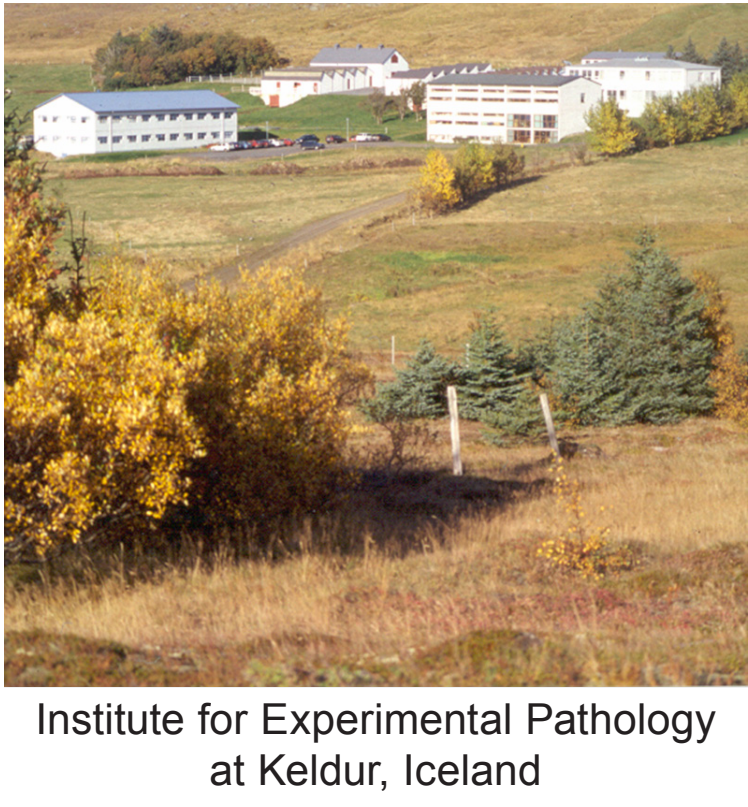


Recent findings of scrapie protective genotypes in Iceland supported by case-control studies in Icelandic scrapie flocks

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Background

Scrapie in sheep is endemic in Northern Iceland and has endured rigorous methods to fight the disease in the past. Breeding for scrapie resistance could not be adopted until recently after a widespread search for scrapie protective prion protein variants in Iceland resulted in the first findings of Icelandic sheep carrying ARR. Furthermore, AT₁₃₇RQ, shown to be protective against scrapie in Sarda sheep in Italy, was found on several farms. The purpose of this study was to access the influence of possibly protective genotypes in Icelandic sheep.

Scrapie surveillance in Iceland—historical overview

1878 Scrapie believed to be imported to Iceland
1878–1978 Passive surveillance (clinical suspects). Notifiable disease
1930–1940 Quarantine zones and stamping out (Karakul diseases)

Active scrapie surveillance. Steps taken towards scrapie eradication

1978 Testing of healthy slaughter by histopathology (HP), culling of scrapie flocks in newly infected areas
1986 Culling of all scrapie flocks and farmers get compensated
1993 Cleaning and disinfection of premices, period without sheep (2–3 years), restocking from scrapie-free areas
2004 Rapid testing of abattoir samples by elisa and typing by WB
2012 Atypical/Nor98 scrapie flocks not culled (spontaneous, not infectious)
2023 Only sheep with susceptible genotypes culled on scrapie farms

Material and Methods

A case-control study was performed on 18 Icelandic scrapie flocks detected in the period: 1997–2024, originating from six different movement protection zones. Total number of sheep analysed was 4978 of which 406 tested positive for scrapie. Scrapie positive and negative sheep were compared in relation to PRNP genotypes. Typing of six codons: 136, 137, 138, 151, 154, 171. Results were analysed by using Fisher’s exact test/Odds ratio (OR).

