Prion diseases in animals

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Identification of the presence of a scrapie resistance gene in sheep on the territory of the Republic of Serbia

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Monitoring of scrapie in sheep and goats has been carried out for many years in Serbia. Despite many years of monitoring, only one case of this disease has been reported in sheep, but given that, there are numerous cases of the disease in some neighboring countries, it is very important to determine the presence of resistant genes in the sheep population in Serbia. Genetic susceptibility to scrapie is influenced mainly by the prion protein polymorphisms of codons136, 154, and 171. The ARR allele is considered to provide very strong resistance against classical scrapie and the VRQ genotype is the most susceptible. In order to examine the genetic makeup of sheep in Serbia related to scrapie, we optimized TaqMan probes of real-time polymerase chain reaction (qPCR) techniques for three codons. We analyzed blood samples from 100 sheep using qPCR and the results showed that AA homozygous for the 136 codon were the most common. For codon 154 the most frequent genotype was RR and for codon 171 the most frequent genotype was QQ.

IDENTIFICATION OF THE PRESENCE OF A SCRAPIE RESISTANCE GENE IN SHEEP ON THE TERRITORY OF THE REPUBLIC OF SERBIA

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INTRODUCTION

Monitoring of scrapie in sheep and goats has been carried out for many years in Serbia. Despite many years of monitoring, only one case of this disease has been reported in sheep, but given that, there are numerous cases of the disease in some neighboring countries, it is very important to determine the presence of resistant genes in the sheep population in Serbia.

MATERIAL AND METHODS

We analyzed blood samples from 100 clinically healthy sheep of the Württemberg breed (Fig. 1) using qPCR. Polymorphism determination of codons 136, 154 and 171 was performed on a StepOne™ Real-Time PCR System. The sequencing reaction was performed using a primer flanking the region of codons 136 , 154 and 171 (Table 1). Quantitative PCR (qPCR) amplification was performed using the TaqMan probe method.

RESULTS

The ARQ allele was the most prevalent, and the predominant genotype was ARQ/ARQ. Sheep with ARR/ARQ genotype form the second most represented group in this study. The VRQ/VRQ genotype is very rare and has been reported in only one case. Sheep with ARR/ARR genotype are considered the most genetically resistant, which was recorded in 6 cases (Table 2).

136 (A/V)	**Primers**	PrP-136F: 5′-GCCTTGGTGGCTACATGCT-3
		PrP-136R: 5′-CGGTCCTCATAGTCATTGCCAAAAT-3
	Probes	PrP-136-Ala-VIC: 5′-CTCATGGCACTTCC 3
		PrP-136-Val-FAM: 5′-CTCATGACACTTCC 3
154 (R/H)	**Primers**	PrP-154 F: 5′-TGGCAATGACTATGAGGACCG-3
		PrP-154 R: 5′-TGGTCTGTAGTACACTTGGTTGGG-3
	Probes	PrP-154-Arg-FAM:5′-ACTATCGTGAAAACAT-31
		PrP-154- His-VIC: 5′-TACTATCATGAAAACATG-3
171 (R/Q)	**Primers**	PrP-171F: 5′-ACCCCAACCAAGTGTACTACAGA-3
		PrP171R: 5′-GTCATGCACAAAGTTGTTCTGGT-3
	Probes	PrP-171-Gln-VIC: 5′-CCAGTGGATCAGTATAGT-3′
		PrP-171-Arg-FAM: 5′-CAGTGGATCGGTATAGT-3

Table 1. Sets of primers and probes for the determination of sheep prnp alleles (Zabavnik et al., 2017)

Figure 1. Sheep of Württemberg breed of which blood samples were taken

PrP genotype	n	%
ARQ/ARQ	34	34
ARR/ARQ	29	29
ARR/ARR	6	6
AHQ/AHQ	10	10
ARQ/AHQ	18	18
VRQ/VRQ	1	1
ARQ/VRQ	2	2
Summary	100	100

Table 2. The frequency of PrP genotypes

CONCLUSION

Although no case of scrapie has been registered in the last ten years, the obtained results indicate that the examined sheep population has genetically little resistance to classical scrapie.

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