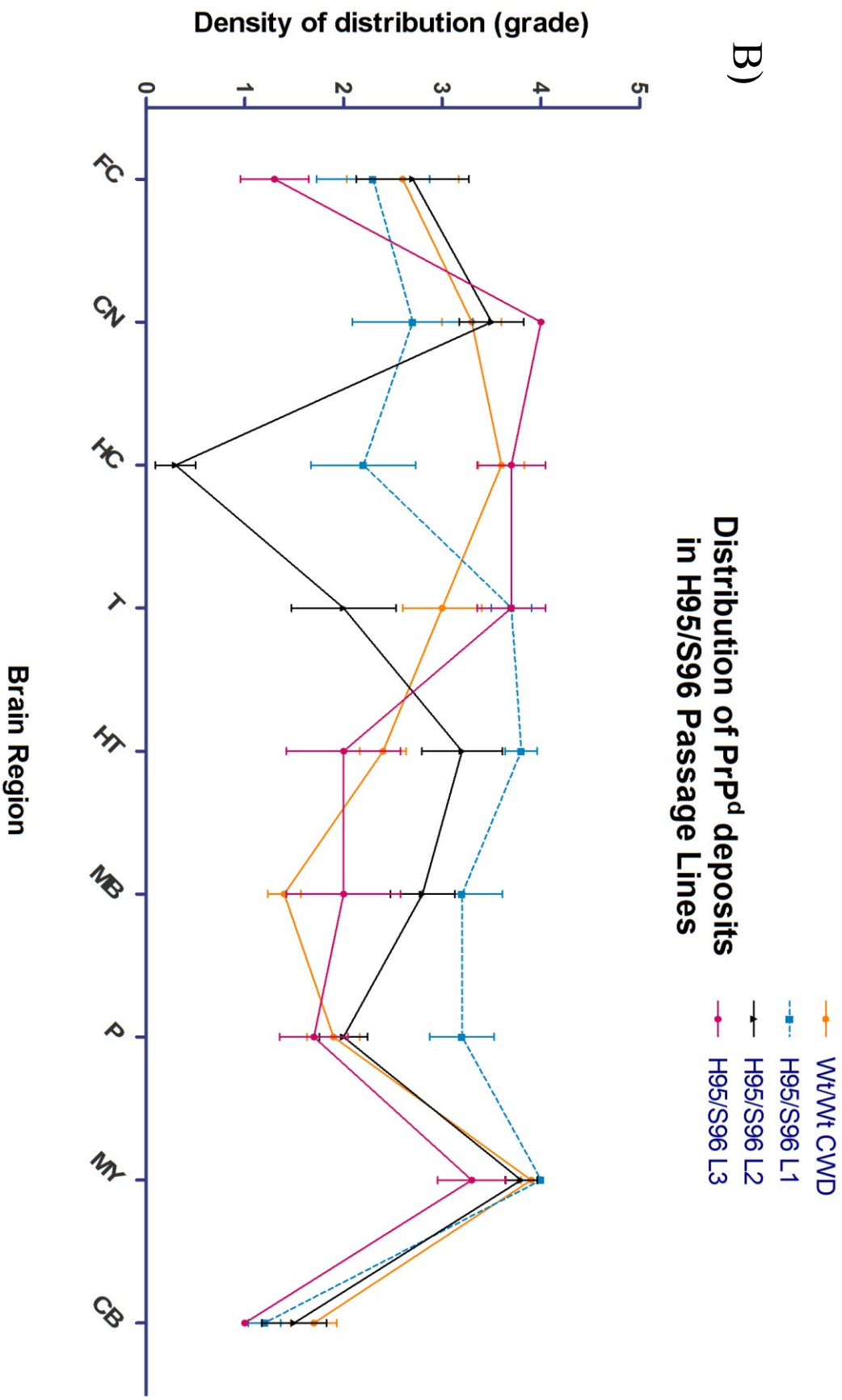
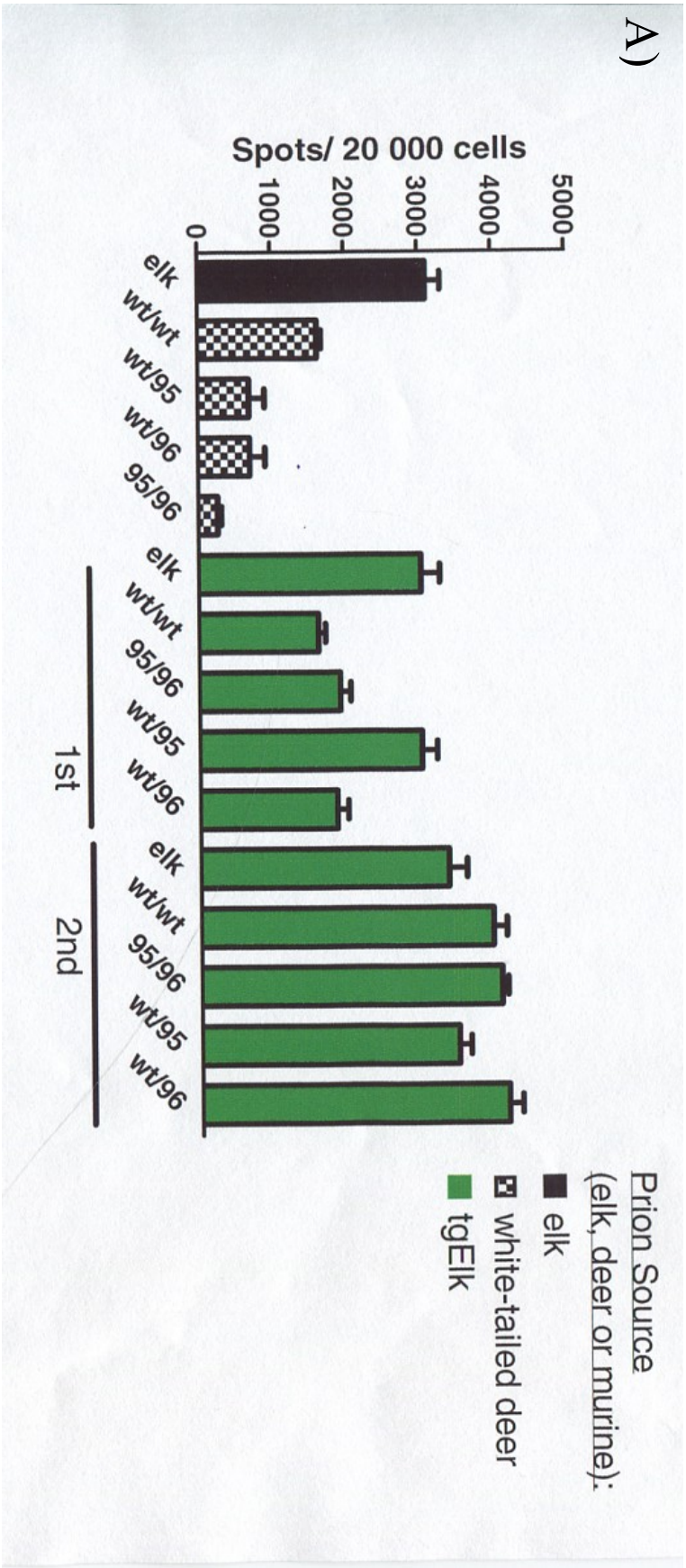
