

Article

Evaluation of Scrapie Test Results of Native and Endangered Hungarian Sheep Breeds for Further Breeding

Eszter Ilona Bácsi ^{1,2} , Renáta Klein ^{3,*} , András Lévai ⁴ , Fiona Kenyon ⁵  and János Oláh ¹

¹ Farm and Regional Research Institute of Debrecen, Institutes for Agricultural Research and Educational Farm, University of Debrecen, Böszörményi Street 138, 4032 Debrecen, Hungary; bacsi.eszter@mailbox.unideb.hu (E.I.B.); olahja@agr.unideb.hu (J.O.)

² Doctoral School of Animal Science, University of Debrecen, Böszörményi Street 138, 4032 Debrecen, Hungary

³ Department of Animal Husbandry, Institute of Animal Science Biotechnology and Nature, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Böszörményi Street 138, 4032 Debrecen, Hungary

⁴ Hungarian Sheep and Goat Breeders' Association, Lóportár Street 16, 1134 Budapest, Hungary; levaiandras@mjksz.hu

⁵ Moredun Research Institute, Bush Road Loan, Penicuik EH26 0PZ, UK; fiona.kenyon@moredun.ac.uk

* Correspondence: klein.renata@agr.unideb.hu

Abstract: In this study, we analysed scrapie test results of Hungarian indigenous sheep breeds (Cikta, Tsigai, Dairy Tsigai, Transylvanian Racka, and Hungarian Racka in white and black colour variants) and the endangered Hungarian Merino rams during the period from 2019 to 2023. In Hungary, it is mandatory to perform scrapie testing for every ram intended for breeding. These results were subsequently compared with data from analyses conducted in 2004 and between 2013 and 2015, which served as control samples. The test results were given by the Hungarian Sheep and Goat Breeders' Association. The employees collected ear cartilage tissue samples during the identification of the lambs using TypiFix™ by Agrobiogen GmbH. We determined the frequencies of alleles, genotypes, and risk groups, and calculated the proportion of each within the studied population. The scrapie test results were evaluated using the SPSS 23 software package and a Chi²-test. Samples were categorised into one of five risk groups (R1 (lowest)–R5 (highest)) based on the degree of resistance observed. In conclusion, we found that there was a significant improvement in scrapie susceptibility for all breeds except the Cikta. However, the potential impact of this improvement on other important traits remains undetermined. Regarding susceptibility to scrapie, the Hungarian Merino is the most resistant group, as 68.8% of the rams in this breed belonged to the R1 risk group, while the Cikta sheep is in the least favourable position, as only 3.3% of the examined individuals belonged to this category.

Keywords: scrapie; indigenous breeds; scrapie resistance; zoonosis



Academic Editors: András Gáspárdy and Ante Ivanković

Received: 26 February 2025

Revised: 12 April 2025

Accepted: 13 April 2025

Published: 18 April 2025

Citation: Bácsi, E.I.; Klein, R.; Lévai, A.; Kenyon, F.; Oláh, J. Evaluation of Scrapie Test Results of Native and Endangered Hungarian Sheep Breeds for Further Breeding. *Agriculture* **2025**, *15*, 880. <https://doi.org/10.3390/agriculture15080880>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Scrapie or TSEs (Transmissible Spongiform encephalopathies) is a generally lethal, infectious, notifiable disease caused by a prion, which is manifested in a degenerative change in the brain substance in sheep [1–3].

These genetic tests enable the selective elimination of scrapie-susceptible genotypes from the breeding population, thereby enhancing overall flock resistance to the disease. Consequently, the performance of scrapie diagnostic tests is of great importance not only for intensive breeds, but also for indigenous and endangered Hungarian sheep breeds. Their milk is a unique ingredient and their meat is used to make the centuries-old mutton

stew from Karcag, which has been awarded as part of the Collection of Hungarian Values also known as Hungarikums. This collection was established by Act number XXX of 2012 concerning Hungarian National Values as enacted and declared by the Hungarian Government [4].

Scrapie was first described in England in 1735 [5,6]. In Hungary, the disease was first reported in 1964; since then, only a few individuals have been diagnosed with the atypical form of the disease during compulsory testing [7].

The typical form is a notifiable disease. Upon detection, complete eradication of the flock is required, followed by official quarantine measures [1,2,8–11].

The atypical form was described first in Norway; it usually occurs in animals older than 5 years [11,12].

The method of prevention is inspection (annual examination of brain tissue) of a specified number of dead/slaughtered animals older than 18 months or animals with more than two permanent incisors [2,13,14].

Sampling can be used to determine susceptibility to scrapie from ear cartilage, blood, or even semen [11,15,16].

Regulation of European Community No. 999/2001 lays down rules for prevention with programmes, among others [17]. The Hungarian national program was started in 2003. All prospective rams must be sampled to determine prion genotypes. Only individuals belonging to risk groups R1–R3 are eligible for further breeding, while those carrying the VRQ allele are designated for slaughter [18,19].

Hungary has been classified as a “low-risk” Scrapie country since 2014. To maintain this status, it is crucial to use prevention to breed less susceptible sheep breeds and classify the animals into less sensitive breeds and bloodlines based on genetic testing [17]. The test involves testing the existing prion protein (PrPC) gene. It is present in all individuals in the form of two alleles, one maternal and one paternal. During breeding, it is important to amplify alleles that are less susceptible to the disease and to reduce the occurrence of alleles that amplify susceptibility.

The susceptibility of small ruminants to scrapie is determined by the polymorphism of the prion gene and the susceptibility of the animal’s host. In the coding section of the prion gene, which contains 771 base pairs, a base exchange can be observed in three places. On place codon 136 there is alanine (A) instead of valine (V), on place codon 154 there is arginine (R) instead of histidine (H), and on codon 171 there is an arginine (R) instead of glutamine (Q) or histidine (H) [2,20–29]. If codon 171 encodes glutamine (Q), the animal should be selected [2,21–24].

The most resistant animals are A136R154R171/A136R154R171, and the most susceptible animals are V136R154Q171/V136R154Q171 [11,17,18,29].

In Hungary, goats are significantly more sensitive to scrapie than sheep [30].

Indigenous breeds of sheep bred in small numbers are generally more susceptible to the disease, and in their case, due to the number of people, it is not possible to select only individuals with the most resistant genotype [19,31–35].

According to the 2023 EFSA report, 93.4% of sheep diagnosed with classical scrapie belonged to the susceptible animals [36]. In Hungary, 12 animals susceptible to scrapie were registered; in the EU, the highest number of animals were registered in this category in Greece (188) and Spain (149) during sampling in 2023 [37].

In the United States of America, the Animal and Plant Health Inspection Service (APHIS) established 17 registered laboratories for scrapie testing. The test is conducted on blood samples, and approximately 30% of the sheep there are susceptible to scrapie.

From the perspective of determining susceptibility, codon 171 is primarily decisive. If Q (glutamine) occurs at this position, the animals are quite susceptible, but H (histidine)

and K (lysine) can also indicate susceptibility in animals. In the USA, this is called valine-independent scrapie. Studies have shown that in the case of classical scrapie, the QQ allele was present at codon 171 in 99% of cases [38].

Codon 136 plays a role in susceptibility to the less common type of classical scrapie found in the United States, valine-dependent scrapie.

In most cases, A (alanine) or V (valine) occurs most frequently, while in rarer instances, T (threonine) may also be present. The V allele leads to increased susceptibility [38].

The aim of this research is to determine the difference in scrapie results among Hungarian breeds and to demonstrate which breeds have shown the most significant improvement.

2. Materials and Methods

According to the Hungarian Sheep and Goat Breeders' Association's (HSGBA) breeding plan, all prospective rams must be sampled to determine prion genotypes [30]. Only individuals belonging to risk groups R1–R3 are eligible for further breeding, while those carrying the VRQ allele (risk categories 4–5) are designated for slaughter [29].

