

PRION PROTEIN GENE POLYMORPHISMS AND ACCUMULATION OF PATHOGENIC PRION PROTEIN (PrP^{Sc}) IN A HERD WITH PREVIOUSLY CONFIRMED SCRAPIE CASES

POLIMORFISMOS DO GENE DA PROTEÍNA PRIÔNICA CELULAR (PRNP) E DISTRIBUIÇÃO DA PROTEÍNA PRIÔNICA ALTERADA (PrP^{Sc}) EM REBANHO COM SURTO ANTERIOR DE SCRAPIE

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ABSTRACT: Scrapie in sheep is associated with at least three polymorphisms in the prion protein gene (PRNP) on codons 136, 154, and 171. Countries where scrapie is endemic have been using breeding programs based on selection for the most resistant alleles. There are some PRNP genotyping data on sheep in Brazil, and scrapie has sporadically been observed since 1978. Paraná is the Brazilian state where most of the cases of scrapie have been diagnosed. A flock that had three clinical scrapie cases in 2003 and 2004 was genotyped (128 sheep: 53 pure Hampshire Down and 75 crossbred) and slaughtered (111 sheep: 47 pure Hampshire Down and 64 crossbred) in 2006. Samples of lymphoid and central nervous tissues were examined by immunohistochemistry (IHC) for altered prion protein (PrP^{Sc}). Six genotypes were detected in the 128 genotyped animals: ARR/ARQ was the most frequent (45.3%), followed by ARQ/ARQ (28.1%), ARR/ARR (14.1%), and ARQ/VRQ (8.6%). ARR/VRQ and ARQ/AHQ showed less than 2.5% genotype frequency. IHC identified 16 positive sheep. Palatine tonsil tissue had the highest percentage of reactive samples: 81.25% of the total positive samples. Of these 16 positive animals, nine (56.25%) had genotype ARR/ARQ, five (31.25%) had genotype ARQ/ARQ, and the remaining two (12.5%) had genotype ARQ/VRQ. All the positive animals were clinically healthy, and therefore represented 14.14% of pre-clinical cases of scrapie in this flock.

KEYWORDS: *Ovis aries*. Prion. Immunohistochemistry. Genotyping.

INTRODUCTION

Scrapie is a fatal, neurodegenerative disease that affects sheep and goats and belongs to the group of transmissible spongiform encephalopathies (TSE) or prion diseases. In these prion diseases the normal cellular form of the prion protein (PrP^C) undergoes a conformational change to the infectious form (PrP^{Sc}). The deposition of PrP^{Sc} in tissues of the central nervous system and lymphoreticular system characterizes the disease (PRUSINER, 1998).

For sheep exposed to the scrapie infectious agent, allelic variation at codons 136 (alanine, A/valine, V), 154 (histidine, H/arginine, R), and 171 (histidine, H/glutamine, Q/arginine, R) of the prion protein gene (PRNP) is associated with an increased risk of disease development (HUNTER et al., 1989, 1997a,b; BELT et al., 1995; DAWSON et al., 1998). It is well established that the ARR/ARR genotype's susceptibility to the agents causing scrapie is extremely low, although no longer considered null (GROSCHUP et al., 2007). On the other hand, the

VRQ allele is considered to be associated with high susceptibility (HUNTER et al., 1997a; ELSER et al., 1999; ACÍN et al., 2004). The PRNP genotype also affects the pathogenesis of scrapie and the tissue levels and distribution of PrP^{Sc} in affected sheep. For instance, in ARR/VRQ sheep, there is little or no involvement of the lymphoid tissue in agent replication (ANDRÉOLETTI et al., 2000; VAN KEULEN et al., 2008).

Even though the first record of scrapie in Brazil occurred in a Hampshire Down ovine imported from England (FERNANDES et al., 1978), and other cases were reported sporadically (RIBEIRO, 1996; DRIEMEIER, 1998) or notified at World Organization for Animal Health - OIE (<http://www.oie.int>), the clinical cases in 2003 and 2004 (POHL DE SOUZA et al., 2005) from the flock reported here were the first cases considered autochthonous in Brazil. After this, other scrapie cases have been reported and published (<http://www.oie.int>; RIBEIRO et al., 2007; ANDRADE et al., 2011). Some of these cases were on the animals from Paraná State (SOTOMAIOR et

al., 2012).

