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Caio Bruno Ribeiro Falcão

Os Cervídeos Brasileiros e a Doença da Debilidade Crônica: Verificação de Polimorfismos Genéticos Associados à Susceptibilidade

Vitória de Santo Antão

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Caio Bruno Ribeiro Falcão

Os Cervídeos Brasileiros e a Doença da Debilidade Crônica: Verificação de Polimorfismos Genéticos Associados à Susceptibilidade

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Dissertação de Mestrado apresentada por **Caio Bruno Ribeiro Falcão** a Pós-Graduação em Saúde Humana e Meio Ambiente do Centro Acadêmico de Vitória da Universidade Federal de Pernambuco, sob o título "OS CERVÍDEOS BRASILEIROS E A DOENÇA PRIÔNICA CWD (*CHRONIC WASTING DISEASE)*: VERIFICAÇÃO DE POLIMORFISMOS GENÉTICOS ASSOCIADOS À SUSCEPTIBILIDADE" orientada pelo Prof. José Eduardo Garcia e aprovada pela Banca Examinadora composta pelos professores:

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Ao Único que é digno de receber a honra e a glória, a força e o poder.

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CWD	Chronic Wasting Disease
EET	Encefalopatia Espongiforme Transmissível
TSE	Transmissible Spongiform Encephalopathy
CJD	Creutzefeldt-Jakob Disease
BSE	Bovine Spongiform Encephalopathy
SNP	Single Nucleotide Polymorphism
PCR	Polymerase Chain Reaction

RESUMO

As doenças priônicas são um grupo de doenças neurodegenerativas e irremediavelmente fatais que afetam humanos e algumas outras espécies de mamíferos. Entre estas, as mais conhecidas são a doença de Creutzfeldt-Jakob (CJD) que acomete humanos, o *scrapie* em ovinos e a encefalopatia espongiforme bovina (BSE). A *Chronic Wasting Disease* (CWD) é a forma priônica que acomete os cervídeos e tem sido considerada como problema de saúde pública nos EUA e Canadá. Pesquisas recentes têm mostrado que alguns animais apresentam maior resistência à infecção da doença. Esta resistência tem sido comumente associada com alterações na sequência de nucleotídeos do gene que codifica a proteína priônica (*PRNP*). Polimorfismos de nucleotídeo único (SNPs) em códons específicos alteram a susceptibilidade ao desenvolvimento da CWD na família *Cervidae*. Objetivando identificar a presença/ausência dos polimorfismos, e consequentemente a potencial susceptibilidade ao desenvolvimento da doença em cervídeos neotropicais, no presente trabalho foram analisadas 60 amostras de três espécies: Veado catingueiro (*Mazama gouazoubira,* n:11), Veado campeiro (*Ozotoceros bezoarticus,* n:18) e Cervo do Pantanal (*Blastocerus dichotomus,* n:31). Um fragmento de aproximadamente 800pb do exon 3 do gene PRNP foi amplificado por PCR e posteriormente sequenciado. As sequências foram alinhadas e os resultados mostraram que a sequência nucleotídica dos animais neotropicais é altamente conservada. Todos os animais apresentaram os polimorfismos associados à susceptibilidade nos códons de interesse (Q95, G96, M132 e S225). Apesar disto, a CWD nunca foi notificada em espécies neotropicais.

Palavras-Chave: Encefalopatia Espongiforme Transmissível, Príon, Cervídeos Neotropicais, *Mazama, Ozotoceros* e *Blastocerus.*

ABSTRACT

Prion diseases are a group of neurodegenerative, invariably fatal disorders that affects humans and non-humans mammals. The most studied prion disorders are Creutzfeldt-Jakob disease (CJD) from humans, scrapie in sheep and goats and the bovine spongiform encephalopathy (BSE). Chronic wasting disease (CWD) is a prion disease of cervids that has been regarded as a health public problem in USA and Canada. Recent researches have shown that some animals present more resistance to the infection. This resistance has been commonly associated with polymorphisms at the nucleotide sequence of the gene which codify the prion protein (*PRNP*). Single nucleotides polymorphism (SNP's) at specific codons has been highly associated with development of neurodegenerative disease on *Cervidae* family. In order to identify the presence/absence of those polymorphisms, and consequently the potential susceptibility to develop CWD, we analyzed samples from three neotropical deer: Gray brocket deer (*Mazama gouazoubira*, *n:11*), Pampas deer (*Ozotoceros bezoarticus, n:18)* and Marsh deer (*Blastocerus dichotomus, n:31)*. A fragment of 800bp from the exon 3 of *PRNP* was amplified by PCR and sequenced. Sequences were aligned and the results have shown that sequence of exon 3 of *PRNP* from Brazilian deer's are highly conserved. All animals encoded glutamine on 95 and glycine on 96, methionine at codon 132 and serine at codon 225 although Brazilian deer's have never been notified as CWD-positive.

Keywords: Transmissible Spongiform Encephalopathy, Prion, Neotropical Deer, *Mazama, Ozotoceros* and *Blastocerus*.

CAPÍTULO 1

1.1 Introdução

No final da década de 1960, um veado da espécie *Odocoileus hemionus* mantido em uma fazenda de criação de cervídeos nos Estados Unidos apresentou um comportamento que, a princípio, parecia uma reação agressiva ao cativeiro, caracterizado por perda de apetite, emaciação, pneumonia e óbito. Esses sinais se repetiram em outros animais da mesma espécie e tal condição patológica passou a ser denominada *"Chronic Wasting Disease"* (CWD), em português como: "Doença da Debilidade Crônica". Uma década depois, a CWD foi listada no rol de doenças neurodegenerativas denominadas encefalopatias espongiformes (WILLIAMS & YOUNG, 1980) cujos representantes mais comuns são a Encefalopatia Espongiforme Bovina (do inglês, Bovine Spongiform Encephalopathy (BSE)), a Paraplexia Enzoótica Ovina (do inglês, *scrapie*) em ovinos e a Doença de Creutzfeldt-Jacob em humanos (BOURNE, 2004). Tais doenças são desencadeadas por uma proteína, denominada *príon* ou proteína priônica, naturalmente presente em vários tecidos e que assume, após a tradução, uma conformação anormal, insolúvel, e que se deposita nos tecidos nervosos causando as encefalopatias (PRUSINER, 1982). Sabidamente a CWD acomete cervídeos norte-americanos (*Odocoileus hemionus*, *Odocoileus virginianus*, *Cervus elaphus* e *Alces alces*) sendo possível a transmissão entre eles por diversas vias, incluindo saliva, fezes e carcaças em decomposição (MILLER et al., 2004; SIGURDSON & AGUZZI, 2007; HALEY et al., 2009). A proteína priônica é codificada pelo gene *PRNP*, e alguns polimorfismos de base única (SNPs) vêm sendo fortemente associados à susceptibilidade dos cervídeos à doença (GOLDMANN et al. 1990; WHITE et al. 2010; JOHNSON et al. 2011). Atualmente a CWD é considerada epidêmica nos Estados Unidos. No Brasil, apesar de ainda não existirem relatos da doença, a possibilidade de ocorrência da CWD não deve ser descartada, uma vez que *O. virginianus*, sabidamente susceptível à doença, ocorre em simpatria com *Mazama sp*. ao norte do Amazonas.

1.2 Objetivos

1.2.1. Objetivo geral

Verificar a ocorrência de polimorfismos no exon 3 do gene *PRNP* em três espécies de cervídeos brasileiros: Veado catingueiro (*Mazama gouazoubira*), Veado campeiro (*Ozotoceros bezoarticus*8) e Cervo do Pantanal (*Blastocerus dichotomus*).

1.2.2. Objetivos específicos

Buscar polimorfismos no gene *PRNP* dos cervídeos brasileiros que possam estar relacionados à susceptibilidade dessas espécies à *Chronic Wasting Disease* (CWD).

Identificar os polimorfismos do gene *PRNP* característicos dos cervídeos neotropicais que os diferenciem dos demais cervídeos ao redor do mundo.

Montar árvores de similaridade genética, baseadas em nucleotídeos e em aminoácidos, entre os cervídeos brasileiros e os demais ao redor do mundo.

Oferecer informações técnicas necessárias para que órgãos governamentais possam propor medidas de manejo sanitário para minimizar o impacto da entrada da doença no Brasil, caso nossas espécies sejam potencialmente sensíveis.

1.3. Revisão da Literatura

Os Príons e as Encefalopatias Espongiformes Transmissíveis

As doenças priônicas correspondem a um grupo de desordens neurodegenerativas irremediavelmente fatais, que acometem humanos e outros mamíferos. O termo Encefalopatia Espongiforme Transmissível (EET) é comumente utilizado para designar as doenças priônicas, dentre as quais, as mais conhecidas são o *scrapie*, em ovinos; a Doença de Creutzfeldt-Jakob (CJD), Kuru, Insônia Familiar Fatal (FFI) e Síndrome Gerstmann-Straussler-Scheinker (GSS) em humanos; a Encefalopatia Espongiforme Bovina (BSE) e a *Chronic Wasting Disease* ou Doença da Debilidade Crônica, em cervídeos. A característica patognomônica que agrupa todas as EEts é a presença de vacúolos neurais na análise anátomo-patológica e morte celular do sistema nervoso central (SNC) fazendo com que a massa encefálica assuma característica morfológica semelhante a uma esponja (BOURNE 2004; AGUZZI et al., 2007).

Estudos histológicos têm mostrado que o acometimento da doença causa vacuolização e degeneração neural no núcleo do trato solitário, hipotálamo, tálamo e no córtex olfatório e também está presente, de forma menos acentuada, no mesencéfalo, metencéfalo e mielencéfalo. Perda neuronal e astrocitose também foram identificadas no tálamo e no hipotálamo, sendo a astrocitose também presente na região óbex da medula oblonga. Os tecidos linfóides apresentaram moderada depleção dos linfócitos foliculares, mas os demais tecidos apresentaram-se livres das lesões histológicas (SPRAKER et al., 2002).

Após a realização de experimentos com radiação que mostraram a inexistência de ácido nucléico nos agentes infecciosos do *scrapie*, Griffith (1967) especulou que uma proteína poderia ser o agente causador das infecções que geravam as encefalopatias espongiformes transmissíveis. Stanley Prusiner, em 1982, publicou o modelo mais aceito de explicação das doenças priônicas, denominada "*Protein-Only Hypothesis*", após seus experimentos mostrarem que os agentes das EETs eram resistentes aos procedimentos que inativavam as propriedades dos ácidos nucléicos e parcialmente sensíveis aos procedimentos que desnaturavam proteínas. Estudos posteriores mostraram que as

complicações decorrentes das doenças priônicas provêm de uma alteração, da naturalmente produzida, proteína celular priônica: PrP^{C} (STAHL, 1987).

A proteína priônica é uma glicoproteína da membrana celular de função pouco conhecida que é codificada pelo gene *PRNP*. A forma normal da proteína (PrP^{C}) é expressa em muitos tecidos mas apresenta significativa expressão no sistema nervoso central e em tecidos linforreticulares. A PrP^{C} se liga à superfície celular através de uma âncora glicosilfosfatidilinositol (GPI) (STAHL, 1987; DIAS CORREIA & DIAS CORREIA, 2005) mas ainda não se sabe ao certo que papel esta GPI desempenha nas EETs (CHESEBRO et al., 2005).

O evento central na patogenicidade da doença priônica, é a alteração conformacional da PrP^{C} em uma isoforma insolúvel e parcialmente resistente à ação de proteases que se propaga "por si própria" segundo Aguzzi & Calella (2009). Portanto, o agente causador das EETs, denominados príons (do inglês, *proteinaceous infectious particle*) são uma isoforma modificada (PrP^{Sc} ou PrP^{res}) que são convertidas através de um mecanismo onde a porção estrutural α-hélice da proteína é transformada em folha-β (PAN et al., 1993). Esta transformação estrutural é acompanhada por alterações nas propriedades físico-químicas da PrP^{C} (PRUSINER, 1997). Estudos com animais modificados geneticamente mostraram que as isoformas patogênicas (PrP^{Sc}) agem como um molde que continuam reformulando as formas naturais não-patogênicas (PrP^{C}) em novas moléculas PrP^{Sc}, em um processo contínuo de infecção (PRUSINER, 1998). Essa contínua conversão e agregação de proteínas forma grandes complexos proteicos em forma de placas amilóides. Doenças que, assim como as EETs apresentam formação de placas amilóides são conhecidas por atingirem o sistema nervoso (AGUZZI, 2009).