HSGBA employees collected ear cartilage tissue samples from 19,479 animals: from 551 Cikta, 2917 Tsigai, 246 Dairy Tsigai, 619 Transylvanian Racka, and 3609 Hungarian Racka, 1827 white and 1782 black colour variants, and 7928 Hungarian Merino during the identification of the lambs using TypiFix™ by Agrobiogen GmbH (Hilgertshausen-Tandern, Germany) between 2019 and 2023.

The samples were then sent to the company's laboratory in Germany for genotyping; PCR was used to amplify the prion protein gene the variant (haplotype) sequence determined through pyrosequencing. Based on the results, the rams were classified to one of the 5 risk groups (Table 1) [18]. During the examination, the prion gene is marked, then the DNA sequence is amplified using PCR technique, and the polymorphic nucleotides are determined by pyrosequencing [18].

The test results were given to the project by the Hungarian Sheep and Goat Breeders' Association. The required data were taken from the Microsoft Excel database and statistically evaluated using the SPSS 23 statistics software. From the number of individual genotypes of the varieties, the individual genotype frequency, the proportion of alleles, the frequency of occurrence, and the frequency or proportion of risk groups were determined. Using the Chi²-test, the current condition was compared to the former condition (2013 to 2015, and 2004) [9,18,19]. We compared the test results from 2004 (Table 2) and 2013–2015 with those from 2019–2023 and illustrated in Tables 3 and 4 the extent of changes in the proportions of haplotypes, genotypes, and risk groups within the population. There were 780 examinations conducted in 2004 and 10083 between 2013–2015.

Table 1 presents the susceptibility to scrapie risk groups by genotypes [19,24,30].

Table 1. PrP-genotypes of the sheep and their classification into different risk groups.

Risk Group	Genotype	Degree of Resistance/Susceptibility
R1	ARR/ARR	The individual and the offspring have a very low probability of developing the disease. That group is genetically most resistant to scrapie. The chance of infection is extremely low.
R2	ARR/AHQ ARR/ARH ARR/ARQ	The individual and the offspring are unlikely to develop the disease. That group are genetically resistant to scrapie but will need careful selection when used for further breeding. The chance of infection is low if paired with an R1 or R2 individual.

Table 1. *Cont.*

Risk Group	Genotype	Degree of Resistance/Susceptibility
R3	AHQ/AHQ	The individual is less likely to have the disease, but some of the offspring may be at risk depending on the genotype of the other parent. That group genetically have little resistance to scrapie but needs careful selection. Increased risk especially in the case of ARQ animals.
	AHQ/ARH	
	AHQ/ARQ	
	ARH/ARH	
	ARH/ARQ	
R4	ARR/VRQ	Individuals may develop scrapie, and the risk of disease in offspring is higher than in the R3 group. That group are genetically susceptible to scrapie and should not be used for breeding, except in the context of a controlled breeding program. High risk for certain animals and for half of the offspring.
	AHQ/VRQ	
R5	ARH/VRQ	The risk of the disease is the highest for that group. Animals are susceptible to scrapie and should not be used for breeding. The risk of infection is very high.
	ARQ/VRQ	
	VRQ/VRQ	

You can find the characteristics of the breeds in the Supplementary Materials.

3. Results

Table 2 presents the 780 scrapie examinations conducted in 2004. Regarding the haplotypes, approximately 70% of the examined sheep in the Cikta and Hortobágy Racka breeds, 65.63% of Tsigai rams, 42.5% of Hungarian Racka, and 46.11% of Hungarian Merino possessed the ARQ allele during this period. At that time, the Hungarian Merino had the highest proportion of the ARR allele (47%).

Among the 15 possible genotypes, the number of genotypes varied across different breeds: Cikta (5), Tsigai, Transylvanian Racka (6), Hungarian Racka (14), and Hungarian Merino (9). In 2004, the VRQ/VRQ genotype was not present in the indigenous and endangered Hungarian sheep breeds [19].

Regarding the risk groups, it was observed that the Cikta, Transylvanian Racka, and Hungarian Racka breeds were classified into the R3 category, while the R2 category was most characteristic for the Tsigai and Hungarian Merino breeds. In the initial phase of scrapie testing, the prevalence of the least susceptible R1 category was low among the examined breeds. Except for the Hungarian Merino (21%), the occurrence of the R1 category remained below 5% in the other four studied breeds. This proportion was considered extremely low.

Table 2. Frequencies of haplotypes and genotypes of native and endangered Hungarian Sheep Breeds in 2004 as well as the distribution of scrapie risk groups. (1) Cikta; (2) Tsigai; (3) Transylvanian Racka Sheep; (4) Hungarian Racka; and (5) Hungarian Merino Sheep.

Groups	(1)	(2)	(3)	(4)	(5)	
Haplotypes	(n = 138) Chi ² = 7.608; p = 0.55	(n = 128) Chi ² = 9.112; p = 0.058	(n = 114) Chi ² = 10.137; p = 0.038	(n = 280) Chi ² = 7.904; p = 0.095	(n = 900) Chi ² = 11.873; p = 0.018	
	ARR	20.29	28.91	27.19	23.93	
	AHQ	9.42	3.13	0.88	17.68	4.56
	ARH	0.00	1.56	-	12.14	0.56
	ARQ	70.29	65.63	70.18	42.50	46.11
	VRQ	0.00	0.78	1.75	3.57	1.78
Genotypes	(n = 69); Chi ² = 13.768; p = 0.032	(n = 64) Chi ² = 15.574; p = 0.077	(n = 57) Chi ² = 16.017; p = 0.042	(n = 140) Chi ² = 22.221; p = 0.052	(n = 450) Chi ² = 28.272; p = 0.003	
	ARR/ARR	1.45	3.13	5.26	5.00	21.11
	ARR/AHQ	4.35	6.25	-	6.43	7.33
	ARR/ARH	-	-	-	4.29	0.67

Table 2. Cont.

Groups	(1)	(2)	(3)	(4)	(5)
ARR/ARQ	33.33	45.31	42.11	25.00	40.89
AHQ/AHQ	-	-	-	4.29	-
AHQ/ARH	-	-	-	7.14	-
AHQ/ARQ	14.49	-	1.75	12.14	1.78
ARH/ARH	-	-	-	0.71	-
ARH/ARQ	-	3.13	-	10.00	0.44
ARQ/ARQ	46.38	40.62	47.38	17.86	24.22
ARR/VRQ	-	-	1.75	2.14	2.89
AHQ/VRQ	-	-	-	1.43	-
ARH/VRQ	-	-	-	1.43	-
ARQ/VRQ	-	1.56	1.75	2.14	0.67
VRQ/VRQ	-	-	-	-	-
Risk groups	(n = 69) Chi ² = 8.451; p = 0.038	(n = 64) Chi ² = 7.877; p = 0.96	(n = 57) Chi ² = 7.640; p = 0.106	(n = 140) Chi ² = 9.055; p = 0.060	(n = 450) Chi ² = 11.335; p = 0.023
R1	1.45	3.13	5.26	5.00	21.11
R2	37.68	51.56	42.11	35.71	48.89
R3	60.87	43.75	49.13	52.15	26.44
R4	-	-	1.75	2.14	2.89
R5	-	1.56	1.75	5.00	0.67

Table 3 describes the evaluation of the results of the 19,479 tests conducted between 2019 and 2023 compared to 2004. The most favourable ARR allele increased to the greatest extent in the Transylvanian Racka breed. Compared to 2004, there was a 48.73% improvement, which is highly advantageous for eradication purposes. This was followed by Hungarian Merino (+35.93%), Tsigai (+32.87%), the white variant of Hortobágy Racka (+21.53%), and the black variant of Hortobágy Racka (+18.97%). Among the examined breeds, only the Cikta exhibited a decrease in the proportion of the ARR allele (−4.95%). Regarding these breeds, the AHQ allele slightly increased (+0.7–0.8%) in the white variant of Hortobágy Racka and Transylvanian Racka, while it decreased in the other breeds. The ARH allele has decreased except for the Transylvanian Racka breeds. The largest decrease was observed for the ARQ allele. This allele increased only in the Cikta breed (+6.48%). The VRQ allele showed an increase of +0.1% in Cikta. The VRQ allele showed an increase of +0.1% in Cikta. Compared to 2004, new haplotypes emerged in certain breeds: the VRQ allele appeared in Cikta, and the ARH allele emerged in Transylvanian Racka.