The ovine population of Paraná totals about 639,000 animals. Although it represents about 4.5% of the Brazilian sheep population, the Paraná sheep population increased 27.5% from 2006 to 2012 (IBGE, 2012). Several foreign breeds are kept in Paraná such as Suffolk, Hampshire Down, Ile de France, Texel, and in recent years, Dorper. Other indigenous Brazilian breeds such as Santa Inês (McMANUS et al., 2010) are also bred and mixed with the former mentioned breeds. In these breeds, seven different genotypes have been reported in sheep from Paraná (SOTOMAIOR et al., 2008), the most frequently found one being ARQ/ARQ (41%), followed by ARR/ARQ (29%).

Although plenty of data demonstrate the association between genotype and resistance or susceptibility to scrapie, differences among breeds and herds are evident, indicating that the use of uniform criteria for selection cannot necessarily be applied for different sheep breeds, flocks, or even countries (LÜHKEN et al., 2004; ACÍN et al., 2004). In Brazil, although there is not yet a breeding program to control scrapie, some data on PRNP polymorphisms have been published (LIMA et al., 2007; PACHECO et al., 2007; SOTOMAIOR et al., 2008; PASSOS et al., 2008; ANDRADE et al., 2011; IANELLA et al., 2012; SANTOS et al., 2012). These articles mainly give the percentages of the different polymorphisms, but do not make any kind of association with the presence of the PrP^{Sc}.

The purpose of this study was to retrospectively analyze the PRNP polymorphisms found in an ovine flock in which three animals had been diagnosed in 2003 and 2004 with the classical form of scrapie, and to determine if these polymorphisms are associated with the presence of PrP^{Sc}. Since the whole flock was slaughtered in 2006, we were able to examine lymphoid and central nervous tissues by immunohistochemistry (IHC) for altered prion protein (PrP^{Sc}).

MATERIAL AND METHODS

Flock

All 128 animals studied belonged to the sheep-breeding herd of the Pontifical Catholic University of Paraná (PUCPR) Experimental Farm located in the city of Fazenda Rio Grande, State of Paraná, Brazil. This flock consisted of two groups of animals: 53 pure Hampshire Down (HD) animals and 75 crossbred (CR) animals, from different breeds such as Texel, Ile de France, Hampshire Down, and even animals with no recognizable breed. Additionally, there were 5 crossbred animals

of the Creolle (2), Polwarth (1), Corriedale (1), and Karakul (1) breeds. Pure HD rams were used in crossings with the crossbred females. In the purebred flock, except for four older ewes (born between 1997 and 1999) and the rams, all other animals had been born at PUCPR. As for the 75 crossbred animals, 33 (44%) of them had been bought from other farms, during 2002 and 2003, and the remaining animals had been born at PUCPR.

Flock history

Until January 2003, no animal had presented signs compatible with scrapie. After this time point, between 2003 and 2004, three animals (females, aged between 6 and 6 ½ years, Hampshire Down) with clinical signs were isolated in the veterinary hospital of PUCPR, the official public veterinary service was informed, and sick animals were euthanized after observation (Pohl de Souza et al., 2005; http://www.agricultura.gov.br/arq_editor/file/Aniamal/programa%20nacional%20dos%20herbivoros/SCRAPIE.pdf). These three animals were officially diagnosed with classical scrapie. After almost 3 years, genotyping and immunohistochemistry were performed on the whole flock in 2006, after the ending of a legal dispute.

DNA extraction and purification

DNA was extracted from frozen whole blood samples. Four mL of blood was collected from the external jugular using Vacutainer[®] tubes with ethylenediamine tetraacetic acid (EDTA) as an anticoagulant. DNA extraction and purification was performed using proteinase K and the phenol/chloroform method.

