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## SHORT COMMUNICATION

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### Are Brazilian cervids at risk of prion diseases?

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**ABSTRACT.** Prion diseases are neurodegenerative fatal disorders that affect human and non-human mammals. Chronic Wasting Disease (CWD) is a prion disease of cervids regarded as a public health problem in North America, and polymorphisms at specific codons in the *PRNP* gene are associated with this disease. To assess the potential CWD susceptibility of South American free-ranging deer, the presence of these polymorphisms was examined in *Mazama gouazoubira*, *Ozotoceros bezoarticus* and *Blastocercus dichotomus*. Despite the lack of CWD reports in Brazil, the examined codons (95, 96, 116, 132, 225, and 226) of the *PRNP* gene showed potential CWD susceptibility in Brazilian deer. Low abundance of deer in Brazil possibly difficult both CWD proliferation and detection, however, CWD surveillance may not be neglected.

**KEYWORDS.** *Blastocercus*, chronic wasting disease, *Mazama*, neotropical deer, *Ozotocercus*, PrP, prion

#### INTRODUCTION

Prion diseases or transmissible spongiform encephalopathies (TSEs), are a group of lethal

neuronal disorders presenting common characteristics. Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats and

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chronic wasting disease (CWD) in cervids, present the same patterns of neuronal vacuums and cell death in the central nervous system.<sup>1</sup> The main event in prion pathogenicity is the conformational alteration from the normal PrP<sup>C</sup> form into a non-normal PrP<sup>Sc</sup>. This isoform is insoluble and partially resistant to proteases. Prion diseases present only the modified isoform (PrP<sup>Sc</sup>), which is converted from  $\alpha$ -helices portions to  $\beta$ -sheets.<sup>2</sup> This structural modification is accompanied by alterations in the physical-chemistry properties of PrP<sup>C</sup>.<sup>3</sup> Studies using transgenic mice have shown that PrP<sup>Sc</sup> acts as a template which transforms PrP<sup>C</sup> into new PrP<sup>Sc</sup> molecules.<sup>4</sup>

Chronic wasting disease is a disorder that affects the Cervidae family, attacking either captive or wild living animals.<sup>5</sup> Among all TSEs, CWD is the most efficiently transmitted and can reach 30% of transmissibility in wild-life. Although not entirely understood, it is supposed that transmission occurs more efficiently in a horizontal way, via direct contact with body secretions,<sup>6</sup> excreta<sup>7</sup> and decomposed carcasses.<sup>8</sup> Prevalence among captive animals can reach ~80% because those animals are kept in restricted areas (research facilities and breeding farms) where the exchange of fluids is constant.<sup>6</sup>

The development of CWD has been commonly associated with polymorphisms at the exon 3 of the *PRNP* gene. Susceptibility and resistance to TSEs follow genetic patterns based on different allelic forms encoded by *PRNP*.<sup>9</sup> Genetic analyses have shown that susceptibility to CWD in white-tailed deer is strongly influenced by polymorphisms at codons 95 and 96.<sup>10,11</sup> Codon 95 encodes Glutamine or Histidine while codon 96 should be Glycine or Serine. An analysis of the codon 96 showed that heterozygosity (G96S) has low frequency in CWD-positive animals, suggesting a reduction on susceptibility or slowdown in disease progress. When both codons (95 and 96) were analyzed together, heterozygosity at codon 95 (Q95H) also presented low CWD-positive frequency, irrespective of homo or heterozygosity in codon 96.<sup>12</sup> In addition, in white-tailed deer, alleles encoding Glycine in codon 116, and alleles encoding Lysine in

codon 226 have been identified as putatively CWD-resistant.<sup>13</sup>

O'Rourke et al.<sup>14</sup> reported a new single nucleotide polymorphism in elks at the codon 132 (M132L) associated with CWD susceptibility. In the following year, the sequence of *PRNP* in CWD-positive and CWD-negative animals was determined to identify any correlation with CWD susceptibility. The results demonstrated that 100% (for wild animals) and 74% (for captive animals) of CWD-positive cases presented 132MM. Animals encoding M132L were not in number significant on captive animals and did not happen on CWD-positive wild living animals. No animals encoding 132LL were CWD-positive.<sup>15</sup> The CWD susceptibility of the M132L and 132MM genotypes was further confirmed by orally dosing with CWD-infected brain elks with the genotypes M132L, 132MM and 132LL.<sup>16</sup>