Entre as doenças atualmente conhecidas, as EETs são tidas como as únicas enfermidades com três possíveis origens: esporádica, genética ou adquirida. Os estudos de Ladogana et al. (2005) em três diferentes continentes mostraram que aproximadamente 85% dos casos de CJD acontecem de forma esporádica, ainda assim, sua etiologia permanece desconhecida. Os demais casos se dão através de mutações no gene *PRNP* em linhagens genéticas familiares ou resultam de alguma forma de exposição a agentes EETs positivos (BÉRINGUE et al., 2008).

Outra característica intrínseca às doenças priônicas, é a grande variabilidade no tempo de incubação e no desenvolvimento da doença entre indivíduos da mesma espécie. Os experimentos de Bartz et al. (1998) mostraram que a inoculação intracerebral de extratos cerebrais de cervídeos CWD-positivos em *ferrets* (*Mustela putorius furo*) levou ao desenvolvimento da patologia. Quando os extratos cerebrais destes *ferrets* infectados foram

transferidos para outros *ferrets*, o tempo de incubação diminuiu e o percentual de animais infectados aumentou. Além disto, a inoculação do homogeneizado de *ferrets* CWD-positivos em hamsters (animais sabidamente resistentes às infecções por agentes causadores das EETs, segundo Gibbs et al. (1996) provocou o desenvolvimento da doença nestes animais (BARTZ et al., 1998). Embora se tenha conhecimento de que as distintas conformações da PrP^{Sc} parecem codificar novas variantes da doença, não está claro como é possível a mutação e adaptação dos príons na ausência de ácidos nucléicos (ANGERS et al., 2010). As variantes priônicas (aparentemente causada pelas infecções em grupos animais diferentes e pelas infecções em gerações diferentes) podem resultar em um aumento da abrangência de espécies susceptíveis, um fator que complica ainda mais as avaliações de riscos-potenciais para novas espécies hospedeiras (BARTZ et al., 1998).

Chronic Wasting Disease (CWD)

A chronic wasting disease (CWD) ou doença da debilidade crônica é uma enfermidade classificada no grupo das EETs que afeta espécies da família *Cervidae.* Comprovadamente presente nos Estados Unidos (WILLIAMS & MILLER, 2002) e Canadá (KAHN et al., 2004), a CWD já foi diagnosticada em cervos-mula (*Odocoileus hemionus*), cervos da cauda branca (*O. virginianus*) e alces das montanhas rochosas (*Cervus elaphus nelsoni*), todas espécies do hemisfério norte. Alguns casos isolados foram reportados na Coréia do Sul em animais importados do Canadá (KIM et al., 2005).

O primeiro registro de caso de CWD data de 1967, acometendo cervos-mula em um centro de pesquisa no estado do Colorado, Estados Unidos. Inicialmente acreditou-se que a patologia pudesse estar relacionada às condições impostas pelo cativeiro, como deficiência nutricional e stress, mas, somente em 1978, com base nas lesões neuropatológicas características a patologia foi reconhecida como pertencente ao grupo das EETs (WILLIAMS & YOUNG, 1980). O primeiro registro confirmado de CWD em animais selvagens aconteceu no ano de 1981 em um alce das montanhas rochosas também no Colorado/USA, em 1985 foi diagnosticada em cervo-mula, e em 1990 no cervo-da cauda branca (WILLIAMS et al., 2002b).

Atualmente, mais de 40 anos após o primeiro registro de CWD, é sabido que a debilidade ataca animais mantidos em cativeiro, bem como os indivíduos de vida livre. A atual distribuição dos casos diagnosticados inclui *hot spots* (locais de grande incidência da doença) que não necessariamente ocupam áreas contíguas. Os casos registrados de CWD ocorrem em estados americanos em lados opostos do mapa o que implica uma origem

independente dos casos da doença ou, por outro lado, um compartilhamento de origem comum da doença que pode datar de muitas décadas passadas (MILLER et al., 2000). Ainda não se sabe qual o curso inicial da doença, se do meio selvagem para os animais em cativeiro, se dos animais cativos para os animais de vida livre ou ainda se a patologia desenvolveu-se separadamente nestes grupos de animais (WILLIAMS & MILLER, 2003).

Embora a origem da debilidade crônica ainda não possa ser determinada, duas teorias vêm ganhando força nesse sentido (WILLIAMS & MILLER, 2003): a primeira teoria remete à possibilidade da doença ter surgido a partir do contato entre animais infectados com o *scrapie* (WILLIAMS & MILLER, 2002). Estudos de Hamir et al. (2004) subsidiam esta teoria mostrando que cervídeos inoculados intracerebralmente com *scrapie* apresentam lesões no sistema nervoso central indistinguíveis dos animais afetados pela CWD.

A outra teoria relaciona o surgimento da debilidade a uma mutação espontânea do gene *PRNP* ocorrida em cervos-mula, que conduziu ao surgimento da doença e posterior disseminação pelos demais animais ou ainda devido a uma alteração conformacional, não induzida, da proteína priônica em sua forma natural (PrP^{C}) para sua forma patogênica (PrP^{Sc}) (SALMAN, 2003).

Entre todas as EETs de mamíferos, a CWD é tida como a mais eficientemente transmitida podendo, em densas populações de vida livre, alcançar 30% de transmissibilidade e, quando em cativeiro, esse índice pode alcançar os 100%. As formas de transmissão da doença ainda não são totalmente esclarecidas, no entanto, acredita-se, e tem sido demonstrado, que as transmissões horizontais acontecem principalmente via contato direto com secreções tais como sangue e/ou saliva (SIGURDSON & AGUZZI, 2007), excretas (urinas e fezes) (HALEY et al., 2009) e até mesmo com carcaças em decomposição (MILLER et al., 2004).

Os estudos de Fox et al. (2006) demonstraram detalhadamente o curso da infecção priônica em animais CWD-positivos inoculados intracerebralmente de forma experimental. Os animais foram acompanhados, e alguns sacrificados a determinados períodos de dias entre o 90º e 785º dia após a inoculação. Os resultados mostraram que o acúmulo da PrP^{Sc} acontece de forma relativamente rápida e disseminada nos tecidos linfáticos, seguido pela deposição nos tecidos nervosos centrais e periféricos e esporadicamente em uma variedade de órgãos e tecidos nos estágios finais da doença. Os resultados mostraram também, que apesar dos locais de deposição serem os mesmos, o tempo de progressão da doença varia de acordo com o genótipo do animal.

Recentemente foi demonstrado o acúmulo de proteína priônica modificada (PrP^{Sc}) em folículos linfoides ectópicos nos rins de cervos da cauda branca inoculados

experimentalmente. Estes acúmulos de proteínas priônicas em vias excretoras aumentam a disseminação da doença e fornecem subsídios para o entendimento das formas de contaminação (HAMIR et al., 2006).

Os riscos de transmissão da CWD para outros animais ainda é alvo de grande discussão. No entanto, as pesquisas não têm obtido sucesso na tentativa de transmissão via oral para outras espécies além dos cervídeos (SIGURDSON, 2008). Os estudos de Kong et al. (2005) com camundongos geneticamente modificados que expressavam a PrP^{C} dos cervídeos apresentaram sucesso na transmissão da CWD para os camundongos. Contudo, quando estes animais modificados geneticamente expressavam a PrP^{C} de humanos, a transmissão da doença não obteve sucesso, isto sugere a existência de uma barreira genética que impede a contaminação dos seres humanos a partir de animais CWD-positivos.

Os animais acometidos pela CWD apresentam sinais característicos como perda de peso, isolamento social, hipersalivação, regurgitação frequente, distensão esofágica e raramente ataxia (SIGURDSON & AGUZZI, 2007).

Nos experimentos de Fox et al. (2006) seis animais foram observados e os sinais clínicos da CWD incluíram opacidade dos olhos, estado de alerta diminuído e alteração de comportamento nos indivíduos entre o 442º e 572º dias após inoculação intracerebral experimental. Os sinais clínicos iniciais foram sutis e inconsistentes e, à medida que a doença progrediu, alterações comportamentais e perda de peso tornaram-se mais pronunciados e consistentes. Alguns sintomas tardios na infecção foram ptialismo, polidipsia e poliúria, que não estavam presentes em todos os casos.

Segundo Williams & Miller (2002) os animais cativos acometidos pela CWD apresentam ainda, comportamento repetitivo, ficam constantemente com a cabeça e orelhas abaixadas, e também demonstram períodos de sonolência e depressão. A diminuição de peso, comumente relatada, parece estar associada com a menor quantidade de alimento ingerido.

Apesar da utilização dos sinais clínicos da doença, diagnósticos definitivos só podem ser afirmados com a realização de exames histopatológicos de detecção das lesões espongiformes ou com a utilização de imuno-histoquímica para detecção do acúmulo da PrP^{Sc}. Além dos tradicionais exames histopatológicos e imuno-histoquímicos, outros métodos comumente usados na comprovação da infecção por agentes priônicos são ELISA e Western Blotting (BOURNE, 2004; WILLIAMS & YOUNG, 1992).

Susceptibilidade Genética à CWD

Os polimorfismos nucleotídicos associados ao gene *PRNP* são comuns nas espécies sujeitas ao desenvolvimento das EETs e estes polimorfismos, por sua vez, são determinantes no que diz respeito ao tempo de incubação e susceptibilidade às doenças priônicas tais como o *scrapie* e a CWD. Susceptibilidade e resistência às doenças seguem padrões genéticos baseados nas diferentes formas alélicas codificadas pelo *PRNP* (GOLDMANN et al., 1990).

O grau de similaridade da seqüência de aminoácidos do gene *PRNP* entre espécies distintas implicará em conseqüências na transmissibilidade das TSEs entre estas espécies. Frequentemente ocorre de os mesmos polimorfismos em duas espécies propiciarem efeitos similares quanto à susceptibilidade às doenças priônicas (GOLDMANN, 2008).

Os estudos de O´Rourke et al. (1998) descreveram um polimorfismo no códon 132 do gene *PRNP* em alces das montanhas rochosas. A seqüência de nucleotídeos deste códon pode, em hetero ou homozigose, codificar Metionina (M) ou Leucina (L). No ano seguinte, foi determinado o genótipo do gene *PRNP* em animais CWD-positivos e CWD-negativos para determinar se este polimorfismo influenciaria na susceptibilidade à CWD. Os resultados mostraram que 100% (para animais de vida livre) e 74% (para animais de cativeiro) dos CWD-positivos, apresentavam homozigose para 132MM. Os casos de heterozigose para 132ML não foram significativos em população em cativeiro e não se apresentaram em animais de vida livre com CWD-positivo. Nenhum indivíduo codificado como 132LL apresentou-se como CWD-positivo (O´ROURKE et al., 1999).

Outro polimorfismo bastante estudado com aparente relação com a susceptibilidade ao desenvolvimento da CWD é do códon 225. Cervos-mula que apresentam heterozigose para Serina (S) e Fenilalanina (F) (225SF) ou homozigose para Fenilalanina (225FF) estão geralmente minimamente representados nos casos de CWD-positivos. Por outro lado, os casos de CWD-positivos para indivíduos que apresentam homozigose para Serina (225SS) são 30 vezes mais frequentes quando comparados aos 225SF. No entanto, apesar de os dados mostrarem que o alelo 225F apresenta ínfima presença nos casos confirmados de CWD, a baixa freqüência deste alelo nas amostras (0.033%) não possibilitou uma conclusão concreta (JEWELL et al., 2005).

Na mesma linha, análises genéticas também mostraram que a susceptibilidade ao desenvolvimento da CWD em cervos da cauda branca é influenciada por polimorfismos nos códons 95 (que pode codificar Glutamina ou Histidina) e 96 (que pode codificar Glicina ou Serina) (JOHNSON et al., 2003; O´ROURKE et al., 2004). A análise baseada apenas no

códon 96 mostrou que a heterozigose neste códon (96GS) aparece com menor freqüência em indivíduos CWD-positivos sugerindo redução da susceptibilidade à CWD ou desaceleração no progresso da doença. Quando analisados conjuntamente, a heterozigose do códon 95 (95QH) mostrou-se ausente nos animais infectados, independente de homozigose ou heterozigose do códon 96 (JOHNSON et al., 2006).