PCR-RFLP

For the analysis of restriction fragment length polymorphisms (RFLP), polymerase chain reaction (PCR) was performed on the DNA samples according to the methods described in Lühken et al. (2004). For amplification of the DNA fragments, reactions were performed in a total volume of 30 µL, using 1X reaction buffer, 1.5 mM magnesium chloride, 0.2 mM dNTP, 10 pmol of each primer, 0.6 U *Taq* DNA Polymerase, and 2.0 µL of sample DNA (variable concentration). PCR amplification conditions were as follows: an initial denaturation temperature of 94°C for 60 s, followed by 40 cycles at 94°C for 20 s, 55°C for 20 s, 72°C for 20 s, and a final extension at 72°C for 5 min. In both PCR reactions, the forward primers were the same (5'-TGTGGCAGGAGCTGCTGCAGCT-3'). The reverse primers were 5'-

TGCACAAAGTTGTTCTGGTTACTATC-3' (RP1), and 5'-GCACAAAGTTGTTCTGGTTACTATAT-3' (RP2), for the first and second reactions, respectively. The amplified regions correspond to nucleotides 342 to 539 (first reaction) and nucleotides 342 to 538 (second reaction) of the PRNP, with lengths of 197 and 196 base pairs (bp) respectively.

Amplified products from each reaction were digested with restriction enzymes *BspDI* and *BspHI*. The first PCR product (197 bp) was cleaved with *BspHI*, while the second (196 bp) was doubly digested with *BspDI* and *BspHI*. Fifteen μ L reactions (1X buffer, 2.5 U enzyme, 8 μ L PCR product, and ultra-pure water) were kept in an oven at 37°C overnight (12h on average). Subsequently, the total volume of the cleavage reaction was separated by electrophoresis in a 2.5% agarose gel prepared with TBE buffer. The electrophoretic run was carried out at 35 V for 6 h. Following staining with ethidium bromide (0.5 μ g/mL), the gel image was processed using Gel-Pro Analyzer 4.0 software (Media Cybernetics, Rockville, MD) for genotype analysis.

Amplification with primer RP1 creates an artificial restriction site for *BspHI*, when the codon for histidine is at position 171. Similarly, amplification with RP2 creates an artificial restriction site for *BspDI*, when the codon for arginine is at position 171. In both the fragments, the codon for valine at position 136 and that for histidine at position 154 are restriction sites for *BspHI*. Therefore, digestion of the 197 bp PCR product with *BspHI* and the double digestion of the 196 bp product with *BspHI* and *BspDI* enzymes generate two band patterns that together define the genotypes of the PRNP for analysis of their alleles.

Diagnosis of PrP^{Sc} by immunohistochemistry

Immunohistochemistry (IHC) was performed in 111 sheep (17 had died of different causes). Those 111 animals were slaughtered in 2006, and their organs were collected for subsequent analysis to determine the presence of PrP^{Sc} through IHC. The collected organs included the brain, the third eyelid, the tonsils, the ileum, and the spleen.

The fragments were kept in 10% formaldehyde and analyzed by immunohistochemistry using monoclonal antibody F89/160.1.5 (O'Rourke et al., 1998) according to the protocol used by the Federal University of Rio Grande do Sul. Samples were treated with

proteinase K (DAKO, Ready-to-use) diluted in distilled water (1:1) to eliminate the PrP^C isoform. Positive and negative controls were available.

Statistical analysis

Statistical analysis of the relationships between the different genotypes and the presence or absence of PrP^{Sc} was performed by building contingency tables and using the chi-square (χ^2) method, with the Yates correction and the Fisher's exact test. Additionally, we used the Z test to compare proportions between the different genotypes in the Hampshire Down animals and in the crossbred animals.