The greatest improvements in the occurrence percentage of the ARR/ARR genotype were observed in Transylvanian Racka (+54.19%), Hungarian Merino (+47.67%), and Tsigai (+37.05%). The most negative VRQ/VRQ allele was not observed in either 2004 or 2019–2023. Changes occurred in the number of genotypes. In Cikta, AHQ/AHQ and ARQ/VRQ appeared. In Tsigai, ARR/ARH, AHQ/AHQ, AHQ/ARQ, and ARR/VRQ emerged. Transylvanian Racka showed ARR/AHQ, ARR/ARH, and ARH/ARQ. In Hungarian Merino, AHQ/AHQ, AHQ/ARH, and ARH/ARH haplotypes appeared.

Regarding risk groups, the most significant improvements in the R1 category were observed in Transylvanian Racka (+54.19%), Hungarian Merino (+47.67%), and Tsigai (+37.05%). The occurrence rate of the R3 risk group decreased in all the examined breeds except for Cikta. Regarding susceptibility to scrapie, it is disadvantageous that the R4 proportion increased in the Tsigai (+0.27%) and Transylvanian Racka (+0.52%) breeds, and that the R5 category proportion increased in the Cikta (+0.2%) breed. This trend developed favourably for the other breeds, as the proportion of these risk groups decreased since the initiation of examinations and eradication programs. Changes also occurred in the risk groups, with the R5 category emerging in Cikta and the R4 category appearing in Tsigai.

Table 3. Changes in frequencies of haplo- and genotypes of native and endangered Hungarian sheep breeds and distribution of scrapie risk groups since 2004. (1) Cikta; (2) Tsigai; (3) Transylvanian Racka Sheep; (4) Hungarian Racka Sheep, white colour variant; (5) Hungarian Merino Sheep; and (6) black-coloured Hungarian Racka Sheep.

Groups	(1)	(2)	(3)	(4)	(5)	(6)
Haplotypes	(n = 1102)	(n = 5834)	(n = 1238)	(n = 3654)	(n = 15,856)	(n = 3564)
ARR	−4.95	+32.87	+48.73	+21.53	+35.93	+18.97
AHQ	−1.62	−0.51	+0.82	+0.71	−2.33	−6.29
ARH	-	−1.34	+0.65	−2.15	−0.19	−5.49
ARQ	+6.48	−30.47	−50.15	−17.46	−31.77	−4.73
VRQ	+0.1	−0.56	−0.05	−2.45	−1.65	−2.28
Genotypes	(n = 551)	(n = 2917)	(n = 619)	(n = 1827)	(n = 7928)	(n = 1782)
ARR/ARR	+1.82	+37.05	+54.19	+16.83	+47.67	+12.62
ARR/AHQ	−2.17	−2.65	+2.42	+9.66	−3.76	+1.54
ARR/ARH	-	+0.31	+1.13	+3.87	−0.16	+2.16
ARR/ARQ	−11.37	−6.3	−15.01	−3.11	−16.92	+10.07
AHQ/AHQ	+0.9	+0.07	-	+0.03	+0.03	−2.89
AHQ/ARH	-	-	-	−3.24	+0.06	−2.88
AHQ/ARQ	−2.88	+1.51	−0.78	−4.37	−1.01	−4.62
ARH/ARH	-	-	-	+0.6	+0.01	−0.01
ARH/ARQ	-	−2.99	+0.16	−4.91	−0.29	−9.05
ARQ/ARQ	+13.51	−25.88	−42.05	−10.47	−22.33	−2.43
ARR/VRQ	-	+0.27	+0.52	−1.04	−2.64	−1.07
AHQ/VRQ	-	-	-	−1.05	-	−1.22
ARH/VRQ	-	-	-	−1.21	-	−1.23
ARQ/VRQ	+0.2	−1.39	−0.62	−1.54	−0.66	−1.02
VRQ/VRQ	-	-	-	-	-	-
Risk groups	(n = 551)	(n = 2917)	(n = 619)	(n = 1827)	(n = 7928)	(n = 1782)
R1	+1.82	+37.05	+54.19	+16.83	+47.67	+12.62
R2	−13.55	−8.64	−11.42	+10.43	−20.83	+13.78
R3	+11.53	−27.29	−42.67	−22.35	−23.54	−21.85
R4	-	+0.27	+0.52	−1.04	−2.64	−1.07
R5	+0.2	−1.39	−0.62	−3.85	−0.66	−3.48

Table 4 shows how the test results from the 2019–2023 period we analysed compare to the 2013–2015 test results of local Hungarian sheep breeds. For the haplotypes, there were favourable changes in the ARR allele frequencies, with the most favourable allele becoming more frequent in all seven varieties. The highest increases were for Transylvanian racka (+18.94%), Tsigai (+15.97%), Hungarian Merino (+15.86%) and Dairy Tsigai (15%). We observed a decrease in the ARQ and VRQ alleles for all the breeds except Cikta. In Cikta, the ARH allele did not occur during the 2019–2023 examination period. The most significant improvement in genotype frequency was observed in the occurrence of the ARR/ARR homozygous genotype in Transylvanian Racka (+24.17%), Hungarian Merino (+23.79%), and Tsigai (+18.13%) breeds, while the smallest increase occurred in Cikta (+0.82%). In the Hungarian Merino breed, the ARH/ARH genotype has emerged since the previous examination period. The VRQ/VRQ genotype, similar to the earlier period, was only observed in Dairy Tsigai and the white variant of Hortobágy Racka, but it showed a decrease in these breeds as well, which is advantageous for breeding purposes.

Regarding the occurrence of risk groups, we observed that due to the eradication program, the proportion of R1 increased in all the examined breeds. Compared to 2013–2015, the most significant increases in R1 proportion were seen in Transylvanian Racka (+24.17%), Hungarian Merino (+23.79%), and Tsigai (+18.13%). The proportion of the R3 risk group decreased in all the breeds. It can be considered favourable that the occurrence rate of the

R4 risk group decreased in all the breeds except for Dairy Tsigai and Cikta. It is extremely advantageous that the proportion of the R5 risk group decreased in all the breeds compared to 2013–2015. This is favourable for reducing susceptibility to scrapie and for the eradication program.

Table 4. Changes in frequencies of haplo- and genotypes of native and endangered Hungarian sheep breeds and distribution of scrapie risk groups since 2013–2015. (1) Cikta; (2) Tsigai; (3) Transylvanian Racka Sheep; (4) Hungarian Racka Sheep, white colour variant; (5) Hungarian Merino Sheep; (6) black-coloured Hungarian Racka Sheep; and (7) dairy Tsigai Sheep.