Extrapolando as fronteiras norte-americanas, um estudo com *Cervus nippon* foi realizado no Japão. O gene *PRNP* foi examinado para determinação dos genótipos dos animais estudados. Com exceção de 3 mutações silenciosas nos códon 63, 255 e 408, a seqüência do gene foi idêntica à seqüência já conhecida de *O. hemionus*. Os já bem conhecidos códons que apresentam maior susceptibilidade à CWD em cervos de cauda branca (95Q, 96G) e em alces de montanhas rochosas (132M) foram observados nas espécies selvagens de *C. nippon*. No entanto, não é sabido se os polimorfismos do gene *PRNP* estão associados com a ocorrência natural de CWD fora dos limites da América do Norte visto que não houve nenhuma indicação de casos CWD-positivos em nenhum dos animais testados (KATAOKA et al., 2005). Além da América do Norte, os únicos casos relatados de CWD aconteceram na Coréia do Sul, no entanto, os animais que apresentaram o desenvolvimento da doença foram importados do Canadá (KIM et al., 2005).

No Brasil, bem como em toda a região neotropical, não existem evidências nem casos relatados de cervídeos acometidos pela CWD. Também não há pesquisas realizadas que indiquem susceptibilidade ao desenvolvimento da doença. Pesquisas de doenças que acometem vida selvagem e estudos descritivos de forma geral são ainda raros, porém extremamente necessários em países neotropicais. Ações de vigilância epidemiológica são de extrema importância para que seja possível lidar com possíveis conseqüências na saúde humana e animal (GORTÁZAR et al., 2007).

Saúde Pública x CWD

Embora algumas pesquisas objetivando estudar a possibilidade de transmissão das doenças priônicas para humanos venham sendo realizadas, pouco se sabe a esse respeito. Os estudos têm mostrado um risco potencial reduzido no que diz respeito à transmissão de CWD para humanos (BELAY et al., 2004) através do consumo da carne do veado (BELAY et al., 2001). As pesquisas têm frisado uma possível barreira genética entre as espécies – humanos e cervídeos – como um entrave que estabelece a segurança da população consumidora da carne de veado (RAYMOND et al., 2000).

Mesmo considerando que no Brasil o consumo deste tipo de carne seja limitado a caçadores e aventureiros esporádicos, é importante ressaltar que o conhecimento é ainda mais restrito quando se trata de transmissão para humanos por outras vias diferentes da ingestão oral. É importante também frisar que no Brasil, o histórico de destruição de florestas e campos para expansão de fazendas pecuaristas, restringe o habitat natural dos cervídeos bem como aproxima a população humana, o gado e demais animais domésticos a áreas dantes habitadas por cervídeos. Esta aproximação aumentaria a exposição humana ao agente causador da CWD. Não existem registros acerca da transmissibilidade da CWD pelo contato humano com materiais biológicos – urina, fezes, sangue, placenta – de animais infectados, contudo a transmissão experimental da CWD para primatas não humanos já ocorreu como relatado por Marsh et al. (2005). A transmissão da CWD para animais domésticos – ovinos, caprinos, suínos – é uma área de grande interesse e conhecimento crescente (SIGURDSON, 2007).

O conhecimento e a informação fazem-se, portanto, indispensáveis para que a população esteja segura quanto ao risco potencial de infecções priônicas. Além disto, é necessário que se conheçam as possibilidades de infecção dos animais brasileiros a fim de municiar os órgãos competentes para o manejo e conservação das – já tão ameaçadas – espécies de cervídeos brasileiros e, se necessário, a definição de um plano de ação que dificulte ou mesmo impeça a entrada da doença no Brasil.

Na ausência de informações precisas, e considerando os possíveis riscos ligados ao consumo de carne de veado, recomendam-se alguns procedimentos básicos de segurança para caçadores e até mesmo taxidermistas, para que se evite a exposição desnecessária à CWD. O consumo da carne de animais que apresentem características das infecções priônicas, bem como das partes mais agudamente afetadas – cérebro, nódulos linfáticos, baço, tonsilas e olhos – mesmo em animais sadios deve ser evitada. O uso de luvas de látex e a profilaxia do material de trabalho – facas, roupas, panelas etc. – também é fortemente recomendado (WILLIAMS & MILLER, 2002).

Implicações de Manejo

A ausência de tratamento para os animais infectados com CWD faz com que o aparecimento dos sinais clínicos da doença sejam inevitavelmente fatais. O longo período de incubação, a inexistência de diagnósticos *ante-mortem* precisos, a resistência reforçada do agente infeccioso, a contaminação *in natura* e a falta de conhecimentos da doença, aumentam a dificuldade de controlar ou erradicar a CWD (WILLIAMS et al., 2002b).

Alguns programas de erradicação da CWD foram elaborados mas não obtiveram sucesso e embora seus fatores de insucesso não tenham sido devidamente relatados, acredita-se que contaminação residual do ambiente e falha na esterilização dos locais de estudo foram os principais deles (WILLIAMS & YOUNG, 1992; MILLER et al., 1998). No entanto alguns zoológicos aonde a CWD foi detectada conseguiram controlar as infecções em suas instalações. Alguns cuidados básicos podem ajudar na prevenção: evitar a introdução de novos animais em ambientes sabidamente ocupados por animais CWD-positivos, realizar exames prévios em animais recentemente adquiridos, colocar os novos indivíduos em quarentena e eliminar rebanhos afetados pela CWD contribuem para a erradicação da doença em populações de cativeiro (WILLIAMS et al., 2002).

O controle da CWD em animais de vida livre é ainda mais complexo do que naqueles criados em cativeiro. No entanto, nas áreas endêmicas de desenvolvimento da doença, são necessários programas de vigilância para monitorar a distribuição e prevalência da CWD além de atualizar a extensão das áreas endêmicas (MILLER & KAHN, 1999).

CAPÍTULO 2

Are Brazilian Cervids at Risk of Prion Diseases?

ABSTRACT

Prion diseases are a group of neurodegenerative fatal disorders invariably fatal that affects humans and non-humans. The most famous prion disorders are Creutzfeldt-Jakob disease (CJD) in humans, scrapie of sheep and goats and bovine spongiform encephalopathy (BSE). Chronic wasting disease (CWD) is a prion disease of cervids that has been regarded as a health public problem in USA and Canada. Recent researches have shown that some animals present more resistance to the infection. This resistance has been commonly associated with the sequence of nucleotides on the gene which codify the prion protein (*PRNP*). Single nucleotides polymorphism (SNP's) at specific codons has been highly associated with CWD development on *Cervidae*. In order to identify the presence/absence of polymorphism, and consequently the potential susceptibility to develop CWD, we analyzed samples from three neotropical deer (*Mazama gouazoubira, Ozotocerus bezoarticus* and *Blastocerus dichotomus*). Genomic DNA was isolated and a fragment of 800bp from the exon 3 of *PRNP* was amplified by PCR and sequenced. Sequences were aligned and the results have shown that sequence of exon 3 of *PRNP* from Brazilian deer's are highly conserved. All animals encoded glutamine on 95 and glycine on 96, methionine at codon 132 and serine at codon 225 although Brazilian deer's have never been notified as CWD-positive.

INTRODUCTION

Prion diseases, as known as transmissible spongiform encephalopathies (TSEs), are a group of lethal neuronal disorders presenting common characteristics among the different known manifestations. Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats, and chronic wasting disease

(CWD) in cervids, presents the same patterns of neuronal vacuums and cells death from central nervous system (CNS) (Aguzzi et al. 2007).

First postulated by Griffith (1967), the idea of a protein as the agent of the infections which produces the TSEs was later remolded and explained by Prusiner (1982). The "Protein-Only Hypothesis" is the most accepted explanation of TSEs regarding that Prusiner showed the agent as resistant to the procedures which inactivated the nucleic acid. Nowadays, it is known that complications of TSEs are caused by an alteration from the naturally produced prion protein PrP^{C}, a glycoprotein little known that is codified by *PRNP* gene (Stahl 1987).

The main event on the prion pathogenicity is the conformational alteration from the normal form PrP^{C} into a non-normal form PrP^{Sc}. This isoform is insoluble and partially resistant to proteases. Prion diseases presents only the modified isoform (PrP^{Sc}) which is converted in a way where the α-helices portions are transformed in β-sheet (Pan et al.1993). This structural modification is accompanied with alterations on physical-chemistry properties from PrP^{C} (Prusiner 1997). Researches using transgenic mouse as a model, have shown that PrP^{Sc} acts as molder which transforms PrP^{C} into new PrP^{Sc} molecules (Prusiner 1998).

The chronic wasting disease is a disorder that affects the *Cervidae* family. United States (Williams & Miller 2002) and Canada (Kahn et al. 2004) have already diagnostic the disease in mule deer (*Odocoileus heminonus*), white-tailed deer (*Odocoileus virginianus*) and rocky mountain elk (*Cervus elaphus nelsoni*), all of them in north hemisphere. Some cases were also reported in South Korea, but the animals were imported from infected farms in Canada (Kim et al. 2005).

The first register of CWD was in 1967 within a wildlife facility in Fort Collins, Colorado where captive mule deer used for nutrition research were reported with a body wasting syndrome (Sigurdson & Aguzzi 2007). In the beginning it was believed that the disease could be related to the conditions in the captivity, such as nutritional deficiency and stress, and only in 1978 the CWD was recognized as TSE (Williams & Young 1980). The first register of CWD in wildlife animals was in 1980 (Williams, Miller & Thorne 2002).

Actually, more than forty years after the first register of CWD, it is known that the disease attacks animals in captivity and wildlife animals equally. The distribution of cases includes hot spots (regions under high incidences of disease) that are in opposite sides of USA territory (Miller et al. 2000). It is not known what was the initial way of CWD, if it is from wildlife to captive animals, from captive to wildlife animals or if the disease developed separately on these groups of animals (Williams & Miller 2003).

Among all TSEs, CWD is the most efficiently transmitted and it can reach 30% of transmissibility in wildlife. Although not entirely understood it is supposed that the transmission happens more efficiently in a horizontal way, via direct contact with body secretions (Sigurdson & Aguzzi 2007), excreta (Haley et al. 2009) and decomposed carcasses (Miller et al. 2004). The transmission among captive animals can reach 100% (Sigurdson & Aguzzi 2007) because, in captivity, the animals are in restricted areas (research facilities and creations' farms) where the change of fluids is constant.

The nucletotide polymorphisms at *PRNP* gene are common on species in risk to the development of TSEs. The development of disease has been commonly associated to the polymorphisms at *PRNP* gene. Susceptibility and resistance to TSEs follow genetics patterns based on different allelic forms codified by *PRNP* (Goldmann et al. 1990).

Genetic analysis have shown that susceptibility to develop CWD in white-tailed deer is strongly influenced by polymorphisms at codons 95 and 96 (Johnson et al. 2003, O'Rourke et al. 2004). Codon 95 can codify Glutamine or Histidine and codon 96 can codify Glycine or Serine. The analysis based only in codon 96 showed that heterozygosis on it (G96S) has low frequency on CWD-positive animals, suggesting reduction on susceptibility or slowdown on diseases' progress. When both codons were analyzed together, it was shown that heterozygosis on codon 95 (Q95H) also presents low frequency on CWD-positive cases, independently of homo or heterozygosis on codon 96 (Johnson et al. 2006).

O'Rourke' et al. (1998) showed a new single nucleotide polymorphism (SNP) on codon 132 in elks associated with CWD susceptibility. The genetic sequence of this codon can, on hetero or homozygosis, codify Methionine (M) or Leucine (L). In the follow year, it was determined the sequence of *PRNP* in CWD-positive and CWD-negative animals to observe if this polymorphism is correlated with the susceptibility to CWD. The results showed 100% (for wildlife animals) and 74% (for captive animals) of CWD-positive cases presenting 132MM. The animals encoding 132ML were not significant on captive animals and did not happen on CWD-positive wildlife animals. No one animal encoding 132LL was CWD-positive (O´Rourke et al. 1999).

