Notas Científicas

Genotypic profile of Pantanal creole sheep regarding susceptibility or resistance to scrapie

Aline Najara Domingos Gonçalves[1], Cleber Oliveira Soares[2], Simone Camargo Sanches[1], Fernando Alvarenga Reis[3] and Grácia Maria Soares Rosinha[2]

[1]Universidade Federal de Mato Grosso do Sul, Faculdade de Medicina Veterinária e Zootecnia, Avenida Senador Felinto Müller, nº 2443, CEP 79070-900 Campo Grande, MS, Brazil. E-mail: aline.ndgoncalves@gmail.com, simonesanches@hotmail.com [2]Embrapa Gado de Corte, Avenida Rádio Maia, nº 830, Zona Rural, CEP 79106-550 Campo Grande, MS, Brazil. E-mail: cleber.soares@embrapa.br, gracia.rosinha@embrapa.br [3]Embrapa Caprinos e Ovinos, Estrada Sobral/Groaíras, Km 4, Caixa Postal 145, CEP 62010-970 Sobral, CE, Brazil. E-mail: fernando.reis@embrapa.br

Abstract – The objective of this work was to determine the genotypic profile specific to scrapie in codons 136, 154, and 171 of the *PRNP* gene of the Pantanal creole sheep. Genomic DNA was extracted from blood samples collected from 66 sheep, and the regions of interest on the DNA strand were amplified by PCR. Five haplotypes were identified: ARR, alanine, arginine, arginine; ARQ, alanine, arginine, glutamine; AHQ, alanine, histidine, glutamine; ARH, alanine, arginine, histidine; and VRQ, valine, arginine, glutamine. The most common genotypes were ARQ/ARQ (27%) and ARR/ARQ (24%). The genotypic profile of the Pantanal creole sheep shows low to moderate susceptibility.

Index terms: genotyping, *PRNP* gene, single nucleotide polymorphisms, transmissible spongiform encephalopathies.

Perfil genotípico de ovinos Crioulos pantaneiros quanto à suscetibilidade ou resistência à scrapie

Resumo – O objetivo deste trabalho foi avaliar o perfil genotípico específico para scrapie, nos códons 136, 154 e 171, no gene *PRNP* de ovinos Crioulos pantaneiros. O DNA genômico foi extraído de amostras de sangue de 66 ovinos, e as regiões de interesse da fita de DNA foram amplificadas por PCR. Foram identificados cinco haplótipos: ARR, alanina, arginina, arginina; ARQ, alanina, arginina, glutamina; AHQ, alanina, histidina, glutamina; ARH, alanina, arginina, histidina; e VRQ, valina, arginina, glutamina. Os genótipos mais frequentes foram ARQ/ARQ (27%) e ARR/ARQ (24%). O perfil genotípico de ovinos Crioulos pantaneiros mostra suscetibilidade baixa à moderada.

Termos para indexação: genotipagem, gene *PRNP*, polimorfismo de nucleotídeo único, encefalopatias espongiformes transmissíveis.

Scrapie is a neurodegenerative, chronic, and fatal disease, from the group of transmissible spongiform encephalopathies, that affects sheep and goats. In Brazil, it was first documented in sheep imported from the United Kingdom and has been sporadically observed since 1978 (Sotomaior et al., 2008). In the state of Mato Grosso do Sul, the first case was reported in 2006 (Martins et al., 2012).

The disease is caused by the accumulation of the abnormal protease-resistant protein, called infectious prion (PrP^{SC}) (Prusiner, 1982). One of the causes of genetic susceptibility to scrapie is the association with polymorphisms in codons 136, 154, and 171, located in exon 3 of the *PRNP* gene (Díaz et al., 2005).

Since raising sheep for meat production is a growing activity in Brazil, it is important to identify animals adapted to the environment. The Pantanal creole sheep is adapted to the adverse conditions of the Pantanal region in the state of Mato Grosso do Sul, and is extensively raised without any selection process and genetic improvement (Vargas Junior et al., 2011a).

Most studies to date have focused on morphometric aspects to define racial patterns and on productive performance compared to exotic breeds, in search of

genetic traits to be included in selection and breeding programs (Costa et al., 2013).

The objective of this work was to determine the genotypic profile specific to scrapie in codons 136, 154, and 171 of the *PRNP* gene of the Pantanal creole sheep.