Groups	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Haplotypes	(n = 1102)	(n = 5834)	(n = 1238)	(n = 3654)	(n = 15,856)	(n = 3564)	(n = 492)
ARR	+1.15	+15.97	+18.94	+9.98	+15.86	+12.03	+15
AHQ	−2.9	−0.68	−1.22	−3.53	−2.25	+3.17	−1.07
ARH	−0.13	−0.2	−1.38	−5.35	+0.09	−2.46	−2.61
ARQ	+1.84	−14.65	−14.74	−0.4	−13.52	−11.08	−9.56
VRQ	+0.05	−0.44	−1.6	−2.49	−0.18	−1.66	−1.76
Genotypes	(n = 551)	(n = 2917)	(n = 619)	(n = 1827)	(n = 7928)	(n = 1782)	(n = 246)
ARR/ARR	+0.82	+18.13	+24.17	+8.56	+23.79	+8.25	+11.72
ARR/AHQ	−0.61	−0.55	−0.63	+2.11	−2.53	+2.45	−0.44
ARR/ARH	-	−0.16	−1.66	−1.44	+0.09	+0.93	+1.42
ARR/ARQ	+1.26	−3.03	−7.16	+5.3	−13.33	+4.51	+5.56
AHQ/AHQ	−0.41	−0.02	-	−2.32	−0.25	+0.89	-
AHQ/ARH	-	-	−0.25	−3.21	+0.04	+2.98	−0.48
AHQ/ARQ	−4.37	−0.66	−1.57	−0.76	−1.49	−0.31	−0.97
ARH/ARH	-	-	-	−0.7	+0.01	−0.33	−0.48
ARH/ARQ	−0.26	−0.24	−0.6	−3.44	+0.03	−7.78	−4.75
ARQ/ARQ	+3.47	−12.59	−9.14	+0.64	−6	−8.32	−8.18
ARR/VRQ	-	−0.58	−1.03	−1.15	−0.09	−0.34	+0.02
AHQ/VRQ	-	−0.09	-	−0.57	−0.02	−0.56	−0.24
ARH/VRQ	-	-	−0.25	−1.2	-	−0.44	−0.44
ARQ/VRQ	−0.11	−0.21	−1.92	−1.53	−0.25	−1.96	−2.61
VRQ/VRQ	-	-	-	−0.24	-	-	−0.12
Risk groups	(n = 551)	(n = 2917)	(n = 619)	(n = 1827)	(n = 7928)	(n = 1782)	(n = 246)
R1	+0.82	+18.12	+24.17	+8.56	+23.79	+8.25	+11.72
R2	+0.64	−3.73	−9.41	+5.97	−15.76	+7.89	+6.54
R3	−1.57	−13.51	−11.56	−9.77	−7.67	−12.83	−14.86
R4	-	−0.58	−1.03	−1.15	−0.09	−0.34	+0.02
R5	−0.11	−0.3	−2.17	−3.59	−0.27	−2.97	−3.42

3.1. The Cikta Sheep

In the period we analysed from 2019 to 2023, the ARQ allele was the most frequent (76.77%). The ARR allele was present in 15.34% and AHQ allele in 7.8% of individuals, while the VRQ allele occurred in only 0.09%. There were no sheep with the ARH allele. Based on that, we determined that, similar to the results of previous years, the ARQ allele was the most common, and the rate of occurrence of the most favourable ARR allele was, as in previous years, much lower than estimated. The Chi²-test indicated that there are statistically significant deviations ($p = 0.026$) in our results and the 2013–2015 results.

Among the 15 known prion genotypes, only 7 were detected between 2019 and 2023. Genotypes carrying the ARQ allele were the most common, followed by sheep carrying the ARR and AHQ alleles. The homozygous VRQ/VRQ genotype, which is most sensitive to scrapie, was not found during the period of our study. There are statistically significant deviations compared to previous years, $p = 0.0032$ (2004) and $p = 0.006$ (2013–2015) in the Chi²-test.

The most common risk group was the R3 (72.4%), then R2 (24.13%). The R1 group had 3.27%, and there were no individual in the R4 risk group neither our results, nor in the past results. Only one Cikta ram (0.2%) was in a R5 group. The percentage slightly increased in the R1 group.

3.2. The Tsigai Sheep

Table 3 describes the most recent test results of the Tsigai rams examined, which we compared to the 2004 test results, shown in Table 2. All five alleles were identified in the tested rams. The ARR allele was the most prominent (61.78%), which is advantageous for susceptibility to scrapie, followed by the ARQ allele with 35.16%, the AHQ allele 2.62%, and the VRQ allele with 0.22%. There were no ARH alleles listed. For the alleles, it was observed that the frequencies of the ARR allele and the ARQ allele shifted in a favourable direction for susceptibility. In 2004, the frequency of the ARR allele was 28.91%, which had increased to 61.78%, and the ARQ allele was 65.63% and, in this study, was 35.16%.

In the rams tested ($n = 2917$) in the analysed period (2019–2023), 10 genotypes were observed from the overall 15. The ARR/ARR and ARR/ARQ genotypes were both present in 40% of the population. The ARQ/ARQ was present in 14.74% which was a decrease from previous years. As in previous years, there were no AHQ/ARH, ARH/VRQ, and homozygote VRQ/VRQ allele.

Most individuals were in the R1 (40.18%) and R2 (42.92%) risk groups. Individuals belonging to group R3 occurred in 16.46% of the samples tested. There were eight rams (0.27%) in the R4 and five (0.17%) in the R5 risk groups. The proportion of the R3 group decreased compared to previous years, while the proportion of the R1 group increased. This is advantageous in terms of the susceptibility of the variety to scrapie.

Based on the Chi²-test, there were statistically significant deviations in the alleles ($p = 0.013$), the genotypes ($p = 0.001$), and the risk groups ($p = 0.015$) between our results and those previously published.

3.3. The Transylvanian Racka Sheep

The scrapie data for the Transylvanian Racka are presented in Table 3. For this breed, the ARR allele was the most frequent (75.92%), followed by the ARQ allele (20.03%). The AHQ and VRQ alleles occurred at a frequency of 1.7%, while the ARH allele was present at 0.65%. The frequency of ARR and ARQ alleles changed similarly to the Tsigai breed compared to 2004, with a significant increase in the proportion of the ARR allele. Significant differences were observed in allele frequencies when compared to the 2004 ($p = 0.038$) and 2013–2015 ($p = 0.011$) analyses.

The examinations were carried out between 2019 and 2023 for 619 individuals. Out of the 15 possible genotypes, 9 were observed. Significant differences were observed compared to previous years, with $p = 0.042$ relative to 2004, and $p = 0.003$ relative to the 2013–2015 period used as a control.

Among the genotypes, ARR/ARR was the most common, occurring in 368 (59.45%) individuals, followed by ARR/ARQ (168 individuals, 27.14%). Similar to previous years, the homozygous genotypes AHQ/AHQ, ARH/ARH, ARH/VRQ, and VRQ/VRQ were not observed in this population. We also found that the proportion of ARR/ARR homozygous genotypes has changed in a positive direction from 5.26% in 2004 to 59.45%, while the ARQ/ARQ prevalence has decreased from 47.38% to 5.33%.

In the case of risk groups, the most favourable R1 category occurred in the highest proportion with 59.45%, followed by R2 in 30.69%. R3 occurred in 6.46%. Adverse R4 and R5 individuals were detected at 2.27% and 1.13%, respectively. Previously, R2 and R3 were

more common, but now this has changed positively, with R1 and R2 individuals becoming more prevalent.

3.4. Hungarian Racka Sheep, White Colour Variant

The results obtained with prion haplo- and genotypes of white-coloured Hungarian Racka Sheep are shown in Table 3. Between 2019 and 2023, 1827 male individuals were examined.

The ARR allele was present in 45.46% of cases, followed by the ARQ allele, which was the second most common at 25.04%, and AHQ was third with a prevalence of 18.39%. Compared to previous years, the proportion of ARR alleles increased considerably compared to 2004 (from 23.93% to 45.46%), while the ARQ allele decreased (from 42.5% to 25.04%).

In this variety, of the 15 genotypes, only the VRQ/VRQ homozygous genotype was not detected. Based on the Chi²-test, significant differences were observed in the genotypes compared to the two previous study periods (2013–2015: $p < 0.001$). ARR/ARR was found in 399 (21.83%) individuals, ARR/ARQ in 400 (21.89%), followed by ARR/AHQ with 294 (16.09%) individuals, ARR/ARH, AHQ/ARQ, and ARQ/ARQ with 140 (7.77%) individuals. The ARR/ARR ratio has increased considerably since 2004, from 5% to 21.83%, while the percentage of ARQ/ARQ individuals showed a decrease.

Regarding the risk groups, R2 was the most common, occurring in 46.14% of individuals, followed by R3 and then R1. R4 and R5 occurred in nearly equal proportions (approximately 1.1%) during the 2019–2023 period.

Since the beginning of the examinations, the occurrence rate has changed somewhat: the R1 category shows an increase, while R3–R5 have decreased significantly; however, R1 still only accounts for 21.8% of the breed.

