

CHRONIC WASTING DISEASE: THE PRION DISEASE OF CERVIDS

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

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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 United 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 Doença 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 Coreia 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. Prions.

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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 Paraná river and Guaporé valley, *Ozotoceros bezoarticus* (pampas deer) in the cerrado region, Pantanal and a relictual population in Paraná, *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 (SZABÓ 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-Jacob 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 α -helix protein is transformed into β -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 (BÉRINGUE 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*)", all northern hemisphere species.

The first recorded case of CWD dates back to 1967 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 442nd and 572nd 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 (GORTÁZAR 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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