Another polymorphism well established and apparently related to the susceptibility to develop CWD is at the codon 225. Mule deer encoding heterozygosis to Serine (S) and Phenylalanine (F) (225SF) or homozygosis to Phenylalanine (225FF) are underrepresented on CWD-positive cases. On the other hand, the CWD-positives cases are overrepresented on animals encoding homozygosis to Serine (225SS) and they can reach 30 times greater when compared to 225SF (Jewell et al. 2005).

The objective of this study was to verify, in three species of neotropical deer (*Mazama gouazoubira, Blastocerus dichotomus e Ozotoceros bezoarticus*), the occurrence of polymorphisms in the *PRNP* gene that were associated with susceptibility of species of the northern hemisphere to the development of CWD.

MATERIAL AND METHODS

Origin of Samples

Blood samples of animals were collected with EDTA by venopunction from *Mazama gouazoubira* (gray brocket deer) (n:11); *Ozotoceros bezoarticus* (pampas deer) (n:18) and *Blastocerus dichotomus* (mash deer) (n:31).

DNA Isolation

Blood samples were maintained in ethylic alcohol until the procedures of DNA isolation. The extraction of total DNA was done following the protocol described by Medrano et al. (1990). DNA quantification and quality verification was done by agarose gel electrophoresis stained with GelRed (Biotium).

Amplification of PRNP gene

A fragment of approximate 800bp from the exon 3 of *PRNP* gene was obtained using primers CWD-13 (5'–TTTTGCAGATAAGTCATCATGGTGAAA–3' [forward]) and CWD-LA (5'–AGAAGATAATGAAAACAGGAAGGTTGC– 3' [reverse]) described by Johnson et al. 2003. Polymerase chain reaction amplification conditions included initial denaturation of the sample at 95 ºC for 5 min, amplification with Taq Polymerase (LGC) for 10 cycles at 95 ºC (45 sec), 58 ºC (45 sec), and 72 ºC (1.5 min) followed by 25 cycles at 95 ºC (45 sec), 57 ºC (45 sec), and 72 ºC (1.5 min), and a final extension at 72 ºC (5 min) following Johnson et al. 2003. Success of amplification was verified using 1,5% agarose gel stained with GelRed. Amplified product was purified using QIAquick PCR Purification Kit (Qiagen).

Sequencing the *PRNP* gene

Sequencing of samples was conducted at Macrogen facilities (www.macrogen.com) (Korea) under BigDyeTM terminator cycling conditions on an ABI 3730xl automatic sequencer (Applied Biosystems, USA).

Sequencing Alignment and Analysis

Polymorphism Analysis

After sequencing the sequences (n: 60) were aligned on BioEdit version 5.0.9 (Hall 1999). The sequences were used to proceed a 'Blast n' on GenBank. After confirming the utilization of correct sequences from *PRNP* gene it was realized a detailed observation base-to-base looking for polymorphisms on the gene. Posteriorly the sequences were translated on BioeEdit and a 'Blast x' was performed on GenBank.

Genetic Similarity

Fifty-three nucleotide sequences of exon 3 of *PRNP* gene, from *Cervidae* family were downloaded from GenBank and aligned within the fragments sequenced in this work (Table 1.1). The selection of those sequences was based in the better scores from Blastx tool (NCBI) obtained by comparing them with those sequenced in present research (*Blastocerus dichotomus, Mazama gouazoubira* and *Ozotoceros bezoarticus*). Alignments were performed by using BioEdit version 5.0.9 (Hall 1999) and Clustal X (Thompson et al. 1997). Additionally, the alignment was visually inspected and a final alignment block at around 500bp was obtained. The alignment was then exported as a Nexus file, and a neighbor-joining (NJ; Saitou and Nei 1987) analysis was conducted by using Paup* 4.0b10 (Swofford 2000) and PaupUp graphic interface (Calendinni and Martini 2005). The ModelTest accessory application (Posadas and Crandall 1998) was used in order to determine the best fitted molecular evolutionary model for the dataset. Thus an unrooted NJ analysis was carried out using the parameters obtained from the HKY+Y model. Posteriorly the nucleotide sequences were translated using the option "translate in selected frame" from BioEdit and another unrooted NJ analysis was computed. At both analyses the branch robustness were assessed by bootstrap and jackknife analyses from a total of 1000 pseudo replicates by a neighbor-joining searching. The last two analyses were also developed by using Paup* v.4.0b10 as described above.

Table 2.1. Data from nucleotide sequences retrieved from GenBank and used for alignment and phylogenetic analyses.

Species	GenBank acc. No.
Alces alces alces	AY639095.1
Alces alces gigas	DQ154297.1
	DQ154298.1
Alces alces shirasi	AY225484.1
	AY225485.1
Capreolus capreolus	AY639096.1
	AY769955.1
Cervus dama	AY286007.1
	AY639094.1
Cervus elaphus	Y09761.1
Cervus elaphus elaphus	AY748454.1
	AY748455.1
	AY748456.1
	FJ436714.1
	FJ436716.1
Cervus elaphus nelsoni	AF156182.1
	AF156183.1
	EU082265.1
	EU082274.1
	EU082280.1
	EU082284.1
	EU082285.1
	EU082286.1
	EU0822.90.1
	EU082291.1
	U21210.1
Cervus elaphus scoticus	FJ436713.1
	FJ436715.1

Table 2.1. (Cont.) Data from nucleotide sequences retrieved from GenBank and used for alignment and phylogenetic analyses.

Cervus nippon	AY655756.1
	AY679695.1
	AY679696.1
	DQ358970.1
	EF057409.1
Dama dama	EF139175.1
	EF165089.1
Odocoileus hemionus hemionus	AF009181.2
	AY228473.1
	AY771349.2
	U25965.1
Odocoileus virginianus	AF156184.1
	AF156185.1
	AY275711.1
	AY275712.1
	AY286008.1
	AY425673.1
Rangifer tarandus	EU032303.1
Rangifer tarandus granti	DQ154292.1
	DQ154293.1
	DQ154294.1
	DQ154295.1
	DQ154296.1
	AY769956.1
Rangifer tarandus tarandus	AY639093.1

Analysis of Genetic Similarity (Nucleic Acid/Amino Acids)

Aiming to create the tree of genetic similarity (nucleotides based) it was used a 504bp fragment (nucleotides positions 200-704). The choice for this size of fragment occurred because this size contemplates the most number of nucleotides with minimal loss of neotropical sequences. The loss of some animals (six *O. bezoarticus* and one *M.*

gouazoubira) happened because these animals presented lack of large fragments. In the end, fifty three neotropical animals and fifty-three around-the-world (sequences deposited on GenBank) animals were used on this analysis.

Aiming to create the tree of genetic similarity (amino acids based) it was used the same fragment used on creation of nucleotide based tree, but with some adaptations. The first and second nucleotides of each sequence were removed because they were respectively the second and third nucleotides of codon 67. However, it is necessary to insert (on Bioedit) a fragment which begins on the first nucleotide of a codon to guarantee a correct and precise translation to amino acids. Regarding the exclusion of second and third nucleotide of codon 67, the fragment inserted began on the first nucleotide of codon 68.

RESULTS

Polymorphisms Analysis of Neotropical Cervids

We examined sixty samples of three neotropical species of deer distributed like follow: eleven samples of *M. gouazoubira*; eighteen of *O. bezoarticus* and thirty one of *B. dichotomus*. The sequences were examined focusing on the known polymorphisms which are related to susceptibility of development of CWD. The polymorphisms mostly associated are at codons 95, 96, 132 and 225.

Regarding the animals were examined, in one sample of *M. gouazoubira* and in one sample of *O. bezoarticus* it was not possible identify the central sequence of *PRNP* but all codons of interest were able to analysis. Regarding *B. dichotomus* it was not possible analyze the codon 132 in one sample and, in four sequences, was not possible identify the codon 225.

The results have shown that exon 3 of sequences from *PRNP* gene from Brazilian deer are highly conserved, even among the three different species of Brazilian cervids. All animals were analyzed encoded Glutamine at 95, Glycine at 96, Methionine at codon 132 and Serine at codon 225.

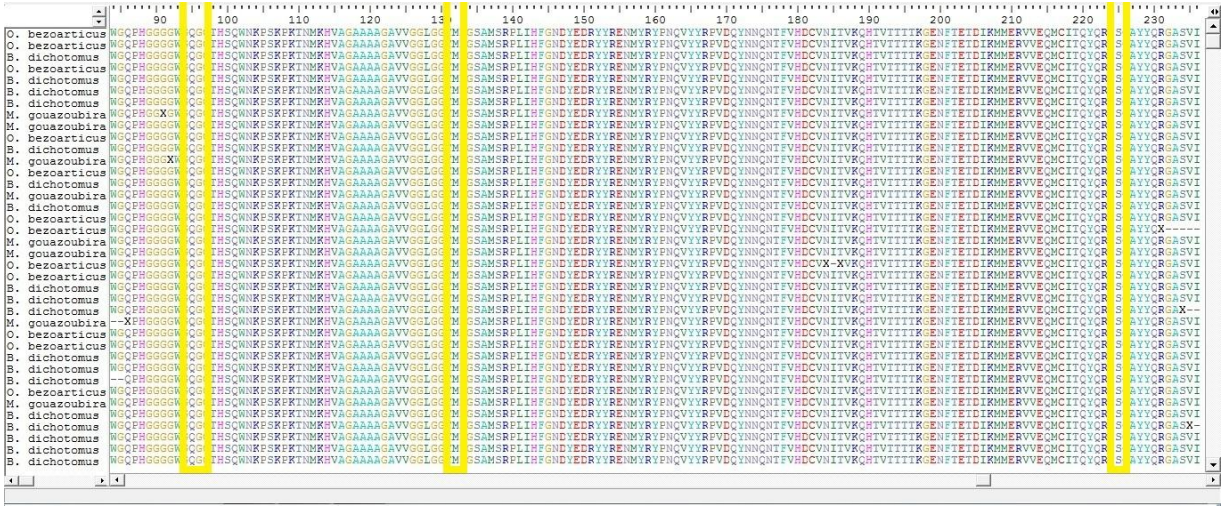

Figure 2.1. Alignment of translated *PRNP* gene from Neotropical cervids (*Mazama gouazoubira, Ozotocerus bezoarticus and Blastocerus dichotomus*) emphasizing codons 95, 96, 132 and 225.

On nine samples of *O. bezoarticus* it was possible access the nucleotide 87 (the last nucleotide of codon 29). All nine samples presented a silent mutation on the last nucleotide at codon 29 which substitutes adenine to guanine.

One sample of *M. gouazoubira* presented a heterozygosis on nucleotide position 271 (the first of codon 91). It codified guanine or adenine in a guanine position. In this case, there was a substitution of a Glycine to Arginine (G91R).

Two samples of *M. gouazoubira* presented a silent mutation at codon 92 where the nucleotide of position 276 (the last nucleotide of codon 92) substitutes a cytosine to thymine. One of them presented a second silent mutation at codon 139 where the nucleotide of position 417 (the last nucleotide of codon 139) substitutes a guanine to adenine.

Another *M. gouazoubira* presented a heterozygosis on nucleotide position 276 (the last of codon 92). It codified cytosine or thymine in a cytosine position.

One *O. bezoarticus* presented a substitution of a cytosine to a thymine on nucleotide position 584 (the second of codon 195). In this case, there was a substitution of a Threonine to an Isoleucine (T195I).

Table 2.2. Polymorphisms identified on *PRNP* gene for Neotropical Cervids.

Animal	Frequency of Affected	Nucleotide Position					Alteration on Amino Acid
		A87	C271	G276	G417	C584	
O. bezoarticus	9/9	G	_	_	_	_	No
M. gouazoubira	1/11	_	A/G		_	_	Yes (G to R)
M. gouazoubira	1/11	_		T		_	No
M. gouazoubira	1/11	_		T	A	_	No
M. gouazoubira	1/11	_		C/T		_	No
O. bezoarticus	1/18					T	Yes (T to I)

Analysis of Genetic Similarity

Comparing neotropical cervids and the northern hemisphere cervids it is possible identify many different polymorphisms along the *PRNP* sequence. Two of these polymorphisms call special attention for being present only in neotropical cervids. The polymorphism of nucleotide 87 (A87G) on *O. bezoarticus* and the polymorphism of nucleotide 684 (T684C), presented in all neotropical animals who was possible access this codon (Annex 1).