Another well established polymorphism apparently related to CWD susceptibility is found at codon 225 of the *PRNP* gene. Mule deer heterozygous for Serine (S) and Phenylalanine (F) (S225F) or homozygous for Phenylalanine (225FF) are poorly represented in CWD-positive cases. Conversely, the CWD-positive cases are overrepresented (30 times higher in incidence) on homozygous to Serine (225SS).<sup>17</sup>

The incidence of CWD has been reported in the United States (USA)<sup>18</sup> and Canada<sup>19</sup> in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*) and rocky mountain elk (*Cervus elaphus nelsoni*). Some cases were also reported in South Korea, but the animals were imported from infected farms in Canada.<sup>20</sup> More recently, the first European CWD infection has been reported in Norway in a free-ranging reindeer (*Rangifer tarandus tarandus*).<sup>21</sup> In the USA, CWD is considered an epidemic, whereas in Brazil there are no records of this disease. However, the potential occurrence of CWD should not be overlooked because the white-tailed deer, known to be susceptible to the disease, occurs in sympatry with *Mazama* spp. in the Setentrional Amazon.<sup>22</sup>

The goal of this study was to examine the potential CWD susceptibility in 3 species of Neotropical deer (*Mazama gouazoubira*, *Blastocercus dichotomus* and *Ozotoceros bezoarticus*), based

on the occurrence of polymorphisms in the *PRNP* gene associated with the development of CWD in North American deer. Together with *O. virginianus* and *O. hemionus*, and other new world deer, the species examined in this study form a distinct clade separate from the old-world deer.<sup>23,24</sup>

## RESULTS AND DISCUSSION

Alignment analysis demonstrated that the exon 3 of the *PRNP* gene from the 3 studied Brazilian deer species was highly conserved, and BLASTx (see methods) showed that the amino acid sequences in these species remained very similar or even identical to that of white-tailed deer (97–100% identity). All analyzed animals were homozygous for the 4 loci, encoding Glutamine, Glycine, Methionine and Serine at codon 95, 96, 132 and 225, respectively. All these genotypes have been reported to be associated with CWD-positive cases in white-tailed deer, and other North American deer species.<sup>10-12,14,15,17</sup> Except for one *M. gouazoubira* individual, which was homozygous for the putative CWD-resistant 116G allele, all sequenced animals were 116AA. The putative CWD-resistant 226K allele was not detected in the analyzed Brazilian deer. One *M. gouazoubira* individual, and one *O. bezoarticus* individual showed the 226LL and 226QQ genotypes, respectively, whereas all the other sampled individuals of the 3 species showed the 226EE genotype.<sup>13</sup> These results suggest a potential susceptibility to CWD in Brazilian deer.<sup>22</sup> However, no cases of CWD have been reported in Brazil. The absence of registered cases of CWD-positive animals despite the existence of susceptibility-related genotypes was also identified by Kataoka et al.<sup>25</sup> in *Cervus nippon*. Therefore, the presence of the susceptibility-associated genotypes and the lack of infected animal records in Brazil are of interest because it suggests new putative threats for the conservation of these animals in the Neotropics.

A recent CWD case has been reported in a free-ranging reindeer individual in Norway - the first case ever reported in Europe - raising the question whether it was an extremely rare

(perhaps isolated) case, or whether this is a subtle disease hardly detected in free-ranging animals.<sup>21</sup> If the second scenario is correct, it possibly explains why CWD has never been detected in Brazil, despite potential genetic susceptibility to this disease in Brazilian deer.