RESULTS

Genotyping

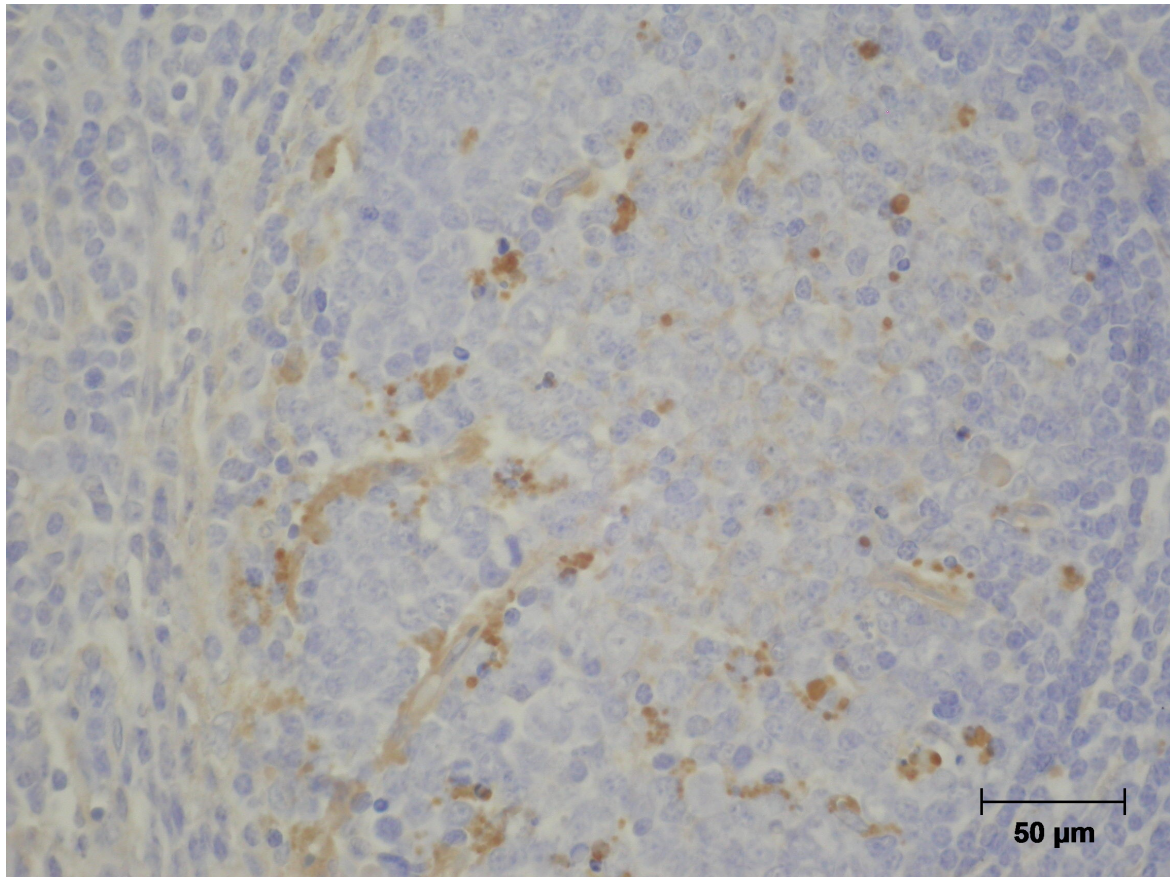
Six genotypes were found in the 128 genotyped animals: ARR/ARR, ARR/ARQ, ARQ/ARQ, ARR/VRQ, ARQ/VRQ, and ARQ/AHQ (Table 1). The genotype ARR/ARQ was the most frequent (45.3%) followed by ARQ/ARQ (28.1%), ARR/ARR (14.1%), and ARQ/VRQ (8.6%). ARR/VRQ and ARQ/AHQ had a genotype frequency of less than 2.5%. When comparing crossbred animals (CR) with Hampshire Down (HD) animals, CR showed a greater percentage of ARR/ARR genotypes than HD (21.3% vs. 3.8%, respectively), the same with ARQ/VRQ (2.7% vs. 1.9%) and ARQ/AHQ (2.7% vs. 0%). The HD breed had greater proportion of the genotypes ARR/ARQ (49.1% vs. 42.7%), ARQ/ARQ (32.1% vs. 25.3%) and ARQ/VRQ (13.2% vs. 5.3). Results from 25 samples were validated by DNA sequencing (data not shown), and in all cases, the results confirmed the genotype suggested by RFLP analysis.