Between March and April 2011, blood samples were collected from 66 Pantanal creole sheep, with 2 years of age, from a controlled breeding herd, belonging to the genetic conservation center of Embrapa Gado de Corte, in the state of Mato Grosso do Sul, Brazil. The samples were collected via the cranial cephalic vein using BD Vacutainer tubes (BD, São Paulo, SP, Brazil) containing the anticoagulant ethylenediaminetetraacetic acid (EDTA), then were aliquoted in 2.0-mL tubes, labeled, and stored at -80°C for 48 hours.

Genomic DNA extraction was performed from whole blood using the Easy DNA kit (Invitrogen, ThermoFischer Scientific, Waltham, MA, USA). The quality of DNA samples was verified by electrophoresis on 0.8% agarose gels stained with SYBR Gold (Invitrogen, ThermoFischer Scientific, Waltham, MA, USA) and examined in a ultraviolet transilluminator (Loccus Biotecnologia, Cotia, SP, Brazil). Samples were quantified on a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) at the wavelengths of 260 and 280 nm. The samples had a 260/280 ratio between 1.8 and 2.0, which is considered pure, and were diluted to a concentration of 60 ng μL^{-1}.

The region of interest on the DNA strand was amplified by PCR using specific forward and reverse primers (5'-CACATGGTGGTGGAGGCTGG-3' and 3'-GGAGCGAGTGGTGGAGCAAA-5', respectively), developed from the sequence under GenBank accession number M31313.1 (National Center for Biotechnology Information, 2015b). The PCR reaction was performed at a final volume of 50 µL containing 60 ng genomic DNA, 1X buffer [50 mmol L^{-1} Tris, 1.5 mmol L^{-1} $MgCl_2$, 10 mmol L^{-1} KCl, and 50 mmol L^{-1} $(NH_4)_2SO_4$, pH 8.3], 0.5 mmol L^{-1} dNTP, 5.0 pmol of each oligonucleotide primer, and 1.0 U *Taq* DNA polymerase, all from Invitrogen (ThermoFischer Scientific, Waltham, MA, USA), to amplify a 388-base pair (bp) fragment. PCR reactions were initially incubated at 94°C for 3 min for denaturing of the DNA strands, followed by 30 cycles containing three stages each: denaturation at 94°C for 30 s, annealing temperature at 67°C for 45 s, and extension at 72°C for 30 s; the reactions were subjected to a final extension at 72°C for 10 min.

After the confirmation of the amplification of the region, the PCR product was purified and the samples were sequenced. The BigDye Terminator v3.1 kit (Applied Biosystems, ThermoFischer Scientific, Waltham, MA, USA) was used in the sequencing reaction, containing: 1.0 µL forward primer and 1.0 µL reverse primer at 5.0 pmol, in separate reactions; 2.0 µL BigDye; 2.0 µL 5X buffer; and 5.0 µL purified PCR product.

The obtained sequences were analyzed using the Blastn program (National Center for Biotechnology Information, 2015a), in which they were compared to the entry under GenBank accession number M31313.1, to search for homologies. The chromatograms generated by the sequencer were observed with the BioEdit program (Ibis Biosciences, Carlsbad, CA, USA).

The sequences were analyzed individually and manually. Polymorphisms in codons 136 (alanine/valine), 154 (arginine/histidine), and 171 (glutamine/arginine/histidine) were used to determine the genotypes of each animal. The distribution of genotypes and their classification regarding risk of scrapie were carried out according to Dawson et al. (2008).

Single 388-bp bands were amplified by PCR reaction. Considering the polymorphisms at codons 136, 154, and 171, five haplotypes were identified: ARR, alanine, arginine, arginine; ARQ, alanine, arginine, glutamine; AHQ, alanine, histidine, glutamine; ARH, alanine, arginine, histidine; and VRQ, valine, arginine, glutamine. This shows the high genetic variability of the Pantanal creole sheep, in which the most frequent alleles were ARR and ARQ; the ARR allele is present in 39% of the animals analyzed. This result is in agreement with Corbière et al. (2007), who evaluated the pure breed red-faced Manech. In comparison, the Dorper sheep breed has a low frequency – of only 7% – of the ARR allele, whereas the Ile de France and Creole breeds have frequencies of 63 and 65%, respectively (Sotomaior et al., 2008).

In the present study, the VRQ haplotype was detected in only one of the 66 animals analyzed. However, the occurrence of this haplotype was interestingly reported in Corriedale, Dorper, Hampshire, Suffolk, and Ile de France sheep commercialized in Brazil (Lanella et al., 2012).

As for genotypes, the most frequent was ARQ/ARQ. Although this genotype is associated with a moderate risk, outbreaks in some countries suggest that it may provide a risk similar to VRQ (Goldmann, 2008). It is important to note that there are cases, such as those observed in Suffolk sheep and in some French breeds, in which the V_{136} allele is rare. Moreover, the polymorphism that determines the degree of susceptibility is located in codon 171 (Moreno et al., 2008).