3.5. The Hungarian Merino Sheep

Table 4 illustrates the prion haplo- and genotypes of the endangered Hungarian Merino Sheep as well as the distribution of scrapie risk groups. From the examined breeds, Hungarian Merino had the highest (82.93%) ARR allele occurrence. After the ARR, the second was the ARQ with 14.34%. The other three alleles were present in only 2.73% of the tested sheep. The occurrence of the ARR allele increased significantly compared to both 2004 and 2013–2015. This fact is highly favourable in terms of resistance. The ARQ allele significantly decreased.

Twelve of the fifteen possible genotypes were identified. There are statistically significant deviations compared to both 2004 ($p = 0.003$) and 2013–2015 ($p = 0.001$). The sample size was 7928 individuals, of which 5453 rams (68.78%) had the ARR/ARR genotype, and 1900 sheep (23.97%) had the ARR/ARQ genotype. Compared to the results of 2004, the most favoured genotype increased. At that time, ARR/ARQ (40.89%) homozygous rams were the most common. The most sensitive ARH/VRQ and VRQ/VRQ genotypes were not detected in the ram population like in the previous years.

From the examined breeds, Hungarian Merino had the most favourable distribution of risk groups in relation to scrapie resistance, because the R1 was 68.8%, and the R2 risk group was 28.1%. Moreover, there was only one individual in Risk Group 5. Based on the Chi²-test, there were statistically significant deviations compared to 2004 ($p = 0.023$) and 2013–2015 ($p = 0.009$). The ratio of R1 individuals increased threefold, while the other risk groups showed a large decrease.

3.6. Hungarian Racka Sheep, Black Colour Variant

Table 3 presents the prion haplo- and genotypes of black-coloured Hungarian Racka Sheep as well as the distribution of scrapie risk groups. Like Tsigai, Dairy Tsigai, and the white-coloured Hungarian Racka, the most common alleles are the ARR (42.9%) and the

ARQ (37.77%). We found that the ratio of the ARR allele within the herd is constantly increasing, while the ratio of the ARH and VRQ alleles decreased since the beginning of the tests.

Results from 1782 individuals were evaluated. There were 14 from the 15 possible genotypes. The most common was the ARR/ARQ genotype with 35.04%, the next was ARR/ARR (containing 60 rams (17.62%), and the third one was ARQ/ARQ with 15.43%. Based on the Chi²-test, there were statistically significant deviations compared to both 2004 ($p = 0.021$) and 2013–2015 ($p = 0.001$). The homozygous VRQ/VRQ allele was not detectable. In the last period, the percentage of ARR/ARR and the ARR/ARQ alleles increased. The R4 and R5 groups were only 1–1.5%. The proportion of R1 and R2 individuals shows an increasing trend, which will hopefully continue in the upcoming period.

3.7. The Dairy Tsigai Sheep

The prion haplo- and genotypes of Dairy Tsigai Sheep, as well as the distribution of scrapie risk groups, are summarised in Table 4. In the examined period, there were only 246 rams with test results, fewer than the 826 examinations carried out in the period between 2013 and 2015.

The ARQ allele was found in 47.76% and the ARR allele in 42.48% of the samples tested. The AHQ was only at 0.2%. Currently, the ARQ allele is still the most common, but since the start of the studies, the proportion of ARR alleles has increased, which is favourable for resistance.

There were 9 from the 15 possible genotypes, the greatest percentage of which was the ARR/ARQ genotype with 92 individuals (37.4%), followed by ARQ/ARQ with 60 rams (24.39%), and the third one was the ARR/ARR with 47 rams (19.11%). The homozygous VRQ/VRQ allele was not detectable. There are statistically significant deviations ($p < 0.001$) compared to 2004 and 2013–2015 as well. The occurrence rate of the homozygous ARR/ARR genotype increased compared to previous years. The AHQ/AHQ allele was still not present in the examined subpopulation.

The highest rate of change was in the R2 (44.31%) and the R3 (32.11%) risk groups, but the R1 group was moderately high (19.11%) too. The R4 (2.44%) and the R5 (2.03) groups were under 3%. For all the study periods, the R2 and R3 were the most relevant. The proportion of R1 showed an increase, but it remains very low within the population.

4. Discussion

Our study confirmed that the examined Hungarian sheep breeds exhibit different levels of genetic susceptibility to scrapie. Eradication programmes have significantly increased the proportion of resistant sheep, especially in intensive breeds [39]. Maintenance of genetic diversity is important for indigenous breeds; however, during selection, care must be taken to maintain the production of the breeds too. Their meat and milk are ingredients which form one of the foundations of the local identity [40,41].

Our work confirmed that Hungarian sheep breeds exhibit varying degrees of genetic susceptibility to scrapie. One of the reasons for the difference between the breeds may be the different sample size. The analyses did not confirm the effect of the number of breeding flocks on the evolution of risk groups. In the case of dairy Tsigai sheep ($n = 246$), there are currently only 4 breeders, yet a more significant improvement has occurred compared to the Cikta breed, which was examined with a larger number of individuals ($n = 551$) and has 15 breeding flocks. The value increased from 1.45% to 3.27%.

We found that there was a significant improvement in the reduction in scrapie susceptibility for all breeds except the Cikta. The Hungarian Merino is in the most advantageous status, as 68.8% of the tested rams in this breed belonged to the lowest (R1) risk group. The

next is the Transylvanian Racka with 59.45%, and the Tsigai with 40.18%; the other breeds are under 20%. The most unfavourable results in terms of susceptibility were obtained with the Cikta sheep; only 3.27% of the individuals were in the R1 risk group. This result had not improved significantly compared to the previous studies.

Since the start of the eradication programme, the following changes have occurred for the analysed Hungarian breeds. The most significant improvement in native breeds in susceptibility to scrapie occurred in the Transylvanian Racka breed. The proportion of the R1 category was 5.26% at the start of the studies, while 59.45% of the ram population is now in this category. This is a huge positive increase. Regarding Tsigai in 2004, 3.13% of individuals belonged to the R1 group, while 40.18% of rams belonged to the R1 group in the period 2019–2023. The other three breeds increased between 12 and 17%. The Hungarian Racka was 5% in 2004 in the black and white colour too; the black increased to 17.62%, and the white to 21.98%. The endangered Hungarian Merino had showed an increase in the proportion of animals in the lowest risk group, increasing from 21.11% to 68.78%.

The Dairy Tsigai population has increased by 11.72% since 2013–2015. During this period, 7.39% of individuals belonged to the R1 risk group, which has now risen to 19.11%.

The R2 risk group was the biggest (around 40–50%) in every breed except the Hungarian Merino and Cikta. Increasing the proportion of R1 individuals in these breeds and in this case by large amounts would lead to a higher inbreeding coefficient. Because of the fact that Cikta is indigenous and inbreeding could result in lower genetic diversity, selective breeding is not recommended.

Despite the results, these Hungarian breeds do not rank among the most at risk. In a Canadian study from 2014, 15% of individuals in a very small sheep population had the VRQ allele [42]. Scrapie is an existing threat. In the EU27 and the UK (Northern Ireland), there were 284686 tests conducted in sheep and 538 scrapie cases were reported in 2023. A total of 462 of these were typical and 70 atypical.

Typical scrapie was reported by four member states, including Romania, which is a neighbouring country of Hungary. The atypical version was reported by 12 member states, including Hungary and neighbouring Austria and Slovenia [36].

The EU is continuously monitoring the prion protein disease in sheep and goats. Data published by EFSA show that the number of scrapie cases has decreased significantly over the last 10 years. While in 2013 there were still around 3000 cases of scrapie in small ruminants, by 2023 this number had dropped to a fraction of that [37].

We compared the results of the local Hungarian breeds analysed by us from 2019 to 2023 with the scrapie testing data of intensive breeds raised in Hungary. We observed that the proportion of the ARR allele was significantly higher in the more intensive breeds. The highest frequencies of the ARR allele were observed in the following breeds: Hampshire (ARR: 97.5%), Bleu du Maine (ARR: 97.4%), English Suffolk (95.9%), Ile de France and Texel (95.1%), and Berrichon du Cher (ARR: 95%) [30].