PrP^{Sc} diagnosis by immunohistochemistry

The results of the IHC analyses of the 111 animals of the infected flock showed that the 95 samples (85.6%) were negative for the presence of PrP^{Sc} in all the examined organs and tissues. Sixteen animals (14.4%) were positive for one or more tissues, 8 of which were pure Hampshire Down (17% of 47 pure animals examined) and 8 were crossbred (12.5% of 64 crossbred animals examined). Figure 1 shows the labeling by IHC in the tonsil of one of the positive animals. It is important to observe that at the moment that they were killed, no animals presented any clinical signs.

Table 1. Number of animals (n), according to genotype and breed of the scrapie-infected flock

Breed	Genotype					
	ARR/ARR	ARR/ARQ	ARQ/ARQ	ARR/VRQ	ARQ/VRQ	ARQ/AHQ
Hampshire Down	2	26	17	1	7	0
Crossbred	16	32	19	2	4	2
Total	18	58	36	3	11	2

**Figure 1.** Immunohistochemical assay of a tonsil lymphoid follicle showing positive PrP^{Sc} immunolabeling, Scale bar = 50 μm .

The 16 positive sheep showed differences in their reactive organs and tissues (Table 2). No animal was positive for all the tissues examined, and tonsil was the tissue with the highest percentage of reactive samples: 81.25%. Only one animal was positive in the brain (6.25%). The average age was 4.3 years, ranging from 3 years up to 7 years. Only one (6.25%) of the positive samples was from a male, but males represented less than 15% of the total animals examined. From the 16 positive animals, 5 (31.25%) were not born in the PUCPR's farm (Table 2).

Relationship between genotyping and IHC

When the 16 positive samples by IHC are compared to the animals' genotype, it is possible to

observe that animals from three genotypes were positive for PrP^{Sc} . The highest percentage of positive animals had the ARR/ARQ genotype, representing 56.25% of the positive samples ($n = 9$), followed by the ARQ/ARQ genotype with 31.25% ($n = 5$). Genotype ARQ/VRQ represented 12.5% of the PrP^{Sc} positive animals ($n = 2$). In genotypes ARR/ARR, ARR/VRQ, and ARQ/AHQ, there were no positive cases (Table 3).

When we compare the proportion of each genotype in the total number of positive and negative samples (Table 3), there was a significant difference only for genotype ARR/ARR. The frequencies of genotypes ARR/VRQ and ARQ/AHQ were insufficient for comparison.

Table 2. Immunohistochemistry (IHC) results from 16 positive animals for the presence of PrP^{Sc}, according to breed (Hampshire Down (HD) and crossbred (CR)), genotype, positive tissues, year of birth, and location of birth

Breed	Genotype	Positive tissues in IHC	Year of birth	Location of birth
HD	ARQ/ARQ	Spleen and brain	1999	Other
HD	ARQ/ARQ	Spleen and tonsil	2002	PUCPR
HD	ARQ/ARQ	Spleen and third eyelid	2003	PUCPR
HD	ARR/ARQ	Tonsil	2003	PUCPR
HD	ARR/ARQ	Spleen and ileum	2003	PUCPR
HD	ARR/ARQ	Tonsil	2003	PUCPR
HD	ARQ/VRQ	Tonsil	2001	PUCPR
HD	ARQ/VRQ	Tonsil	2001	PUCPR
CR	ARQ/ARQ	Tonsil	1999	Other
CR	ARQ/ARQ	Tonsil	2002	PUCPR
CR	ARR/ARQ	Tonsil	1999	Other
CR	ARR/ARQ	Spleen and tonsil	2001	PUCPR
CR	ARR/ARQ	Tonsil	2001	Other
CR	ARR/ARQ	Tonsil	2001	Other
CR	ARR/ARQ	Tonsil	2002	PUCPR
CR	ARR/ARQ	Tonsil and ileum	2003	PUCPR

HD = Hampshire Down CR = crossbred

In the chi-square (χ^2) method, we could not find an influence by genotype in the positive and negative animals, when considering all data together (HD + CR), or only HD breed data. For the crossbred animals, there was a statistically significant influence of genotype in the IHC result.

The ARR/ARQ genotype was significantly different ($p < 0.05$) from the other genotypes. The ARR/ARQ genotype corresponds to 39.3% of the negative and to 75% of the positive results by IHC (Table 3). The proportional differences between the remaining genotypes were not statistically significant.