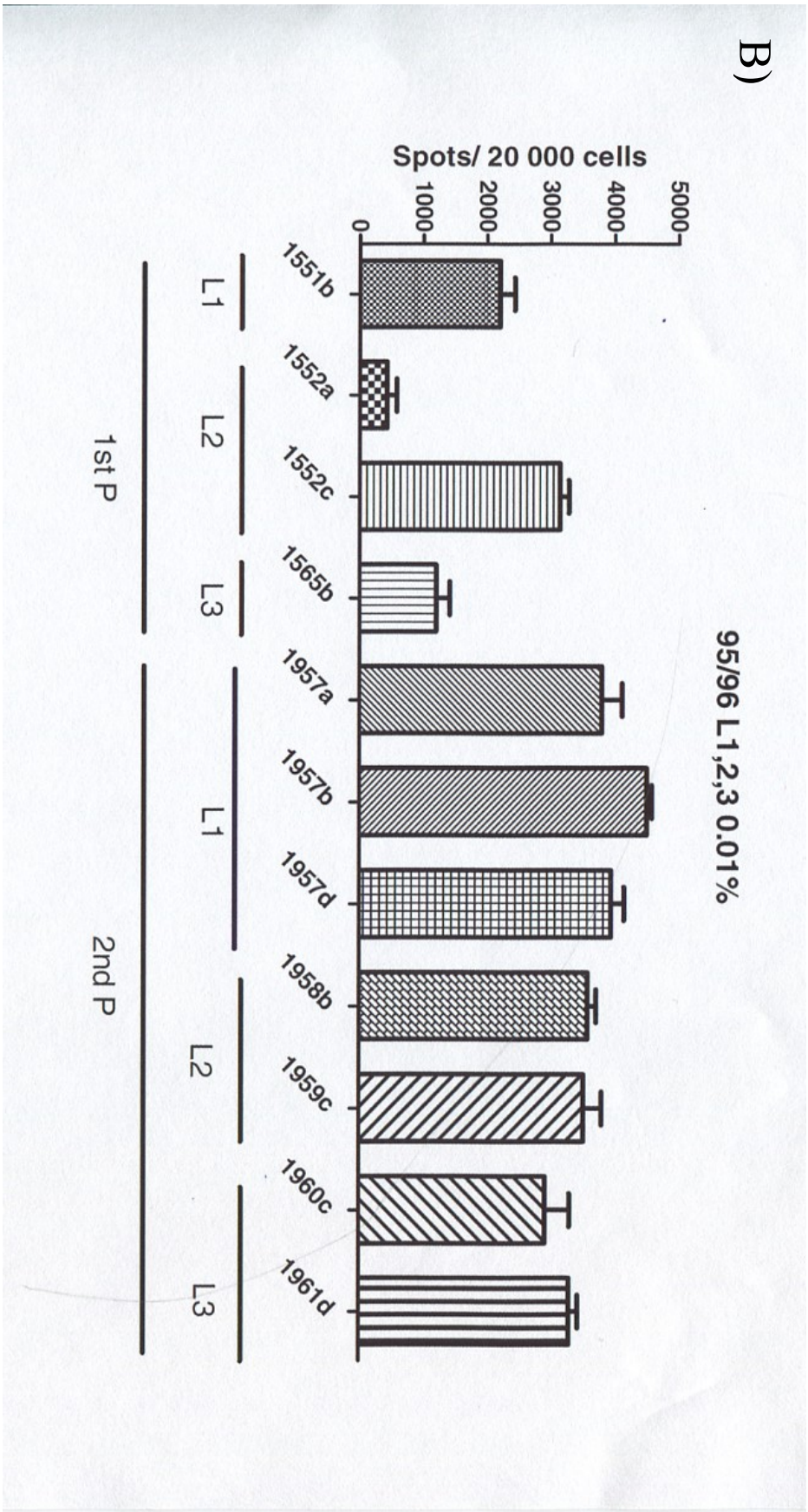