The construction of a nucleotide based tree of genetic similarity presents a wide range of separation of animals in different clusters, mainly when comparing neotropical and around-the-world cervids. However, neotropical cervids remains grouped in a unique cluster, even comparing the three different species.

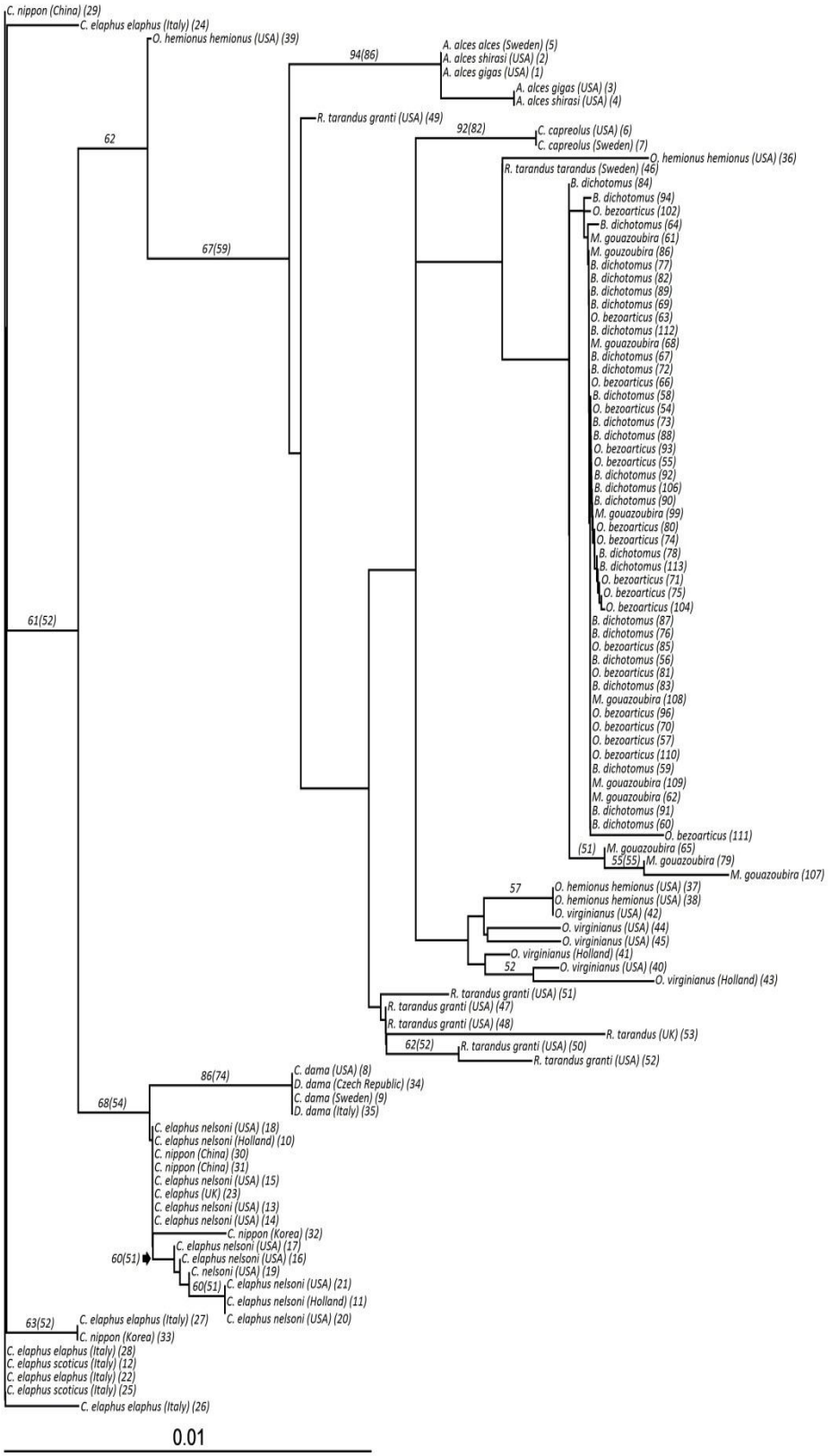

Figure 2.2. Nucleotide based tree of genetic similarity of *PRNP* gene among different species of cervids from South America and North Hemisphere

On the other hand the comparison among the sequence of amino acids shows us a well conserved region. Neotropical and around-the-world cervids presents grouped all together with a little exception.

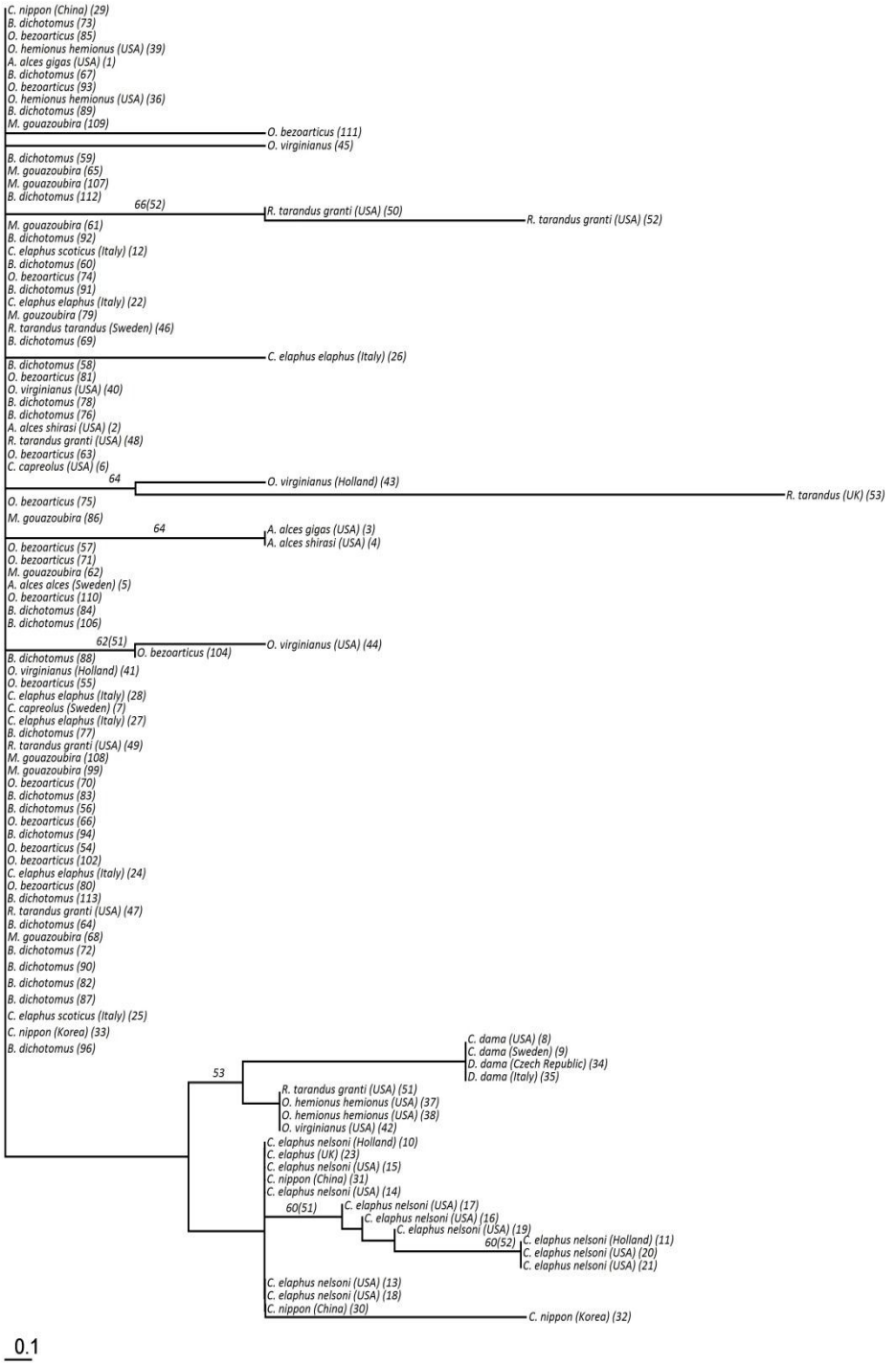

Figure 2.3. Amino acids based tree of genetic similarity of *PRNP* gene among different species of cervids from South America and North Hemisphere

The distance of individuals on amino acids based tree analysis showed the polymorphisms among animals which separate them on different clusters.

Table 2.3. Analysis of polymorphisms in each cluster of amino acid based tree of genetic similarity.

Cluster 66(52)	
	Codon G129 (Glycine)
R. tarandus granti USA (50)	Serine (S)
R. tarandus granti USA (52)	Serine (S)
Cluster 64:	
	Codon G96 (Glycine)
O. virginianus Holland (43)	Serine (S)
R. tarandus UK (53)	Cysteine (C)
Cluster 64:	
	Codon M209 (Methionine)
A. alces gigas USA (3)	Isoleucine (I)
A. alces gigas USA (4)	Isoleucine (I)
Cluster 62(51):	
	Codon A116 (Alanine)
O. virginianus USA (44)	G (Glycine)
O. bezoarticus (104)	Problems on sequencing
Cluster 53:	
	Codon S138 (Serine)
C. dama USA (8)	N (Asparagine)
C. dama Sweden (9)	N (Asparagine)
D. dama Czech Republic (34)	N (Asparagine)
D. dama Italy (35)	N (Asparagine)
O. hemionus hemionus USA (37)	N (Asparagine)
O. hemionus hemionus USA (38)	N (Asparagine)
O. virginianus USA (42)	N (Asparagine)
R. tarandus granti USA (51)	N (Asparagine)

Table 2.3. (Cont.) Analysis of polymorphisms in each cluster of amino acid based tree of genetic similarity.

Cluster 60(51):	
	Codon M132 (Methionine)
C. elaphus nelsoni USA (16)	Heterozygotes M (Methionine) / L (Leucina)
C. elaphus nelsoni USA (17)	Heterozygotes M (Methionine) / L (Leucina)
C. elaphus nelsoni USA (19)	Heterozygotes M (Methionine) / L (Leucina)

Cluster 60(52):	
	Codon M132 (Methionine)
C. elaphus nelsoni Holland (11)	L (Leucine)
C. elaphus nelsoni USA (20)	L (Leucina)
C. elahus nelsoni USA (21)	L (Leucina)

Another Cluster (No significant):	
	Codon Q95 (Glutamine)
O. virginianus USA (45)	H (Histidine)
	Codon T195 (Threonine)
O. bezoarticus (111)	I (Isoleucine)

Secondary Cluster (No significant):	
	Codon Q226 (Glutamine)
C. elaphus nelsoni Holland (10)	E (Glutamate)
C. elaphus nelsoni USA (13)	E (Glutamate)
C. elaphus nelsoni USA (14)	E (Glutamate)
C. elaphus nelsoni USA (15)	E (Glutamate)
C. elaphus nelsoni USA (18)	E (Glutamate)
C. elaphus UK (23)	E (Glutamate)
C. nippon China (30)	E (Glutamate)
C. nippon China (31)	E (Glutamate)
C. nippon Korea (32)	E (Glutamate) / Codon S100G

DISCUSSION

Polymorphisms Analysis of Neotropical Cervids

The presence of these polymorphisms at codon 95, 96, 132 and 225 suggests the potential susceptibility to development of CWD in Brazilian deer (Johnson et al. 2006), although cases of CWD have never been registered in Brazil. The absence of registered cases of CWD-positive animals, contradicting the existence of sequences which demonstrate the susceptibility, also happened in the Kataokas' et al. (2005) researches with *Cervus nippon* in Japan. The presence of the susceptibility-associated polymorphism and the lack of infected animals' description are of interest.

Regarding prion diseases have the majority of cases related as "sporadic" (Ladogana et al. 2005), almost like randomly, it is expected that animals presenting the polymorphism and developing the disease been found anywhere around the world. So, why Brazilian deer did not develop the disease, or could Brazil´s cases have been sub-notified? The necessity of a correct and precise diagnostic and notification is important to understand the patterns of disease (Gortázar et al. 2007) and to avoid the transmissibility to humans, which although improbable, cannot be discarded (Belay *e*t al. 2004).