Table 3. Comparison of animals with positive (+) and negative (-) immunohistochemistry (IHC) for the presence of PrP^{Sc} by genotype of Hampshire Down (HD) and crossbred (CR) ovine in the PUCPR flock

Genotype	HD	CR	Total	HD	CR	Total
	+ IHC n (%)	+ IHC n (%)	+ IHC n (%)	- IHC n (%)	- IHC n (%)	- IHC n (%)
ARR/ARR	0 (00.0)	0 (00.0)	0 (00.0)	2 (05.1)	16 (28.6)	18 (18.9)
ARR/ARQ	3 (37.5)	6 (75.0)	9 (56.2)	19 (48.7)	22 (39.3)	41 (43.2)
ARR/VRQ	0 (00.0)	0 (00.0)	0 (00.0)	1 (02.6)	2 (03.6)	3 (03.2)
ARQ/ARQ	3 (37.5)	2 (25.0)	5 (31.3)	12 (30.8)	11 (19.6)	23 (24.2)
ARQ/VRQ	2 (25.0)	0 (00.0)	2 (12.5)	5 (12.8)	3 (05.3)	8 (08.4)
ARQ/AHQ	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	2 (03.6)	2 (02.1)
Total number of animals	8 (100.0)	8 (100.0)	16 (100.0)	39 (100.0)	56 (100.0)	95 (100.0)

DISCUSSION

The clinical cases of scrapie of the flock reported here were the first notification of scrapie in native, not imported animals, in a Brazilian flock of sheep

(http://www.agricultura.gov.br/arq_editor/file/Aniamal/programa%20nacional%20dos%20herbivoros/S CRAPIE.pdf) and the further genotyping and immunohistochemistry of the whole flock allowed us to conduct a retrospective study relating genotype and resistance to scrapie in an ovine flock in Brazil.

These cases also led the Brazilian Ministry of Agriculture to reconsider categorizing scrapie as an exotic disease in the country. The disease is now part of the National Encephalopathy Program. Nowadays, in cases of suspect alive animals, samples from third eyelid or other lymphoid tissue are examined. All positive IHC for PrP^{Sc} are considered a scrapie-positive diagnosis (BRASIL, 2008).

In the flock studied here, up to the moment the animals were killed, none had presented any clinical signs. However, out of the 111 animals slaughtered and tested, 16 were positive for scrapie (14.4% of the total). The concern about the presence of such asymptomatic PrP^{Sc} carrier sheep is that they may transmit the disease agent for long periods, before clinical cases appear in the flock. Billinis et al. (2004), also testing healthy animals in a herd where there were positive cases of scrapie, found 25% of animals in the latent phase (without clinical signs) compared to the clinically healthy. Tongue et al. (2005), in a survey of the prevalence of PrP^{Sc} in 14 herds whose animals were slaughtered after the confirmation of cases of scrapie, estimated a prevalence of 6.6%, ranging from zero to 15.4%, in clinically healthy appearing animals.

In cases of classical scrapie, infection occurs by the oral route via infection of the Peyer's patches followed by replication in the gut-associated lymphoid tissues (GALT). It may then spread to the central nervous system through the autonomic nervous fibers innervating the digestive tract (ANDRÉOLETTI et al., 2000). Environmental contamination can also occur through the placenta and infected fetal fluids (ANDRÉOLETTI et al., 2002; DETWILER; BAYLIS, 2003; TOUZEAU et al., 2006). It is known that during the scrapie incubation period, ewes with the susceptible genotypes accumulate large quantities of PrP^{Sc} and may transmit the agent through the placenta even in the first gestation, well before the beginning of the clinical signs of the illness (ANDRÉOLETTI et al., 2002). There is also the possibility of milk transmission, as described by Konold et al. (2008). The environmental contamination in the case of this flock reported here could have been caused by the three ewes, earlier diagnosed with scrapie, since they gave birth many times before the appearance of any clinical signs. Thus, the infection of all the animals born on the farm probably occurred soon after their birth, due to the contaminated environment. However, there were also positive animals from the group that was bought from other farms, and were contaminated only as adults. Working with the introduction of adult, scrapie-free