A 2023 study examined the susceptibility of Awassi sheep to scrapie in three regions (Palestine, Saudi Arabia, and Turkey) using blood samples. There were 50 animals in each group. Comparing this, it can be concluded that for the majority of Hungarian examined sheep breeds (except for the black variant of the Hungarian Racka), better results were observed compared to the three regions. In Turkey, the ARQ/ARQ genotype was the most common among the examined sheep, like the Hungarian indigenous Cikta breed. In the Palestinian Authority and Saudi Arabia, the ARQ/ARH allele occurred in the highest proportion. In Saudi Arabia, not a single individual belonged to the R1 category and the R2 and R3 categories were the most common. The R5 category was present only in Turkey at 2% [43]. The Hungarian native and endangered breeds show more promising results.

In Italy, 12 local Italian sheep breeds (Juraschaf, Tiroler Steinschaf, Tiroler Bergschaf, Plezzana, Schnalser Bergschaf, Schwarzbraunes Bergschaf, Villnoesser Brillenschaf, Alpago, Brogna, Foza, Lamon, and Carsolina) were compared based on risk groups and genotypes. The proportion of the ARR/ARR genotype was most favourable in the case of Lamon and Carsolina, ranging between 11–14%. The proportion of R2 and R3 risk groups was more than 75% for all breeds. Overall, the local Hungarian breeds, apart from Cikta, are in a more advantageous position than the local Italian breeds, which is also reflected in the prevalence of the R1 category. In the case of Tiroler Bergschaf and Plezzana breeds, the R4 risk group was absent, similar to the Cikta breed in Hungary. However, for the other breeds, this proportion was similar to that of the Hungarian breeds [44].

A 2022 Irish study analysed the scrapie test results of Belclare, Charollais, Suffolk, Texel, Vendeen, and crossbred sheep. The lowest occurrence rate of the R1 risk group was observed in the Texel breed (44.7%), which is worse than in the indigenous Transylvanian Racka (59.45%) and the Hungarian Merino. The best results were observed in the Suffolk (85.93%), Charollais (81.08%), and Vendeen (75.58%) breeds. A total of 69.58% of the Belclare breed individuals and 62.64% of the crossbred sheep could be classified into this category. Based on that, most breeds examined in Ireland were in a significantly better position regarding susceptibility to scrapie. However, it can also be noted that the R1 group occurred at approximately the same rate (69%) in the Belclare and the endangered Hungarian Merino breeds. Similar to the 2019–2023 period we analysed, the VRQ/VRQ genotype did not occur in purebred individuals in Ireland. Additionally, we can conclude that the ARR/ARQ genotype occurred at a higher rate in the breeds we examined compared to the Irish study. In this study, it was also found that ewes with the ARH/VRQ allele were 3.79 kg heavier than other genotypes [3].

From a breeding point of view, it is important to note that if a breed is small, it should not be selected as much as in the case of breeds with a larger number of individuals. We may see an improvement in the prevalence of risk groups, but we may also see a higher level of inbreeding. The conservation of genetic variability is of particular importance in the case of the indigenous Hungarian sheep breeds under national protection that we have analysed, and in the case of the endangered Hungarian merino, because of the small population size.

5. Conclusions

Scrapie eradication is important to increase the proportion of more resistant genotypes and reduce the proportion of susceptible genotypes in all breeds. It is critical to increase the proportion of scrapie-resistant genotypes within the Hungarian sheep population to eradicate this highly infectious and fatal disease.

The results from this study confirm that scrapie-resistant genotypes have been enhanced; however, we do not know what other valuable traits (for example: milk yield, growth production, fertility, and hardiness) may have been lost during the selection process focused on this aspect. It would be important to implement additional molecular genetic tests, beyond the mandatory scrapie screening for breeding rams, as prescribed in the breeding program of the Hungarian Sheep and Goat Breeders' Association. These additional tests should be made compulsory within the breeding program framework.

At the moment, we do not yet know what other traits of importance for production and breeding will be lost by selecting for more favourable scrapie risk groups. It would also be important to examine correlations between, for example, birth weight, daily body weight gain, fat thickness, body composition, daily milk production, milk composition parameters, number of lambs born, and scrapie risk groups, in order to obtain more accurate and complex information for selecting our animals and implementing the breeding programme.

Since the start of the examination, we have observed better results for all native varieties, except for the zikta variety and including the endangered Hungarian merino, in the 2019–2023 survey period than in 2004.

Given that the breeds under study are native and/or endangered, their selection requires careful professional work with a breeding approach focused on gene conservation. The breeding programme in Hungary allows the use of individuals from the R1-R2-R3 risk groups. It is important to emphasise that the R3 ram is just as valuable to the population. These individuals in the risk groups have been neglected for breeding in many cases over the last decade, so it is important to not only emphasise their importance but also to breed them in larger harems to maintain genetic balance.

Regarding scrapie, Hungary is a “low-risk” category country; however, it is still important to follow the exemption program and apply a continuous monitoring system. The means for this can be the further breeding of scrapie-resistant animals and brain examination of a sufficient number of dead animals per year.

In the future, the reduction of the presence of the VRQ allele is essential, alongside the further increase in the frequency of the ARR allele. It is also important to preserve the genetic diversity of the local breeds while gradually reducing susceptibility.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture15080880/s1>, Characteristics of the breeds. References [45–63] are cited in the Supplementary Materials.

Author Contributions: Conceptualisation, J.O. and A.L.; methodology, J.O.; software, E.I.B.; validation, E.I.B. and J.O.; formal analysis, A.L.; resources, E.I.B. and J.O.; data curation, A.L.; writing—original draft preparation, E.I.B. and J.O.; writing—review and editing, R.K. and F.K.; visualisation, E.I.B.; supervision, J.O.; and project administration, R.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors thank the breeding organization for their collaboration.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Dexler, H. Traberkrankheit. In *Tierheilkunde und Tierzucht: Eine Enzyklopädie der Praktischen Nutztierkunde*; Stang, V., Wirth, D., Eds.; Urban & Schwarzenberg: Berlin, Germany; Wien, Austria, 1931; p. 807.
- Rusvai, M. Fertőző betegségek—Vírusos eredetű betegségek. In *A Bárány- és a Juhhús Fenntarthatósága*; Kukovics, S., Ed.; Juh Terméktanács-Interovic: Budapest, Hungary, 2021; pp. 171–194.
- McHugh, N.; O’Brien, A.C.; Pabiou, T.; McDermott, K.; Berry, D.P. Association between the prion protein genotype and animal performance traits in a large multibreed sheep population. *Animal* **2022**, *16*, 100587. [[CrossRef](#)]
- Act No. XXX of 2012 Concerning Hungarian National Values and Hungaricums. 11 April 2012. Available online: <https://leap.unep.org/en/countries/hu/national-legislation/act-no-xxx-2012-concerning-hungarian-national-values-and> (accessed on 22 November 2024).
- Dzialas, F. Die Entwicklung und die Bedeutung der Schafhaltung in der Deutschen Landwirtschaft Während des 19 Jahrhunderts. Ph.D. Thesis, Hohen Philosophischen Fakultät der Universität Jena, Budapest, Hungary, 1897.
- Reckzeh, C. Immunhistochemische Untersuchungen zum Vorkommen von Scrapie Prionprotein in Ovinen Geweben. Ph.D. Thesis, Freien Universität Berlin, Berlin, Germany, 2010.
- Áldássy, P.; Süveges, T. A juhok surlókórjának hazai előfordulása. *Hun. Vet. J.* **1964**, *19*, 463–465.
- Soós, F. XII. A juhok egészségtana. In *Juhtenyésztés A-tól Z-ig*; Jávora, A., Kukovics, S., Molnár, G., Eds.; Mezőgazda Kiadó: Budapest, Hungary, 2006; pp. 288–311.