First case of CWD and the major number of infected animals are described in places where animals are maintained in captivity, for research proposals (Williams & Young 1980) or in big farms that creates animals to use as food resource. In Brazil, it is not common to use cervids as food resource and there are no registers of research facilities of deer. Maybe, this can explain the fact that, in Brazil, had never been registered infected animals. Of course, the low frequency of animals living together could isolate the cases of CWD and, obviously, decrease the number of infected animals and the dissemination of the disease, since it is know that the CWD is transmitted in a horizontal way from animal to animal (Sigurdson & Aguzzi 2007).

The few registers of Brazilian animals living in captivity are related to the animals maintained in Zoos which, in most cases, are submitted to critical conditions. Some researchers have shown the influence of captivity on the animals' life. Bichard & Sherding (1998) affirms that captivity provides stress and decreased immunity making favorable the emergence of helminthiasis. Manson (1991) says that low environmental wealth triggers the stress causing dietary and health problems. Duarte & Merino (1997) affirms that captivity causes stress, not only for the absence of freedom but also because the imposed number of animals living together. Probably because the stress conditions, and the natural stressful

vulnerability, these animals are submitted, they do not reach long ages and dye earlier than the necessary time that would be required to the development of disease, since it is well established that CWD is a disease of long incubation time.

Another point is the fact that recent researchers have identified Brazilian deers as solitary animals, for *M. gouazoubira*, or restricted to small group of animals, for *O. bezoarticus* and *B. dichotomus*. Pinder and Leeuwenberg (1997) identified *M. gouazoubira* as solitary animals but two or more individuals can be observed together occasionally in periods of restriction or concentration of food resources. Tomas et al. (1997) have shown that *B. dichotomus* are mostly solitary, yet they are usually observed in a small familiar groups composed of an adult pair and one or more offspring. The only species that presents less solitary behavior is *O. bezoarticus* which was reported by Rodrigues & Monteiro-Filho (1996) who observed that even when resources are abundant these animals live in small groups, joining and dispersing continuously as the individuals roam freely among different groups. This behavior can be crucial for avoid or reduce the spread of CWD since transmission happens more efficiently in a horizontal way, via direct contact with body secretions (Sigurdson & Aguzzi 2007), excreta (Haley et al. 2009) and decomposed carcasses (Miller et al. 2004) and solitary animals presents less probability to change body fluids among themselves.

Beside it, the absence of CWD-positive cases registered in Brazil makes the chronic wasting disease unknown of veterinarians, biologists and mainly people in general. The number of wildlife professionals who are able to identify infected animals is really reduced and this can cause a sub-notification of cases in Brazil. Although deer are not commonly used as food resource, it is known some few cases where hunters have done it. The notification of infected animals by hunters could be really valuable since they are constantly looking for animals and “near” of them but regarding that to hunt is strictly forbidden in Brazil they cannot be trained and provide information of possible infected animals.

Analysis of Genetic Similarity

The distribution of animals on nucleotide based tree of genetic similarity in a wide range of clusters can easily be explained for the great number of nucleotide polymorphisms. Regarding the animals compared belongs to different species around the world, it is plausible this differentiation along the sequence of *PRNP* gene. This distribution corroborates the previous researches of Rongyan et al. (2008) about evolution and differentiation of *PRNP* gene. But, when analyzing only neotropical cervids, even comparing nucleotide sequences of

three different species of South America, it is possible to observe the great conservation of this region. It is clearly identifiable that neotropical cervis shares much more genetic similarity when compared to around-the-world animals. The great number of cluster does not present a regular distribution in which been possible separate animals on “susceptible” and “not susceptible”.

On the other hand, the analysis of the distribution of animals on amino acids based tree of genetic similarity, shows a completely conserved region. Confronting the nucleotide based tree (randomly distributed) and the amino acids based tree (well conserved) it is possible to affirm that nucleotide polymorphisms are silent mutations with no effect on translational procedures to amino acids. According to Vorberg et al. (2003) species barriers to the TSE agent are strongly influenced by the *PRNP* amino acid sequence of both the donor and the recipient animals. Some specific regions in the prion protein have been implicated in function, pathogenicity, and species barrier. The evolutionary maintenance of *PRNP* across vertebrate classes (in this case: *cervidae* family) suggests that it has an important and conserved function, perhaps promoting protein–protein interactions (van Rheede et al. 2003). This distribution suggests that no dramatic sequence changes have occurred to avoid cross-species TSE infectivity.

The distance of individuals on amino acids based tree analysis could be explained analyzing individually each amino acid along the *PRNP* gene in each animal (Table 2.3). The analyses of codons of interest which can influence the susceptibility to CWD, showed an interesting standard of distribution when analyzed the similarity among *C. elaphus nelsoni* USA (13, 14, 15 and 18) codifying Methionine; *C. elaphus nelsoni* USA (11, 20 and 21) codifying Leucine and *C. elaphus nelsoni* codifying Methionine/Leucine. *C. elaphus nelsoni* it is one of species who has commonly been associated to development of CWD. The distribution grouped animals codifying Methionine in a different cluster than animals which encode Leucine, and the heterozygotes for Methionine/Leucine appears like a transitional group.

Another interesting fact is the presence of *C. elaphus nelsoni* from Holland and United Kingdom and *C. Nippon* from China grouped together to *C. elaphus nelsoni* (USA) which encodes Methionine. The high degree of genetic similarity and “not-development” of CWD in animals from Holland, UK and China has to be better studied.

Finally, many animals from *Cervidae* family were shown to share the polymorphisms associated to development of CWD (Kataoka et al. (2005) in Japan, Meng et al. (2005) in China) and presents high degree of genetic similarity to animals from North America [Schettler et al. (2004) in Germany, Sieber et al. (2008) and Sieber et al. (2010) in

Switzerland and Peletto et al. (2009) in Italy and Scotland], however, even presenting these forms which are associated with high susceptibility, CWD remains to happen only in countries of North America. Which kind of factors could be associated to the development of chronic wasting disease besides the genetic predisposition to it? Or could these associated polymorphisms not been necessarily associated with development of CWD as proposed by Perucchini et al. (2008)? Obviously, chronic wasting disease and TSEs, in general, needs more researches to elucidate all uncertain about it.

CONCLUSION

The presence of polymorphisms which are commonly associated to development of CWD (in North America) and the high degree of genetic similarity of *PRNP* gene among Neotropical and around-the-world cervids makes Brazilian deer highly susceptible to CWD. These polymorphisms in animals living in Europe and South America together to the absence of positive cases of CWD in these continents bring a relevant question of endemism of CWD in USA and Canada and all possible factors involved on TSEs.

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ANEXOS

Annex 1.1. Alignment of *PRNP* gene from Neotropical and around-the-world Cervids.

	G	C	T	C	G	A	C	T	C	A	T	T	G	C	A	G	C	A	G	A	A	A	C	A	G	C	G	A	C	G	C	G	T	C	G	A	C	C	C	C	G	C	T	C	C	C	G
	0	0	0	0	0	0	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	4	4	4	4	4	4	5	5	5	5	6	6	6	6	6	7	7	7	7
	0	2	4	6	6	8	7	8	8	9	2	3	3	3	5	7	7	8	8	9	9	2	4	6	6	8	8	9	0	1	1	1	3	6	0	2	5	8	0	1	2	7	8	3	4	5	7
	4	4	4	0	3	7	5	3	9	5	2	1	4	7	5	1	6	5	6	2	8	4	7	0	7	4	5	4	8	3	4	7	8	8	5	6	5	4	6	8	7	6	4	8	1	7	1
1	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	G	•	•	•	•	•	•	C	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•
2	•	•	•	•	•	•	•	•	T	•	•	G	•	•	G	•	•	•	•	•	•	•	C	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	A	•	•	•
3	•	•	•	•	•	•	•	•	•	•	•	G	•	•	G	•	•	•	•	•	•	•	C	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	T	T	•	•	•	•	•	•
4	•	•	•	•	•	•	•	•	•	•	•	G	•	•	G	•	•	•	•	•	•	•	C	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	T	T	•	•	•	•	•	•
5	•	•	•	•	•	•	•	•	T	•	•	G	•	•	G	•	•	•	•	•	•	•	C	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	A	•	•	•
6	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	C	•	•	•	•	•	•	T	•	•	•	A	•	•	
7	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	C	•	•	•	•	•	•	T	•	•	•	A	•	•	
8	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	T	•	•	•	•	•	•	•	•	G	•	•	•	•	•		
9	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	T	•	•	•	•	•	•	•	•	G	•	•	•	•	•		
10	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•		
11	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•		

12	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
13	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
14	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	—
15	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
16	•	•	•	•	Y	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	W	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
17	•	•	•	•	Y	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	W	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
18	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
19	•	•	•	•	Y	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	W	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
20	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
21	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
22	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
23	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
24	•	•	•	•	C	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
25	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
26	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
27	•	•	•	•	C	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
28	•	•	•	•	C	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
29	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
30	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
31	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
32	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•

33	•	•	•	•	C	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
34	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	T	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
35	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	T	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•
36	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	T	•	•	•	•	•	•	C	T	•	•	•	•	T	T	•	•	•	•	•	•	•
37	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	T	•	•	•	A	•	A	C	•	•	•	•	•	•	T	•	•	•	•	•	•	•
38	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	T	•	•	•	A	•	A	C	•	•	•	•	•	•	T	•	•	•	•	•	T	A
39	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
40	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	T	•	•	•	•	•	A	C	•	•	•	T	•	•	T	•	•	•	•	•	•	•
41	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	T	•	•	•	•	•	A		•	•	•	•	•	•	T	•	•	•	•	•	•	•
42	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	T	•	•	•	A	•	A	C	•	•	•	•	•	•	T	•	•	•	•	•	•	•
43	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	A	•	•	•	•	C	•	T	•	•	•	•	•	A		•	•	•	T	•	•	T	•	•	•	•	•	•	•
44	•	•	•	T	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	G	C	•	T	•	•	•	•	•	A	C	•	•	•	•	•	•	T	•	•	•	•	•	•	•
45	•	•	•	T	•	•	•	•	•	•	•	•	•	•	G	•	•	T	•	•	•	•	•	C	•	T	•	•	•	•	•	A	C	•	•	•	•	•	•	T	•	•	•	•	•	•	•
46	•	•	•	•	•	•	•	•	•	G	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	T	•	•	•	•	•	•	C	•	•	•	•	•	•	T	•	•	•	•	•	•	•
47	•	•	•	•	•	•	•	•	•	G	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	T	•	•	•	•	•	•	•
48	•	•	•	•	•	•	•	•	•	G	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	T	•	•	•	•	•	•	•
49	•	•	•	•	•	•	•	•	•	G	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•
50	•	•	•	•	•	•	•	•	•	G	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	•	A	•	•	•	•	•	C	•	•	•	•	•	•	T	•	•	•	•	•	•	•
51	•	•	•	•	•	•	•	•	•	G	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	•	•	•	•	A	•	•	C	•	•	•	•	•	•	T	•	•	•	•	•	•	•
52	A	•	•	•	•	•	•	•	•	G	•	•	•	•	G	•	•	•	•	•	•	•	•	C	•	•	A	•	•	•	•	•	C	•	A	•	•	•	•	T	•	•	•	•	•	•	•
53	•	T	•	•	•	•	•	•	•	G	•	•	•	•	G	•	•	•	T	•	•	•	•	C	A	•	•	•	•	•	•	•	C	•	•	G	•	•	•	T	•	•	•	•	•	•	•

54	— — — — • G • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
55	— — — — — G • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • • —
56	— — — — — • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • — —
57	— — — — — G • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • • •
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61	— — — — — • • • • • • • • N G R • • • • • • • C • T • • • • • • C • • • • • • T • • C • • • •
62	— — — — — • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
63	— — — — — G • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • •
64	— — — — — — — — — — — — — — G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
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66	— — — — — • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • • •
67	— — • • • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • • •
68	— — — • • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • • •
69	— — — • • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • • •
70	— — — • • G • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • — —
71	— — — — — — — — — — • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
72	— — — — • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • — —
73	— — — — — • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • — —
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75	— — — — — — — — — — • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
76	— — — • • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
77	• • • • • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
78	— — — — — — — — — — • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
79	— — — — — — — — — — — — — — — • T • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
80	— — — — — — — — — — • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
81	— — — — — • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
82	— — — • • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
83	— — — — — • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
84	— — — — — — — — — — — — — — G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
85	— • • • • G • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
86	— — — — — • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
87	— — — — — — — — • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
88	— — — — — • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
89	— — — — — — — — • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
90	— — — — — • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
91	— • • • • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
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95	— — — — — — — — — — — — — — — — — • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —

96	— — — • • G • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • • •
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99	— — — • • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C • • • •
100	— — — — — • • • • • • • • • G • • • • • • • • C • T • • • • • — — — — — — • • T • • C — — — —
101	— — — — — • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • — — — — — —
102	— — — — — — — — — — — — • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
103	— — — • • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • — — — — — — — — —
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107	— — — — — — — — — — — — — — — • T • • • • • • C • T • • • • • A C • • • • • • T • • C — — — —
108	— — — — — — — — • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
109	— — — • • • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
110	— — — — — — — — • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
111	— — — • • G • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • T • T • • C — — — —
112	— — — — — • • • • • • • • • G • • • • • • • • C • T • • • • • • C • • • • • • T • • C — — — —
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CHRONIC WASTING DISEASE: THE PRION DISEASE OF CERVIDS

(DOENÇA DA DEBILIDADE CRÔNICA: A DOENÇA PRIÔNICA DOS CERVÍDEOS)

C. B. R. FALCÃO[1], J. E. GARCIA[1]*

SUMMARY

Transmissible spongiform encephalopathy (TSE), or prion diseases, are a group of infectious neurodegenerative diseases, which leads the hosts to death. Without available healing and prevention programs, TSE becomes more important as new researches have shown its comprehensive global spread and the large number of animal groups susceptible to developing TSEs. The most popular TSEs are Creutzfeldt-Jacob disease in humans, Bovine Spongiform Encephalopathy (BSE), and *scrapie* in ovine. Another prion disease less known, but not less important, is the Chronic Wasting Disease (CWD) which attacks animals from *Cervidae* family, and is the only form of TSE that attacks wild animals. Recent studies from the Unites States, Canada and South Korea have shown a well-established pattern of CWD development associated with specific nucleotide polymorphisms in the *Prnp* gene. Despite being considered an epidemic in the United States and with many programs of wildlife vigilance being developed to detect and reduce the spread of CWD around the world, in Brazil, there are no registered cases of CWD yet, and only one research group is working with this disease at this time.

KEY-WORDS: Chronic wasting disease. Cervids. Prion.

RESUMO

As encefalopatias espongiformes transmissíveis (EETs), ou doenças priônicas, são um grupo de doenças infecciosas neurodegenerativas que conduzem seus portadores à morte. Ainda sem cura e formas de prevenção, as EETs ganham cada vez mais importância à medida que novos estudos têm mostrado sua abrangente disseminação mundial e a grande quantidade de grupos animais susceptíveis ao desenvolvimento das doenças. As EETs mais popularmente conhecidas são a doença de Creutzfeldt-Jacob em humanos, a Encefalopatia Espongiforme Bovina (BSE) e o *scrapie* em ovinos. Outra forma de encefalopatia menos conhecida, mas não menos importante, é a DoenÁa da Debilidade Crônica (CWD) que acomete espécies da família *Cervidae*. A CWD diferencia-se das demais EETs por ser a única forma conhecida da doença que ataca animais selvagens. Estudos nos EUA, Canadá·e Coréia do Sul têm mostrado um padrão bastante preciso do desenvolvimento da CWD associado a polimorfismos específicos no gene *Prnp*. Apesar de ser considerada uma epidemia nos Estados Unidos, e diversos programas de vigilância de vida selvagem estarem em curso com intuito de detectar e minimizar a propagação da CWD ao redor do mundo, no Brasil ainda não foram registrados casos da doença e até o momento apenas um grupo de pesquisa vêm se dedicando a estudá-la.

PALAVRAS-CHAVE: Cervídeos. Doença da debilidade crônica. Príons.

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INTRODUCTION

In the late 60¥s, a deer of the *Odocoileus hemionus* species kept in a deer research facility in the USA showed a behavior that at first seemed like an aggressive reaction to captivity, characterized by loss of appetite, emaciation , pneumonia and death. These symptoms repeated in other animals of the same species and the pathological condition was named Chronic Wasting Disease (CWD). A decade later, CWD was listed as a neurodegenerative disease called Spongiform Encephalopathy (WILLIAMS & YOUNG, 1980), whose most common representative is Bovine Spongiform Encephalopathy (BSE), *scrapie* in ovine and Creutzfeldt-Jacob Disease in humans (BOURNE, 2004). Such diseases are triggered by a protein called prion, naturally present in various tissues and which assumes, after translation, an abnormal conformation, insoluble, which deposits on the nerve tissues causing encephalopathy (PRUSINER, 1982). It is known that CWD affects north-American cervids (*Odocoileus hemionus*, *Odocoileus virginianus*, *Cervus elaphus* and *Alces alces*) and can be transmitted by several routes, including saliva, feces and decaying carcasses (MILLER et al., 2004; SIGURDSON & AGUZZI, 2007; HALEY et al., 2009). The prion protein is encoded by the *Prnp*, and some single nucleotide polymorphisms (SNPs) have been strongly associated with cervid susceptibility to the disease (GOLDMANN et al., 1990; WHITE et al., 2010; JOHNSON et al., 2011). Currently, CWD is considered an epidemic in the USA. Although in Brazil, there are no reports of the disease, the possibility of the occurrence of CWD should not be overlooked, since *O. virginianus*, known to be susceptible to the disease occurs in sympatry with *Mazama sp*. north of Amazonas.

State of conservation of Brazilian deer

Brazil is rich in deer species, but it is not known exactly how many species exist in the country. The most widely classification accepted currently indicates the occurrence of eight species, occupying almost all Brazilian ecosystems: *Blastocerus dichotomus* (marsh deer) in Pantanal, Mato Grosso, in the floodplains of Paran· river and GuaporÈ valley, *Ozotoceros bezoarticus* (pampas deer) in the cerrado region, Pantanal and a relictual population in Paran·, *Mazama gouazoubira* (gray brocket) distributed throughout the country except in the north, *Mazama americana* (red brocket) across the South, *Mazama nana* (Brazilian dwarf brocket) and *Mazama bororo* (small red brocket) in the south and southeast, *Mazama nemorivaga* (Amazonian brown brocket) in the Amazon region and *Odocoileus virginianus* (white-tailed deer), the latter occupies the left bank areas of the Amazon river all the way to the northern United States (DUARTE et al., 2001). Despite their great ecological importance and wide distribution over the entire national territory, the *Cervidae* family is a group little studied in Brazil and many gaps still remain, especially with regard to taxonomy and species evolution. This lack of knowledge further increases the threat to preservation of the species in nature. Other important factors contributing to the declining deer population is the habitat fragmentation caused by dam construction, agricultural activities (BECCACECI, 1994; DUARTE et al., 2003) and exposure of wildlife to diseases transmitted by domestic animals (SZAB'' et al., 2003; TORRES et al., 2003). All these factors contribute to classify *B. dichotomus* and *O. bezoarticus* as Vulnerable (VU) and as Near Threatened (NT), respectively, in the Red List of Threatened Species of International Union for Conservation of Nature - IUCN (BAILLIE et al., 2004). While all species of the *Mazama* genus are currently described as Data Deficient (DD) in the same list, which shows that despite having little knowledge about the actual state of conservation of these species, there is a general agreement that all are at risk.

Prions and Transmissible Spongiform Encephalopathy

Prion diseases are a group of fatal neurodegenerative disorders that affect humans and other mammals. The term Transmissible Spongiform Encephalopathy (TSE) is commonly used to describe prion diseases, the best known among them are, *scrapie* in sheep; Creutzfeldt-Jakob Disease (CJD), Kuru, Fatal Familial Insomnia (FFI) and Gerstmann-Straussler-Scheinker Syndrome (GSS) in humans; Bovine Spongiform Encephalopathy (BSE) and Chronic Wasting Disease, in cervids. The pathognomonic feature that groups all TSE together is the presence of neural vacuoles in antomo-pathologic analysis and cell death of the central nervous system (CNS) causing the brain mass to assume a morphology similar to sponge (BOURNE 2004; AGUZZI et al., 2007).

Histological studies have shown that the onset of the disease causes vacuolation and neural degeneration in the nucleus of the solitary tract, hypothalamus, thalamus and olfactory cortex and is also present, to a lesser extent, in the midbrain and hindbrain. Astrocytosis and neuronal loss were also found in the hypothalamus and thalamus, while astrocytosis was also present in the obex region of the oblong medulla. The lymphoid tissues showed moderate depletion of follicular lymphocytes, but other tissues had no histological lesions (SPRAKER et al., 2002).

After conducting radiation experiments that showed lack of nucleic acid in scrapie infectious agents, Griffith (1967) speculated that a protein could be the causative agent of infections that generated transmissible spongiform encephalopathy. Stanley Prusiner, in 1982, published the most accepted model to explain human prion disease called Protein-Only Hypothesis, after his experiments showed that the

agents of TSE were resistant to procedures that inactivated the properties of nucleic acids and partially sensitive to the procedures to denature proteins. Later studies showed that the complications resulting from prion disease come from a change in the naturally produced cellular prion protein, PrP^C (STAHL, 1987).

The prion protein is a glycoprotein of the cellular membrane with a little known function that is encoded by the gene *Prnp*. The normal form of the protein (PrP^C) is expressed in many tissues, but has significant expression in the CNS and lymphoreticular tissues. The PrP^C binds to the cell surface via the glycosylphosphatidylinositol anchor (GPI) (STAHL, 1987; DIAS CORREIA & DIAS CORREIA, 2005), but it is not yet clear what role this GPI plays in TSEs (CHESEBRO et al., 2005).

The central event in prion pathogenicity is the conformational change of PrP^C to an insoluble isoform partially resistant to the protease action that spreads "itself" according to Aguzzi & Calella (2009). Therefore, the causative agent of TSEs, known as prion (proteinaceous infectious particle) is the modified isoform (PrP^{Sc} or PrP^{res}), which is converted by a mechanism where the structural portion of the ·-helix protein is transformed into ¸-sheet (PAN et al., 1993). This structural change is accompanied by changes in the physicochemical properties of PrP^C (PRUSINER, 1997). Studies with genetically modified animals have shown that pathogenic isoforms (PrP^{Sc}) act as a mold that continues reshaping the natural non-pathogenic forms (PrP^C) into new PrP^{Sc} molecules, in a continuous infectious process (PRUSINER, 1998). This continuous protein conversion and aggregation forms large protein complexes in the form of amyloid plaques. Diseases that, as well as the TSEs present amyloid plaques, are capable of reaching the nervous system (AGUZZI, 2009).

Among the diseases currently known, the TSEs are the only diseases with three possible origins: sporadic, genetic or acquired. Studies by Ladogana et al. (2005) in three different continents have shown that approximately 85% of CJD cases occur sporadically, yet its etiology remains unknown. The remaining cases occur through mutations in the *Prnp* gene in family bloodlines or result from some form of positive exposure to TSEs (B...RINGUE et al., 2008).

Another intrinsic characteristic of prion diseases, is the great variability of incubation time and the development of the disease among individuals of the same species. The experiments by Bartz et al. (1998) showed that the intracerebral inoculation of brain extracts from CWD-positive deer in ferrets (*Mustela putorius furo*) led to the development of pathology. When extracts of brain infected ferrets were transferred to other ferrets, the incubation time decreased and the percentage of infected animals increased. Moreover, the homogenized inoculation of CWD-positive ferrets in hamsters (animals known to be resistant to the infection causative agents of TSEs, according to Gibbs et al., 1996), according to Gibbs et al. (1996) led to the development of disease in these animals (BARTZ et al., 1998). Although it is known that different conformations of PrP^{Sc} seem to encode new variants of the disease, it is unclear how prion mutation and adaptation is possible in the absence of nucleic acids (ANGERS et al., 2010). Prion variants (apparently caused by infections in different animal groups and different generations) can result in an increased range of susceptible species, a factor that further complicates assessment of potential risk of new host species (BARTZ et al., 1998).