ovine in contaminated flocks, Ryder et al. (2004) showed the possibility of horizontal transmission in adult animals. Their work also shows that, even if young animals are more susceptible to scrapie than adult animals, sheep are susceptible to infection at different ages, including adulthood. Such data corroborate the results found in the present work, in which animals born in other farms and introduced in the contaminated flock were positive by IHC. In the crossbred flock, 33 (44%) were over 1-year old when they were brought into the flock. Four of them were positive by IHC, indicating that these animals (12.12%) were probably infected when they were adults. When adult animals are infected, the slower PrP^{Sc} dissemination would be due to the important role of the ileal Peyer's patch. In experimental infections, Heggebo et al. (2003) showed that 5 weeks after the challenge, lambs with the susceptible genotypes already presented coloration in the Peyer's patch, indicating the important role of that lymphoid tissue in the absorption and dissemination of the scrapie agent. If infection occurs after the involution of this organ, it may be less effective and lead to slower development of the illness (ERSDAL et al., 2003).

Of the tissues and organs examined from the 16 confirmed cases, the tonsil presented the highest positive reaction percentage (81.25%). Schreuder et al. (1998) and Andréoletti et al. (2000) report PrP^{Sc} accumulation in biopsies of tonsils, independent of the animal's age. It is interesting to observe that one animal presented a positive result from the obex, without any clinical signs. In the clinical phase of the illness, Andréoletti et al. (2000) found PrP^{Sc} not only in the central nervous system (CNS) but also in the lymphoid tissues of VRQ/VRQ animals. However, in ARR/VRQ animals, the PrP^{Sc} deposition occurred only in the CNS. Other authors have reported similar data in natural infections in Texel ovine (Van KEULEN et al., 1996; SCHREUDER et al., 1998), which led them to suggest that ovine carrying an ARR allele would not accumulate PrP^{Sc} in the lymphoid tissues (ANDRÉOLETTI et al., 2000; Van KEULEN et al., 2008). These data are not in accordance with the results found in the present work, which showed that 56.25% of the positive results were from animals heterozygous for the ARR allele, and that all had positive signals in the tonsil or spleen. Ersdal et al. (2003) also found a scrapie positive ARR/VRQ lamb in the Peyer's patch on day 86 of life, the same age found for another VRQ/VRQ lamb, indicating that even in the presence of the ARR allele, there can be prion accumulation in the lymphoid tissue.

In the analysis of the genotypes associated with PrP^{Sc} accumulation, the presence of PrP^{Sc} in ARR/ARR, which represented 14.1% of animals, was not found. Although there are confirmed cases of ARR/ARR animals with classical scrapie (Groschup et al., 2007), the susceptibility of the ARR/ARR genotype to classical scrapie is considered very low (Hunter et al., 1997a; Elsen et al., 1999; Acín et al., 2004).

Analyzing the data on pure-HD animals, the genotypes with positive cases were ARQ/ARQ (37.5% of the cases), ARR/ARQ (37.5%), and ARQ/VRQ (25%). In the HD breed, as in the Suffolk breed, alanine is the amino acid most frequently found at codon 136 (Dawson et al., 1998), and the presence of the VRQ allele is absent or rare. In sheep from Paraná, Sotomaior et al. (2008) found 2% of ARR/VRQ and 10% of ARQ/VRQ genotypes in the HD breed. In other Brazilian HD breed samples, Passos et al. (2008) found a frequency of 6% for allele VRQ and Ianella et al. (2012) also found the ARR/VRQ genotype (4.2%). Andrade et al. (2011), working with Suffolk, found the VRQ allele at a frequency of 3%. Therefore, in these breeds, the ARQ allele is considered to be the most susceptible to scrapie (HUNTER et al., 1997a; DAWSON et al., 1998), as seen in this study, where all the positive animals were at least heterozygous for the ARQ allele.

