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STUDY OF THE FREQUENCY OF SCRAPIE-RESISTANT GENOTYPES IN ȚURCANĂ SHEEP BREED

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Abstract

Scrapie tests were performed on a total of 416 adult sheep and young sheep of the Țurcană breed from a private farm in Sibiu County for two consecutive years. Blood was collected on EDTA and the samples were sent to the National Reference Laboratory for Diagnosis in Bucharest. From the three batches of tests performed, it was concluded that resistant young sheep represent only half of the flock. Among adults, the proportion of resistant sheep used for breeding is 57.07%. In exceptional cases, sheep with low resistance may also be used for breeding. The 10.34% of susceptible and highly susceptible sheep must be culled.

•Introduction

Transmissible spongiform encephalopathies are caused by prions, which are pathological isoforms of a normal prion protein (PrP) encoded by host organisms. Certain codons of the prion gene (particularly 136, 154, 171) are important for resistance or susceptibility to scrapie. The ARR allele (especially ARR/ARR) is usually considered to confer resistance, while others such as ARQ, VRQ, and AHQ differ in their degree of susceptibility.

•Material and method

Blood samples were taken from Țurcana adult sheep and lambs born in 2021. The next batch of samples was collected from lambs aged 3-4 months, born in 2022. Blood samples were collected from the jugular vein, on EDTA, in a quantity of 6 ml/animal, and refrigerated at 4^0C for transport to the laboratory. The samples were identified by recording: Sample code, Sample/sub-sample type, Animal code, Sample details/Animal details, Farm code, Owner, Target group. The analysis was performed in the molecular genetics laboratory at the DSVSA Sibiu and at the National Reference Laboratory for Animal Health and Diagnosis in Bucharest. The samples were genotyped at the PRNP locus using the primer extension technique, which involves the following steps: collection of K3 EDTA blood samples; DNA extraction from samples; determination of DNA template quality and quantity; pre-Snap Shot PCR reaction for DNA amplification; purification of the PCR product on columns to remove NTP primers and inhibitors;

Genetic analysis. Determining sensitivity to scrapie involves performing capillary electrophoresis and determining polymorphism at the three loci after the primer extension reaction. This is carried out in the presence of fluorescently labeled dideoxynucleotides (ddNTP) on an ABI Prism313 x l Applied Biosystem genetic analyzer.

Analysis of results. To analyze the results of the electrophoretic profiles, use the GeneMapper R software provided with the device or the PeakScanner program was used. The PCR product was purified using the High Pure purificationKit (Roche) method.

The primers used in the reaction are fluorescently labeled, with each nitrogenous base corresponding to a peak and a characteristic color. During electrophoresis of the PCR product in capillaries filled with gel, the fluorescently labeled DNA passes in front of a laser beam with which the device is equipped. This stimulates each labeled molecule and converts the fluorescent values into a nucleotide sequence in the form of a matrix. The polymorphism existing at the level of the 3 codons (136, 154, and 171) is detected at the level of the electropherogram.

• Results and discussions

Frequency of genotypes for the PRNP locus in young sheep of the Turcana breed in Sibiu County, 2021

RISK INTENSITY			
Risk specification	**Genotypes**	**Number of lambs**	**Share %**
I. Sheep - genetically highly resistant to scrapie	ARR /ARR	4	12.5
II. Sheep genetically resistant to scrapie but requiring attention for use in selection programs	ARR / AHQ	1	3.125
	ARR / ARH	0	0
	ARR /ARQ	11	34.375
III. Sheep with low genetic resistance to scrapie used in selection schemes to be avoided	AHQ / AHQ	0	0
	AHQ /ARH	1	3.125
	AHQ /ARQ	3	9.375
	ARH /ARH	0	0
	ARH / ARQ	1	3.125
	ARQ /ARQ	8	25
IV. Sheep susceptible to scrapie	ARR / VRQ	1	3.125
V. Sheep highly susceptible to scrapie that must be castrated or slaughtered	AHQ /VRQ	1	3.125
	ARH / VRQ	0	0
	ARQ / VRQ	1	3.125
	VRQ/VRQ	0	0

Frequency of genotypes for the PRNP locus in adult sheep of the Turcana breed in Sibiu County, 2021

RISK INTENSITY			
Risk specification	**Genotypes**	**Number of lambs**	**Share %**
I. Sheep - genetically highly resistant to scrapie	ARR /ARR	44	13.8
II. Sheep genetically resistant to scrapie but requiring attention for use in selection programs	ARR / AHQ	5	1.57
	ARR / ARH	4	1.26
	ARR /ARQ	129	40.44
III. Sheep with low genetic resistance to scrapie used in selection schemes to be avoided	AHQ / AHQ	-	0
	AHQ /ARH	-	0
	AHQ /ARQ	13	4.07
	ARH /ARH	1	0.31
	ARH / ARQ	6	1.88
	ARQ /ARQ	84	26.33
IV. Sheep susceptible to scrapie	ARR / VRQ	12	3.76
V. Sheep highly susceptible to scrapie that must be castrated or slaughtered	AHQ /VRQ	1	0.31
	ARH / VRQ	-	0
	ARQ / VRQ	19	5.96
	VRQ/VRQ	1	0.31

Frequency of genotypes for the PRNP locus in young sheep of the Turcana breed in Sibiu County, 2022

RISK INTENSITY			
Risk specification	**Genotypes**	**Number of lambs**	**Share %**
I. Sheep - genetically highly resistant to scrapie	ARR /ARR	9	13.85
II. Sheep genetically resistant to scrapie but requiring attention for use in selection programs	ARR / AHQ	4	6.15
	ARR / ARH	-	0
	ARR /ARQ	20	30.77
III. Sheep with low genetic resistance to scrapie used in selection schemes to be avoided	AHQ / AHQ	-	0
	AHQ /ARH	-	0
	AHQ /ARQ	-	0
	ARH /ARH	-	0
	ARH / ARQ	2	3.07
	ARQ /ARQ	22	33.85
IV. Sheep susceptible to scrapie	ARR / VRQ	-	0
V. Sheep highly susceptible to scrapie that must be castrated or slaughtered	AHQ /VRQ	1	1.54
	ARH / VRQ	-	0
	ARQ / VRQ	7	10.77
	VRQ/VRQ	-	0

Following the results obtained from the reform of adult sheep susceptible to the disease, measures were taken to purchase rams that are highly resistant to scrapie (group I) and to replace the rams in the flock that belonged to genotypes III, IV, and V.

Conclusions

In the two years analysed, both in lambs and adults, resistant genotypes represented approximately 50%–57% of the population, those with low resistance between 32.59% and 40.63%, and those sensitive between 9.37% and 12.31%. We recommend testing for scrapie resistance at the first case of the disease, selecting sheep according to genotype, and purchasing rams with a highly resistant genotype for breeding.