Chronic Wasting Disease (CWD)

Chronic wasting disease (CWD) is a disorder of the TSE group that affects species of the *Cervidae* family. Demonstrably present in the USA (WILLIAMS & MILLER, 2002), Canada (KAHN et al., 2004) and South Korea (KIM et al., 2005), CWD has already been diagnosed in mule-deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*) and moose (*Alces alces*)**î**, all northern hemisphere species.

The first recorded case of CWD dates back to1967 and affected a mule-deer at a Research Center in Colorado, USA. Initially, it was believed that the disease could be related with the conditions imposed in captivity, such as nutritional deficiency and stress, only in 1978, based on the characteristic neuropathological lesions, the disease was recognized as belonging to the TSE group (WILLIAMS & YOUNG, 1980). The first confirmed record of CWD in wild animals happened in 1981 in a Rocky Mountain elk in Colorado, USA, in a mule-deer in 1985 and in a white-tailed deer in 1990 (WILLIAMS et al., 2002b).

Currently, more than 40 years after the first CWD record, it is known that the weakness attacks animals kept in captivity, as well as animals living in the wild. The current distribution of diagnosed cases includes the hot spots (great incidence of disease) that do not necessarily occupy contiguous areas. The CWD cases recorded in the USA occur on opposite sides of the map, which implies an independent source of the disease or, on the other hand, a common origin that may date to many decades ago (MILLER et al., 2000). The initial course of the disease is not known, if it started with animals in captivity and spread to wildlife or vice-versa, or it developed at the same time in both groups of animals (WILLIAMS & MILLER, 2003).

Although the origin of CWD can not be determined yet, two theories have gained strength (WILLIAMS & MILLER , 2003). The first theory postulates the possibility of the disease to come from contact with animals infected with scrapie (WILLIAMS & MILLER, 2002). Studies by Hamir et al. (2004) reinforce this theory by showing that deer intracerebrally inoculated with scrapie exhibited central nervous system injury that was indistinguishable from animals affected by CWD. The second theory relates the appearance of CWD to a spontaneous mutation of the *Prnp* gene occurred in mule-deer, which led to the emergence of the disease and further dissemination by other animals or due to a non-induced conformational change of the prion protein in its natural form (PrP^C) to a pathogenic form (PrP^{Sc}) (SALMAN, 2003).

Among all mammalian TSEs, CWD is thought to be the most efficiently transmitted and may, in dense free-living populations, achieve 30% transmission and when in captivity, this rate can reach 100%. The forms of disease transmission have not been fully elucidated yet, however, it is believed and has been shown that horizontal transmission take place primarily by direct contact with secretions such as blood and/or saliva (SIGURDSON & AGUZZI, 2007), excreta (urine and feces) (HALEY et al., 2009) and even decaying carcasses (MILLER et al., 2004).

Studies by Fox et al. (2006) show in detail the course of a prion infection in CWD-positive animals that were experimentally intracerebrally inoculated. The animals were monitored and some euthanized within periods that varied between 90 and 785 days after inoculation. The results showed that the accumulation of PrP^{Sc} occurs relatively quick and spreads in the lymphatic tissue, followed by deposition in the central and peripheral nervous tissues and, sporadically in a variety of organs and tissues in the final stages of the disease. The results also showed that despite same deposition sites, the disease progression time varies depending on the genotype of the animal.

Recently, the accumulation of modified prion protein (PrP^{Sc}) in ectopic lymphoid follicles in the kidneys of white-tailed deer experimentally inoculated has been demonstrated. These accumulations of prion protein in excretory pathways fuel the spread of the disease and provide information for understanding the sources of contamination (HAMIR et al., 2006).

The risk of CWD transmission to other animals is still the subject of much discussion. However, studies have not been able to successfully transmit the disease orally in species other than deer (SIGURDSON, 2008). Kong et al. (2005) while studying genetically modified mice that expressed cervid PrP^{C}, successfully transmitted CWD to mice. However, when these animals were genetically engineered to express human PrP^{C}, disease transmission was unsuccessful, thus suggesting the existence of a genetic barrier that prevents human contamination from CWD-positive animals.

Animals affected with CWD have characteristic symptoms such as weight loss, social isolation, hypersalivation, frequent regurgitation, esophageal distension and rarely, ataxia (SIGURDSON & AGUZZI, 2007).

Fox et al. (2006) observed six infected animals and reported that the clinical signs of CWD also included opacity of the eyes, decreased alertness and behavior change between the 442^{nd} and 572^{nd} days after experimental inoculation. Initial clinical signs were subtle and inconsistent, but as the disease progressed, behavioral changes and weight loss became more pronounced and consistent. Some later symptoms in the infection were drooling, polydipsia and polyuria, which were not observed in all cases.

According to Williams & Miller (2002) captive animals affected with CWD also presented repetitive behavior, constant lowering of the head and ears, and also had periods of drowsiness and depression. The weight loss, most commonly reported symptom, seems to be associated with lower amount of ingested food.

Despite the clinical signs of the disease, a definitive diagnosis can only be given after histopathological examination to detect spongiform lesions or by using immunohistochemistry to detect PrP^{Sc} accumulation. In addition to the traditional histopathological and immunohistochemical studies, other methods commonly used for a definitive diagnosis of infection caused by prion agents are ELISA and Western Blotting (BOURNE, 2004; WILLIAMS & YOUNG, 1992).

Genetic susceptibility to CWD

The nucleotide polymorphisms associated with the *Prnp* gene are common in species prone to develop TSE, and these polymorphisms, in turn, are critical with respect to incubation time and susceptibility to prion diseases such as, *scrapie* and the CWD. Disease resistance and susceptibility follow genetic patterns based on different allelic forms encoded by the *Prnp* (GOLDMANN et al., 1990).

The degree of similarity of amino acid sequence of the *Prnp* gene among different species will have consequences on the transmission of TSEs among these species. Often, the same polymorphisms in two species result in similar effects with respect to susceptibility to prion disease (GOLDMANN, 2008).

Studies by O'Rourke et al. (1998) described a polymorphism in the codon 132 of the *Prnp* gene in Rocky Mountain elk. The nucleotide sequence of this codon may, in hetero or homozygous, decode Methionine (M) or Leucine (L). The following year, the genotype of the *Prnp* gene in CWD-negative and CWD-positive animals was determined to check whether this polymorphism would influence susceptibility to CWD. The results showed that 100% (free-living animal) and 74% (captivity animals) of the CWD-positive animals had homozygous 132MM. The cases of heterozygosity for 132ML were not significant for the population in captivity and were not present in free-living animals with CWD-positive. No animal coded as 132LL was CWD-positive (O'ROURKE et al., 1999).

Another widely studied polymorphism, and apparently, related with the susceptibility to develop CWD is codon 225. Mule-deer with heterozygous for serine (S) and phenylalanine (F) (225SF) or homozygous Phenylalanine (225FF) are generally minimally represented in CWD-positive cases. Furthermore, the cases of CWD-positive animals who have homozygous Serine (225SS) are 30 times more frequent compared to 225SF. However, although the data show that the 225F allele has negligible presence in confirmed cases of CWD, the low frequency of this allele in the samples (0.033%) did not allow a definite conclusion (JEWELL et al., 2005).

Genetic analyzes also showed that susceptibility to developing CWD in white-tailed deer is influenced by polymorphisms in codons 95 (which can encode glutamine or histidine) and 96 (which can encode glycine and serine) (JOHNSON et al., 2003;

O'ROURKE et al.,2004). The analysis based only on codon 96 showed that heterozygosity in this codon (96GS) appears less frequently in CWD-positive animals suggesting reduced susceptibility to CWD or slower progress of the disease. When analyzed together, heterozygosity in codon 95 (95QH) was absent in infected animals, regardless of homozygosity or heterozygosity in codon 96 (JOHNSON et al., 2006).

Outside the USA, another study with *Cervus nippon* was performed in Japan. The *Prnp* gene was examined to determine the genotypes of the studied animals. With the exception of three silent mutations in codon 63, 255 and 408, gene sequence was identical to the already known from *O. hemionus*. The well-known codons that are most susceptible to CWD in white-tailed deer (95Q, 96G) and Rocky Mountain Elk (132M) were observed in wild species of *C. nippon*. However, it is not known whether *Prnp* gene polymorphisms are associated with naturally occurring CWD outside the bounds of North America, since there was no indication of CWD-positive cases in any of the tested animals (KATAOKA et al., 2005). In addition to North America, the only reported cases of CWD occurred in South Korea, however, the animals that developed the disease were imported from Canada (KIM et al., 2005).

In Brazil, as well as throughout the neotropical countries, there is no evidence or reported case of deer affected by CWD. There are also no surveys that indicate susceptibility to disease development. Surveys of diseases that affect wildlife and descriptive studies, in general, are still rare, but extremely necessary in neotropical countries. Epidemiological surveillance activities are extremely important to be able to deal with possible consequences for both human and animal health (GORT¡ZAR et al., 2007).

Public Health and CWD

Although some research in order to study the possibility of transmission of prion diseases to humans is being performed, little is known about this subject. Studies have shown a reduced potential risk of CWD being transmitted to humans (BELAY et al., 2004) through the consumption of deer meat (BELAY et al., 2001). Research has pointed out the existence of a possible genetic barrier between the two species, humans and deer, and such an obstacle makes it safe to consume deer meat (RAYMOND et al., 2000).

Even considering that in Brazil the consumption of such meat is limited to sporadic hunters and adventurers, it is important to note that knowledge is even more restricted when it comes to transmission to humans by routes other than oral ingestion. Also in Brazil, the history of forest and field destruction to expand livestock farms, restricts deer natural habitat and approximates the human population, cattle and other livestock to areas formerly inhabited by deer. This approach would increase human exposure to the causative agent of CWD. There are no records about the transmissibility of CWD through human contact with materials such as urine, feces, blood, and placenta of infected animals, but experimental transmission of CWD to primates has occurred as reported by Marsh et al. (2005). CWD transmission to domestic animals - sheep, goats, pigs - is an area of great interest and growing knowledge (SIGURDSON, 2007).

Knowledge and information are, therefore, indispensable so the population can be safe regarding the potential risk of prion infection. Moreover, it is necessary to know the probability of infection of Brazilian animals in order to equip the competent areas for management and conservation of the already endangered species of deer in Brazil and, if necessary, the definition of an action plan to hinder or even prevent the disease entry in Brazil.

In the absence of precise information, and considering the possible risks associated with consumption of venison, a few basic safety procedures are recommended for hunters and even taxidermists, in order to avoid unnecessary exposure to CWD. The meat consumption of animals that exhibit symptoms of prion infections, as well as of parts that are the most acutely affected such as brain, lymph nodes, spleen, tonsils and eyes, even of healthy animals should be avoided. The use of latex gloves and prophylaxis of working equipment, knives, clothes, pots, are also strongly recommended (WILLIAMS & MILLER, 2002).

Management Implications

The lack of healing for animals infected with CWD causes the onset of disease symptoms to be inevitably fatal. The long incubation period, the lack of accurate ante-mortem diagnosis, the enhanced resistance of the infectious agent, contamination *in natura* and lack of knowledge about the disease, increase the difficulty of controlling or eradicating CWD (WILLIAMS et al., 2002b).

Some CWD eradication programs have been developed, but were unsuccessful, and although failure factors have not been properly reported, it is believed that residual contamination of the environment and failed sterilization of study sites were the main reasons (WILLIAMS & YOUNG, 1992, MILLER et al., 1998). However, few zoos where CWD was detected were able to control infections in their facilities. Some basic precautions can help prevention such as, new animals must not be introduced in environments where there are CWD-positive animals, newly acquired animals should be tested for the disease and should be put in quarantine and herds affected by CWD should be eliminated. These measures all contribute to the eradication of the disease in captivity (WILLIAMS et al., 2002).

Controlling CWD in the wild is even more complex than in captivity. However, in endemic areas for the development of the disease, surveillance programs are needed to monitor the distribution and prevalence of

CWD in order to update and extend endemic areas (MILLER & KAHN, 1999).

CONCLUSION

Although CWD is well known in the northern hemisphere, it is still virtually unknown in Brazil. Until the conclusion of this work, only our research group was studying this disease in neotropical cervids. This lack of knowledge can lead to inaccurate diagnosis of the disease and no notification of the disease to the competent sectors, which may ultimately have serious consequences for public health and the deer population as well.

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