A total of 7 out of the 15 possible genotypes for the five alleles observed in the evaluated samples were identified, confirming the dimorphism at codons 136 and 154, and the polymorphism at codon 171.

The seven genotypes detected in codons 136, 154, and 171 of the Pantanal creole sheep were: ARR/ARR, ARR/ARQ, ARR/ARQ, ARR/VRQ, ARQ/ARQ, ARQ/AHQ, and AHQ/ARH. However, Pacheco et al. (2007) observed only four genotypes while conducting a similar survey with both the white and red varieties of the Morada Nova sheep, one of the main hair-sheep breeds in Northeast Brazil.

The genotypic profiling of the Pantanal sheep was carried out by automatic sequencing, which showed that 57% of the analyzed animals were concentrated in the R1 and R2 risk groups (Department for Environment, Food and Rural Affairs, 2010), in which the possibility of developing the disease is low.

The ARR/ARR genotype, responsible for maximum genetic resistance (Nodelijk et al., 2011), was found in 12 animals. However, the VRQ/VRQ homozygous profile, considered the most susceptible to scrapie, was not observed in any of the assessed samples.

Although the expression of ARQ/ARQ was common, as in many breeds, this genotype was not observed by Wisniewska & Mroczkowski (2009) in Ile de France sheep. Lanella et al. (2012) also analyzed Pantanal creole sheep and found genotypic frequencies of 7.8 and 30.8% for genotypes ARQ/AHQ and ARQ/ARQ, respectively, whereas, in the present study, the frequencies were of 12.2 and 24.24%; these genotypes are considered of moderate risk of developing scrapie (Department for Environment, Food and Rural Affairs, 2010).

Considering the efforts of the sheep industry to become a stable and profitable activity, the results obtained in the present study contribute so that this disease does not become a barrier to the strengthening of the evaluated genetic group, which presents excellent qualities. These qualities include, for example, the fact that females do not present seasonal anestrus and may have two deliveries a year; and that lambs exhibit quadratic body growth similar to that of major exotic breeds, which are genetically improved for the traits longitudinal, girth, trochanter, and leg lengths (Vargas Junior et al., 2011b).

In conclusion, the genotypic profile of the Pantanal creole sheep was found to be of low to moderate susceptibility to scrapie. Information obtained from genotyping may help direct selection and breeding in an attempt to establish controlled herds with a higher frequency of the ARR/ARR genotype. In this context, the present study may be considered a starting point for further researches involving these animals, which are not only important economically but also for the conservation of genetic resources.

Acknowledgments

To Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (Fundect) and to Financiadora de Estudos e Projetos (Finep), for financial support; and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for grant.

References

CORBIÈRE, F.; BARILLET, F.; ANDRÉOLETTI, O.; FIDELLE, F.; LAPHITZ-BORDET, N.; SCHELCHER, F.; JOLY, P. Advanced survival models for risk-factor analysis in scrapie. **Journal of General Virology**, v.88, p.696-705, 2007.

COSTA, J.A.A. da; EGITO, A.A. do; BARBOSA-FERREIRA, M.; REIS, F.A.; VARGAS JUNIOR, F.M.; SANTOS, S.A.; CATTO, J.B.; JULIANO, R.S.; FEIJÓ, F.L.D.; ÍTAVO, C.C.B.F.; OLIVEIRA, A.R. de; SENO, L.O. Ovelha Pantaneira, um grupamento genético naturalizado do estado de Mato Grosso do Sul, Brasil. In: CONGRESO LATINOAMERICANO DE ESPECIALISTAS EN PEQUEÑOS RUMIANTES Y CAMÉLIDOS SUDAMERICANOS, 8., 2013, Campo Grande. **Anais**. Campo Grande: UCDB/ALEPRYC´s, 2013. p.25-43.

DAWSON, M.; MOORE, R.C.; BISHOP, S.C. Progress and limits of PrP gene selection policy. **Veterinary Research**, v.39, p.1-12, 2008. DOI: 10.1051/vetres:2007064.

DEPARTMENT FOR ENVIRONMENT, FOOD AND RURAL AFFAIRS. **National scrapie plan for Great Britain**. 2010. Available at: <http://webarchive.nationalarchives.gov.uk/20130402151656/http://archive.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/bse/othertses/scrapie/nsp.htm>. Accessed on: 28 Aug. 2013.