In the analysis of the crossbred animals, although there was no significant difference, there were more positive than negative cases within the ARQ/ARQ genotype, as noted in the HD breed. However, when the ARQ allele is associated with the ARR allele, this genotype represents 75.0% of the cases positive for the presence of PrP^{Sc} and 39.3% of the cases of negative animals ($p < 0.05$). These results differ from that usually presented in the literature (O'DOHERTY et al., 2002; BAYLIS et al., 2004) about the influence of alleles, which must be studied more deeply. The fact that the animals in the present study come from the crossbreeding of various breeds may have

contributed to these results, since there are differences in genotype susceptibility in different breeds. Authors working with the Texel breed found the ARQ allele to be codominant with the VRQ allele, and therefore did not grant protection (Belt et al., 1995), but this is contrary to data from the Ile de France breed (LAPLANCHE et al., 1993).

In modern production, selection of animals that are genetically resistant to diseases is an ongoing effort. Thus, determination of the genetic basis of a disease is needed, as it allows for the definition of more objective criteria for efficient breeding and crossbreeding systems. Since many differences among breeds and herds are seen in their resistance or susceptibility to scrapie, the use of uniform criteria for selection cannot necessarily be applied to different sheep breeds, flocks, or countries (LÜHKEN et al., 2004; ACÍN et al., 2004). Thus, defining the relationship between genotype and resistance or susceptibility is essential before starting any type of controlled breeding program. Even though this is a retrospective study from a flock analyzed in 2006, the present work may contribute to future studies and scrapie control programs, since it associates different genotypes with the presence of PrP^{Sc} in crossbred animals and in the Hampshire Down breed, although the number of positive animals was quite low.

It is also necessary that before a selective breeding program is proposed, the previous structure of the population and the degree of variability of the PRNP among different breeds is known. Currently, data about PRNP polymorphisms in Brazil are being published (LIMA et al., 2007; PACHECO et al., 2007; SOTOMAIOR et al., 2008; PASSOS et al., 2008; ANDRADE et al., 2011; IANELLA et al., 2012; SANTOS et al., 2012). Further studies like these must be carried out in order to identify the resistant and susceptible genotypes in the different breeds, contributing to increase the value of rams and dams, which may be commercialized with the certification of scrapie resistance and will help control the disease.

RESUMO: Scrapie nos ovinos está associada a pelo menos três polimorfismos do gene da proteína priônica celular (PRNP) nos códons 136, 154 e 171. Países onde o scrapie é endêmico têm utilizado programas de melhoramento, com a seleção para os alelos mais resistentes. Há alguns dados disponíveis de genotipagem do PRNP em ovinos no Brasil, e o scrapie tem sido observado esporadicamente desde 1978. O Paraná é o Estado brasileiro onde a maioria dos casos de scrapie foi diagnosticada. Um rebanho, que teve três casos clínicos de scrapie em 2003 e 2004, foi genotipado (128 ovinos - 53 Hampshire Down e 75 mestiços) e abatido (111 ovinos - 47 Hampshire Down e 64 mestiços) em 2006. Amostras de tecido linfóide e sistema nervoso central foram examinadas por imunohistoquímica (IHQ) para presença de proteína priônica alterada (PrP^{Sc}). Seis genótipos foram encontrados nos 128 animais genotipados: ARR/ARQ foi o mais frequente (45,3%), seguido por ARQ/ARQ (28,1%), ARR/ARR (14,1%) e ARQ/VRQ (8,6%). ARR/VRQ e ARQ/AHQ apresentaram menos de 2,5% de frequência do genótipo. Na IHC, 16 animais com exame positivo para a presença da proteína priônica celular alterada (PrP^{Sc}) foram detectados. As tonsilas foram o tecido com a mais alta porcentagem de

amostras reativas: 81,25% do total das amostras positivas. Considerando os 16 animais positivos, nove (56,25%) tinham o genótipo ARR/ARQ, seguido pelo genótipo ARQ/ARQ com 31,25% (n = 5) e ARQ/VRQ com 12,5% (n = 2). Todos os animais positivos estavam clinicamente saudáveis, representando, portanto, 14,14% de casos pré-clínicos de scrapie neste rebanho.

PALAVRAS - CHAVE: *Ovis aries*. Prion. Imunohistoquímica. Genotipagem.

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