The very similar or even identical amino acid sequences observed in the Brazilian deer species here studied, and in CWD-positive white-tailed deer individuals, but the lack of CWD reports in Brazil may suggest the importance of other genetic factors associated with CWD-susceptibility. However, in Brazil, formal attempts to detect CWD have never been performed by governmental authorities, which summed to others factors, perhaps prevented CWD detection in this country. Except for the *Pantanal* region, natural deer populations show extremely low densities and no deer farming exists in Brazil. Thus, the probability of detecting CWD infected animals is very low, and CWD outbreaks may be hampered given the low abundance of most free-ranging deer in Brazil. In contrast, although deer farms are not the cause of CWD, in North America, the high density of animals in these farms have facilitated the occasional detection of novel epidemic foci or have served as harbingers of a low prevalence epidemic in free-ranging species. Although deer have been kept in small-scale captivity for research purposes in Brazil, CWD have never been diagnosed under such conditions. However, because stress or inappropriate raising conditions are high in captivity, premature mortality before CWD development - which has a long incubation period - possibly hinder positive cases.<sup>26</sup> Furthermore, because deer hunting in Brazil is illegal, potential hunters' reports of CWD infected animals - an important source of CWD information in the US where deer hunting is legal - may have been omitted. Also, in contrast to the US, in Brazil deer are found in remote areas far from urban areas, which also reduce chances of detecting infected animals. Another possible factor contributing for the lack of CWD reports in Brazil, despite the evidence of genetic susceptibility to this disease here presented, might be the small number of trained personnel able to identify infected animals.

The potential susceptibility to CWD in Brazilian deer species here reported also raises concerns because deer provide vital protein to subsistence hunters across the Neotropics. Redford<sup>27</sup> estimates that 14 million mammals are killed each year in the Amazonian region, highlighting the staggering extent of subsistence hunting. Although it is not known whether CWD infected animals can transmit the disease to human populations, meat consumption of potentially CWD susceptible deer species by indigenous and traditional peoples, raises an important issue in public health and deserves further investigation. Meanwhile, it is important that public health policies take into consideration the findings here presented to reduce any potential risks of CWD transmission to human populations in South America.

## MATERIALS AND METHODS

Blood samples were collected with EDTA by venopuncture from *Mazama gouazoubira* (gray brocket deer) ( $N = 6$ ); *Ozotoceros bezoarticus* (pampas deer) ( $N = 15$ ) and *Blastocercus dichotomus* (marsh deer) ( $N = 25$ ). Sampling a greater number of deer was not possible given the low abundancy of the studied species along their distribution range, and the high costs involving their capture, which often require the support of a helicopter and a great number of personnel.

Blood samples of the captured animals were stored in ethyl alcohol until DNA isolation. Total DNA was isolated according to Medrano et al.<sup>28</sup> DNA quantification and quality verification was done by agarose gel electrophoresis stained with GelRed (Biotium). A fragment of ~800 bp from the exon 3 of the *PRNP* gene was obtained using primers CWD-13 (5'-TTTTGCAGATAAGT-CATCATGGTGAAA-3' [forward]) and CWD-LA (5'-AGAAGATAATGAAAACAG-GAAGGTTGC- 3' [reverse]) as described by Johnson et al.<sup>10</sup> Polymerase chain reaction amplification conditions included initial denaturation at 95°C for 5 min, amplification with Taq Polymerase (LGC Biotecnologia) for 10 cycles at 95°C (45 sec), 58°C (45 sec), and 72°C (1.5 min) followed by 25 cycles at 95°C (45 sec), 57°C (45

sec), and 72°C (1.5 min), and a final extension at 72°C (5 min) according to Johnson et al.<sup>10</sup> Successful amplifications were verified using a 1.5% agarose gel stained with GelRed. Amplified products were purified using QIAquick PCR Purification Kit (Qiagen). Sequencing of samples was conducted at MacroGen facilities (www.macrogen.com) (Korea) under BigDye™ terminator cycling conditions on an ABI 3730 xl automatic sequencer (Applied Biosystems, USA).

Sequences ( $N = 46$ ) were aligned using BioEdit version 5.0.9,<sup>29</sup> and submitted to the algorithm 'BLASTn' in GenBank to confirm whether they belonged to the exon 3 of the *PRNP* gene. Nucleotide sequences were translated on BioEdit and a BLASTx was performed on GenBank also intending to confirm the nature of the sequences. Polymorphisms at the *PRNP* gene were detected base-to-base by eye, focusing on known polymorphisms related to CWD susceptibility. The polymorphisms mostly associated to CWD occur at codons 95, 96, 132 and 225.<sup>10,11,14,17</sup> In addition, we examined codons 116 and 226, where putative CWD-resistant polymorphisms (116G and 226K) have recently been identified.<sup>13</sup>

## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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