DÍAZ, C.; VITEZICA, Z.G.; RUPP, R.; ANDRÉOLLETI, O.; ELSEN, J.M. Polygenic variation and transmission factors involved in the resistance/susceptibility to scrapie in a Romanov flock. **Journal of General Virology**, v.86, p.849-857, 2005. DOI: 10.1099/vir.0.80412-0.

GOLDMANN, W. *PrP* genetics in ruminant transmissible spongiform encephalopathies. **Veterinary Research**, v.39, 2008. DOI: 10.1051/vetres:2008010.

LANELLA, P.; MCMANUS, C.M.; PAIVA, S.R. PRNP haplotype and genotype frequencies in Brazilian sheep: issues for conservation and breeding programs. **Research in Veterinary Science**, v.93, p.219-225, 2012. DOI: 10.1016/j.rvsc.2011.06.025.

MARTINS, H.M.; CARVALHO, N.M.; RIBAS, N.L.K.S.; DRIEMEIER, D.; LEMOS, R.A.A.; GUIMARÃES, E.G. Scrapie e seu diagnóstico diferencial em ovinos no Mato Grosso do Sul. **Pesquisa Veterinária Brasileira**, v.32 p.1230-1238, 2012. DOI: 10.1590/S0100-736X2012001200004.

MORENO, C.R.; COSSEDDU, G.M.; SCHIBLER, L.; ROIG, A.; MOAZAMI-GOUDARZI, K.; ANDREOLETTI, O.; EYCHENNE, F.; LAJOUS, D.; SCHELCHER, F.; CRIBIU, E.P.; LAURENT, P.; VAIMAN, D.; ELSEN, J.M. Identification of new quantitative trait loci (other than the PRNP gene) modulating the scrapie incubation period in sheep. **Genetics**, v.179, p.723-726, 2008. DOI: 10.1534/genetics.108.088146.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. **BLAST**: basic local alignment search tool. Available at: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. Accessed on: 16 Jun. 2015a.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. **Ovis aries prion protein (PrP) gene, complete cds**. Available at: <http://www.ncbi.nlm.nih.gov/nuccore/M31313.1>. Accessed on: 16 Jun. 2015b.

NODELIJK, G.; ROERMUND, H.J.W. van; VAN KEULEN, L.J.; ENGEL, B.; VELLEMA, P.; HAGENAARS, T.J. Breeding with resistant rams leads to rapid control of classical scrapie in affected sheep flocks. **Veterinary Research**, v.42, 2011. DOI: 10.1186/1297-9716-42-5.

PACHECO, A.C.L.; OLIVEIRA, S.M.P.; GOLVEIA, J.J.S.; DINIZ, M.C.; VASCONCELOS, E.J.R.; VIANA, D. de A.; ROSINHA, G.M.S.; MAGGIONI, R.; COSTA, R.B.; OLIVEIRA, D.M. Analysis of prion protein gene (*PRNP*) polymorphisms in healthy Morada Nova sheep reveals the presence of genotypes susceptible to scrapie. **Ciência Animal**, v.17, p.27-36, 2007.

PRUSINER, S.B. Novel proteinaceous infectious particles cause scrapie. **Science**, v.216, p.136-144, 1982. DOI: 10.1126/science.6801762.

SOTOMAIOR, C.S.; SOTOMAIOR, V.S.; MADEIRA, H.M.F.; THOMAZ-SOCCOL, V. Prion protein gene polymorphisms in sheep in the state of Paraná, Brazil. **Animal Genetics**, v.39, p.659-661, 2008. DOI: 10.1111/j.1365-2052.2008.01775.x.

VARGAS JUNIOR, F.M. de; LONGO, M.L.; SENO, L. de O.; PINTO, G. dos S.; BARBOSA-FERREIRA, M.; OLIVEIRA, D.P. de. Potencial produtivo de um grupamento genético de ovinos nativos Sulmatogrossenses. **PUBVET**: Publicações em Medicina Veterinária e Zootecnia, v.5, n.30, art. 1197, 2011a.

VARGAS JUNIOR, F.M. de; MARTINS, C.F.; SOUZA, C.C.; PINTO, G. dos S.; PEREIRA, H.F.; CAMILO, F.R.; AZEVEDO JUNIOR, N.P. de. Avaliação biométrica de cordeiros pantaneiros. **Revista Agrarian**, v.4, p.60-65, 2011b.

WISNIEWSKA, E.; MROCZKOWSKI, S. Different breeding strategies for scrapie resistance depending on breed-specific PrP allele and genotype frequencies in the Polish sheep. **Züchtungskunde**, v.81, p.180-189, 2009.

Received on November 29, 2014 and accepted on February 25, 2016