B)
Distribution of PrPd deposits in H95/S96 Passage Lines
Wt/Wt CWD
H95/S96 L1
H95/S96 L2
H95/S96 L3
Density of distribution (grade)
0
1
2
3
4
5
FC
CN
HC
T
HT
MB
P
MY
CB
Brain Region

Figure 9: Cervid prion cell assay from CWD infected TgElk. Cells expressing elk PrP (ELK21 cells) were exposed to 0.1% inocula of first and second passage brain homogenates from TgElk mice infected with white-tailed deer or elk CWD isolates. CPCA analysis was performed by Dr. van der Merwe and spot counts/ 20,000 cells reported B) Three animals from H95/S96 inoculum were examined individually and used as separate passage lines 1, 2 and 3 (L1, L2, L3), for second passage studies. 0.01 % brain homogenates from these passage lines after first and second passage in TgElk mice were used to infect ELK21 cells. CPCA analysis was performed by Dr. van der Merwe and spots/ 20,000 cells reported.


A)
Spots/ 20 000 cells
5000
4000
3000
2000
1000
0
elk
wt/wt
wt/95
wt/96
95/96
elk
wt/wt
95/96
wt/95
wt/96
elk
wt/wt
95/96
wt/95
wt/96
1st
2nd
Prion Source
(elk, deer or murine):
elk
white-tailed deer
tgElk


B)
95/96 L1,2,3 0.01%
Spots/ 20 000 cells
0
1000
2000
3000
4000
5000
1551b
1552a
1552c
1565b
1957a
1957b
1957d
1958b
1959c
1960c
1961d
L1
L2
L3
L1
L2
L3
1st P
2nd P

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