9. Kovács, E.; Mitro, S.; Tempfli, K.; Zenke, P.; Maróti-Agóts, Á.; Sáfár, L.; Bali Papp, Á.; Gáspárdy, A. A specific selection programme is required in the autochthonous Cikta Sheep which is endangered by own frequent ARQ prion haplotype? *Appl. Agric. For. Res.* **2017**, *67*, 141–146. [[CrossRef](#)]
10. Rabenau, H.F. Teil III. Spezielle Mikrobiologische Diagnostik, 2 Prionen: 28 Diagnostik prionbedingter Erkrankungen, TSE-Erreger, Übertragungswege bei Tieren. In *Mikrobiologische Diagnostik: Bakteriologie-Mykologie-Virologie-Parasitologie*; Neumeister, B., Geiss, H.K., Braun, R.W., Kimmig, P., Eds.; Thieme Verlag: Stuttgart, Germany, 2009; pp. 635–639.
11. NÉBIH: Fertőző Szivacsos Agyvelőbántalmakkal Kapcsolatos Tájékoztató Anyag. 2024. Available online: <https://portal.nebih.gov.hu/-/fertozo-szivacsos-agyvelobantalmakkal-kapcsolatos-tajekoztato-anyag> (accessed on 25 November 2024).
12. Andréoletti, O.; Orge, L.; Benestad, S.L.; Beringue, V.; Litaïse, C.; Simon, S.; Le Dur, A.; Laude, H.; Simmons, H.; Lugan, S.; et al. Atypical/Nor98 scrapie infectivity in sheep peripheral tissues. *PLoS Pathog.* **2011**, *7*, e1001285. [[CrossRef](#)]
13. FVM (2003): 69/2003. (VI.25.) FVM Rendelet a Fertőző Szivacsos Agyvelőbántalmak Megelőzéséről, az Ellenük Való Védekezésről, Illetve a Leküzdésükről. Available online: <https://net.jogtar.hu/jogszabaly?docid=A0300069.FVM&xtreferer=99700041.FM> (accessed on 13 January 2010).
14. FVM (2009): 179/2009. (XII.29.) FVM Rendelet a Fertőző Szivacsos Agyvelőbántalmak Megelőzéséről, az Ellenük Való Védekezésről, Illetve a Leküzdésükről. Available online: <https://net.jogtar.hu/jogszabaly?docid=a0900179.fvm> (accessed on 1 January 2023).
15. Anton, I.; Zsolnai, A.; Fésüs, L.; Kovács, A.; Kukovics, S.; Molnár, A.; Oláh, J.; Jávör, A. A juhok surlókorja 4. A priongenotípusok gyakorisága muflonban, valamint szőrös és vedlőgyapjas házijuokban. *Hun. Vet. J.* **2007**, *129*, 610–614.
16. Agrobiogen GmbH: Scrapie Resistenz. Agrobiogen GmbH Biotechnologie. Available online: <https://www.agrobiogen.de/leistungen/schaf/scrapie-resistenz> (accessed on 7 November 2024).
17. EK: Regulation (EC) No. 999/2001 of the European Parliament and of the Council of 22 May 2001 Laying Down Rules for the Prevention, Control and Eradication of Certain Transmissible Spongiform Encephalopathies. Available online: <http://data.europa.eu/eli/reg/2001/999/oj> (accessed on 11 April 2024).
18. Kovács, E.; Tempfli, K.; Harmat, L.; Zenke, P.; Maróti-Agóts, Á.; Bali Papp, Á.; Sáfár, L.; Gáspárdy, A. A cikta juh hímvárú részpopulációjának surlókor elleni genetikai rezisztenciája a mentesítés másfél évtizedét követően. *Anim. Breed. Feed.* **2020**, *69*, 101–110.
19. Gáspárdy, A.; Holly, V.; Zenke, P.; Martóti-Agóts, Á.; Sáfár, L.; Bali-Papp, Á.; Kovács, E. The response of prion genic variation to selection for scrapie resistance in Hungarian indigenous sheep breeds. *Acta Vet. Hung.* **2018**, *66*, 562–572. [[CrossRef](#)] [[PubMed](#)]
20. Fésüs, L.; Anton, I.; Zsolnai, A. A juhok surlókorja (genetikai vonatkozások)—Irodalmi áttekintés. *Hun. Vet. J.* **2002**, *124*, 726–729.
21. Fésüs, L.; Zsolnai, A.; Anton, I.; Sáfár, L. Breeding for scrapie resistance in the Hungarian sheep population. *Acta Vet. Hung.* **2008**, *56*, 173–180. [[CrossRef](#)]
22. Sartore, S.; Rasero, R.; Colussi, S.; Acutis, P.L.; Peletto, S.; Soglia, D.; Maione, S.; Spalenza, V.; Sacchi, P. Effect of selection for scrapie resistance on genetic diversity in a rare and locally adapted sheep breed: The case of Sambucana. *Livest. Sci.* **2013**, *157*, 75–80. [[CrossRef](#)]
23. Vitale, M.; Migliore, S.; La Giglia, M.; Alberti, P.; Di Marco, L.; Presti, V.; Langeveld, J.P. Scrapie incidence and PRNP polymorphisms: Rare small ruminant breeds of Sicily with TSE protecting genetic reservoirs. *BMC Vet. Res.* **2016**, *12*, 141. [[CrossRef](#)]
24. Balkema-Buschmann, A. *Verteilung von Infektiosität und Krankheitsassoziiertem Prion-Protein in Geweben von mit Klassischen Oder Atypischen TSE-Erregern Infizierten Rindern und Schafen*; Institut für Neue und Neuartige Tierseuchenerreger am Friedrich-Loeffler-Institut und dem Institut für Virologie der Stiftung Tierärztliche Hochschule Hannover: Hannover, Germany, 2011.
25. Goldmann, W.; Hunter, N.; Smith, G.; Foster, J.; Hope, J. PrP genotype and agent effects in scrapie: Change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *J. Gen. Virol.* **1994**, *75*, 989–995. [[CrossRef](#)]
26. Belt, P.B.; Muileman, I.H.; Schreuder, B.E.; Bos-de Ruijter, J.; Gielkens, A.L.; Smits, M.A. Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. *J. Gen. Virol.* **1995**, *76*, 509–517. [[CrossRef](#)] [[PubMed](#)]
27. Dawson, M.; Hoinville, L.J.; Hunter, N. Guidance on the use of PrP genotyping as an aid to the control of clinical scrapie. Scrapie Information Group. *Veter. Rec.* **1998**, *142*, 623–625.
28. von Distl, O. Züchterische Kontrolle der Empfänglichkeit für die Traberkrankheit (Scrapie) beim Schaf über Molekulargenetische Testverfahren. *Tierärztl. Umschau* **2000**, *55*, 609–623.
29. Baylis, M.; Chihota, C.; Stevenson, E.; Goldmann, W.; Smith, A.; Sivam, K.; Tongue, S.L.; Gravenor, M.B. Risk of scrapie in British sheep of different prion protein genotype. *J. Gen. Virol.* **2004**, *85*, 2735–2740. [[CrossRef](#)] [[PubMed](#)]
30. HSGBA: Hungarian Sheep and Goat Breeders' Association's (HSGBA) Breeding Plan. Available online: <https://mjkszu.hu/tenyesztes/tenyesztesi-program> (accessed on 12 December 2024).
31. NÉBIH: Tájékoztató a Fertőző Szivacsos Agyvelőbántalmakról. Available online: https://portal.nebih.gov.hu/aktualitasok/hirek/friss-hirek/hirek2/-/asset_publisher/4ndba0yRXvQX/content/tajekoztato-a-fertozo-szivacsos-agyvelobantalmakrol/pop_up (accessed on 7 November 2024).

32. Biscarini, F.; Nicolazzi, E.L.; Stella, A.; Boettcher, P.J.; Gandini, G. Challenges and opportunities in genetic improvement of local livestock breeds. *Front. Genet.* **2015**, *6*, 33. [[CrossRef](#)]
33. Gandini, G.; Turri, F.; Rizzi, R.; Crotta, M.; Minozzi, G.; Pizzi, F. Economic evaluation of genetic improvement in local breeds: The case of the Verzaschese goat. *Ital. J. Anim. Sci.* **2017**, *16*, 199–207. [[CrossRef](#)]
34. Perucho, L. Rôle des Pratiques de Gestion Génétique dans L'adéquation Entre Troupeaux de Races Locales et Conduites Pastorales. Ph.D. Thesis, Sciences du Vivant [q-bio], Montpellier SupAgro, Montpellier, France, 2018.
35. Tsiokos, D.; Ligda, C. Monitoring inbreeding and selection on scrapie resistance in a closed nucleus of Florina sheep breed. *Small Rumin. Res.* **2021**, *201*, 106422. [[CrossRef](#)]
36. European Food Safety Authority (EFSA). The European Union summary report on surveillance for the presence of transmissible spongiform encephalopathies (TSE) in 2023. *EFSA J.* **2024**, *22*, e9097. [[CrossRef](#)]
37. European Food Safety Authority (EFSA). Transmissible Spongiform Encephalopathies (TSEs) Effective Monitoring Keeps TSEs Under Control. 21 October 2024. Available online: <https://storymaps.arcgis.com/stories/f3dc669cc2994fca35526ccdb696df2> (accessed on 12 February 2025).
38. Aphis. Available online: <https://www.aphis.usda.gov/livestock-poultry-disease/sheep-goat/scrapie> (accessed on 28 November 2024).
39. Drögemüller, C.; Leeb, T.; Distl, O. PrP genotype frequencies in German breeding sheep and the potential to breed for resistance to scrapie. *Veter. Rec.* **2001**, *149*, 349–352. [[CrossRef](#)] [[PubMed](#)]
40. Kovács, E.; Tempfli, K.; Shannon, A.; Zenke, P.; Maróti-Agóts, Á.; Sáfár, L.; Bali Papp, Á.; Gáspárdy, A. STR diversity of a historical sheep breed bottlenecked, the Cikta. *J. Anim. Plant Sci.* **2019**, *29*, 41–47.
41. Posta, J.; Kovács, E.; Tempfli, K.; Sáfár, L.; Bali-Papp, Á.; Gáspárdy, A. A kis létszámban átmentett Cikta juh származási adatainak értékelése különös tekintettel a családokra. *Magy. Állatorvosok Lapja* **2019**, *141*, 171–180.
42. Cameron, C.; Bell-Rogers, P.; McDowall, R.; Rebelo, A.R.; Cai, H.Y. Prion protein genotypes of sheep as determined from 3343 samples submitted from Ontario and other provinces of Canada from 2005 to 2012. *Can. J. Vet. Res.* **2014**, *78*, 260–266.
43. Rashaydeh, F.S.; Yildiz, M.A.; Alharthi, A.S.; Hani, H.; Al-Baadani, H.H.; Alhidary, I.A.; Meydan, H. Novel Prion Protein Gene Polymorphisms in Awassi Sheep in Three Regions of the Fertile Crescent. *Vet. Sci.* **2023**, *10*, 597. [[CrossRef](#)] [[PubMed](#)]
44. Bordin, F.; Zulian, L.; Granato, A.; Caldon, M.; Colamonico, R.; Mutinelli, F. Prion protein (PRNP) gene polymorphisms analysis and susceptibility to scrapie of native sheep breeds from North-Eastern Italy. *Large Anim. Rev.* **2022**, *28*, 33–40.
45. Jánosi, J.Z.; Matyóka, K.; Sáfár, L. Cikta. In *Régenhonos juh- és Kecskefajtáink*; Sáfár, L., Ed.; HVG Press: Budapest, Hungary, 2017; pp. 106–138; ISBN 978-963-12-8668-7.
46. Schandl, J. *Juhtenyésztés*; Mezőgazdasági Kiadó: Budapest, Hungary, 1955; p. 264.
47. Korom, I. *Juhtenyésztés*; Debreceni Agrártudományi Egyetem: Debrecen, Hungary, 1980; p. 280.
48. Jávor, A. A cikta juh. In *Régi Magyar Juhfajták*; Jávor, A., Ed.; Mezőgazda Kiadó: Budapest, Hungary, 2006; pp. 90–101.
49. Koppány, G. The Cikta sheep. In *Living Heritage: Old Historical Hungarian Livestock*; Bodó, I., Ed.; Agroinform: Budapest, Hungary, 2000; pp. 58–59.
50. Gáspárdy, A.; Sáfár, L. Óshonos Juhfajtáink. In *Cigája és Tejelő Cigája*; HVG Press: Budapest, Hungary, 2014; p. 43.
51. Gáspárdy, A. A cigája vagy berke. (The Tsigai or Hungarian Berke). In *"Eleven örökség" (Living Heritage)*; Bodó, I., Ed.; Agroinform Kiadó és Nyomda Kft: Budapest, Hungary, 2000; pp. 60–62; ISBN 963 502 720 6.
52. Mihálka, T.; Veress, L. Juhtenyésztés. In *Állattenyésztés II. Kötet*; Horn, A., Ed.; Mezőgazdasági Kiadó: Budapest, Hungary, 1976; pp. 229–335.
53. Veress, L.; Jávor, A. *A Juh Tenyésztése és Tartása*; Debreceni Agrártudományi Egyetem: Debrecen, Hungary, 1990; p. 198.
54. Gáspárdy, A.; Eszes, F.; Bodó, I.; Koppány, G.; Keszthelyi, T.; Márton, F. A cigája (berke) juh fajta hazai változatainak alkattani összehasonlító vizsgálata/Type comparison of different Hungarian Tsigai/Berke/sheep variants. *Anim. Br. F-(Állatteny és Tak.)* **2001**, *50*, 33–42.
55. Kukovics, S. A cigája juh. In *Régi Magyar Juhfajták*; Jávor, A., Ed.; Mezőgazda Kiadó: Budapest, Hungary, 2006; pp. 37–89.
56. Gáspárdy, A.; Sáfár, L. Cigája és tejelő cigája. In *Régenhonos juh- és Kecskefajtáink*; Sáfár, L., Ed.; HVG Press: Budapest, Hungary, 2017; pp. 64–105; ISBN 978-963-12-8668-7.
57. Kukovics, S. Tejelő cigája. In *Tenyésztési- és Fajtahasználati Útmutató/Guidebook for Breeding and Breed Utilization*; Jávor, A., Fésűs, L., Eds.; Lícium-Art Könyvkiadó- és Kereskedelmi Kft.: Debrecen, Hungary, 2000; pp. 81–82.
58. Veress, L. Juhfajták. In *Juhtenyésztők Kézikönyve*; Veress, L., Jankowski, S.T., Schwark, H.J., Eds.; Mezőgazdasági Kiadó: Budapest, Hungary, 1982; pp. 79–118.
59. Kukovics, S. A tenyésztett fajták, és a fajtapolitikát befolyásoló tényezők. In *Juhtenyésztés haladónak az Extenziótól a Precízióig*; Jávor, A., Ed.; Debreceni Egyetem–Juh Terméktanács: Debrecen, Hungary, 2022; pp. 78–161.
60. Földi, D.; Földi, G.y.; Sáfár, L. Gyimesi racka. In *Régenhonos juh- és Kecskefajtáink*; Sáfár, L., Ed.; HVG Press: Budapest, Hungary, 2017; pp. 139–179; ISBN 978-963-12-8668-7.
61. Mucsi, I. Fajták. In *Juhtenyésztés és -Tartás*; Mezőgazda Kiadó: Budapest, Hungary, 1997; pp. 30–51.

62. Sáfár, L.; Domanovszki, Á. Magyar merinó. In *Tenyésztési- és Fajtahasználati Útmutató*; Jávor, A., Fésűs, L., Eds.; Lícium-Art Könyvkiadó- és Kereskedelmi Kft.: Debrecen, Hungary, 2000; pp. 17–20.
63. Yousefi, V.; Kóbori, J. *A Merinói Juhok Tenyésztése és Kiválasztása*; Szaktudás Kiadó Ház Rt.: Budapest, Hungary, 2002; p. 200.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.