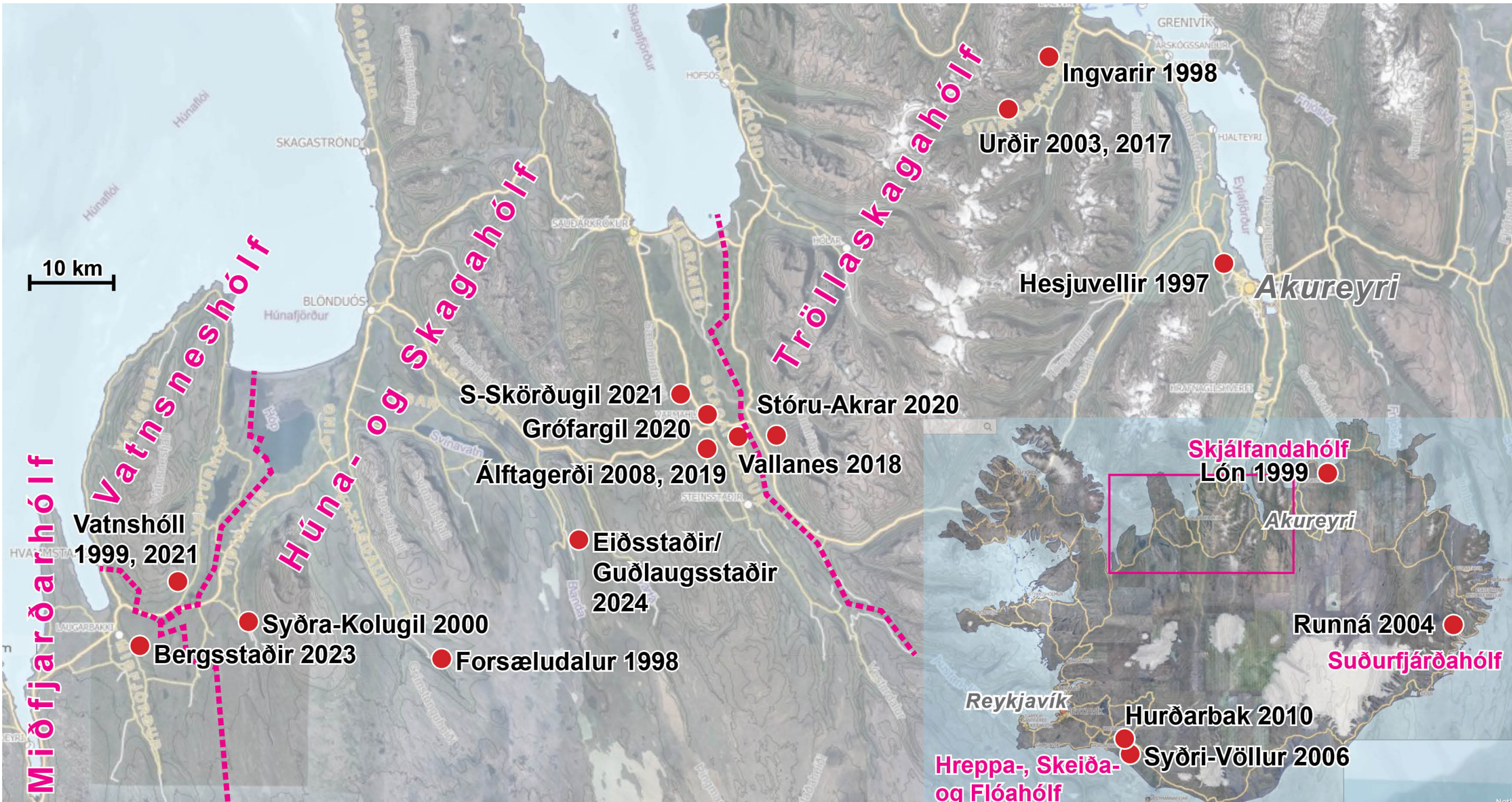


Figure 4. Location of scrapie farms in study

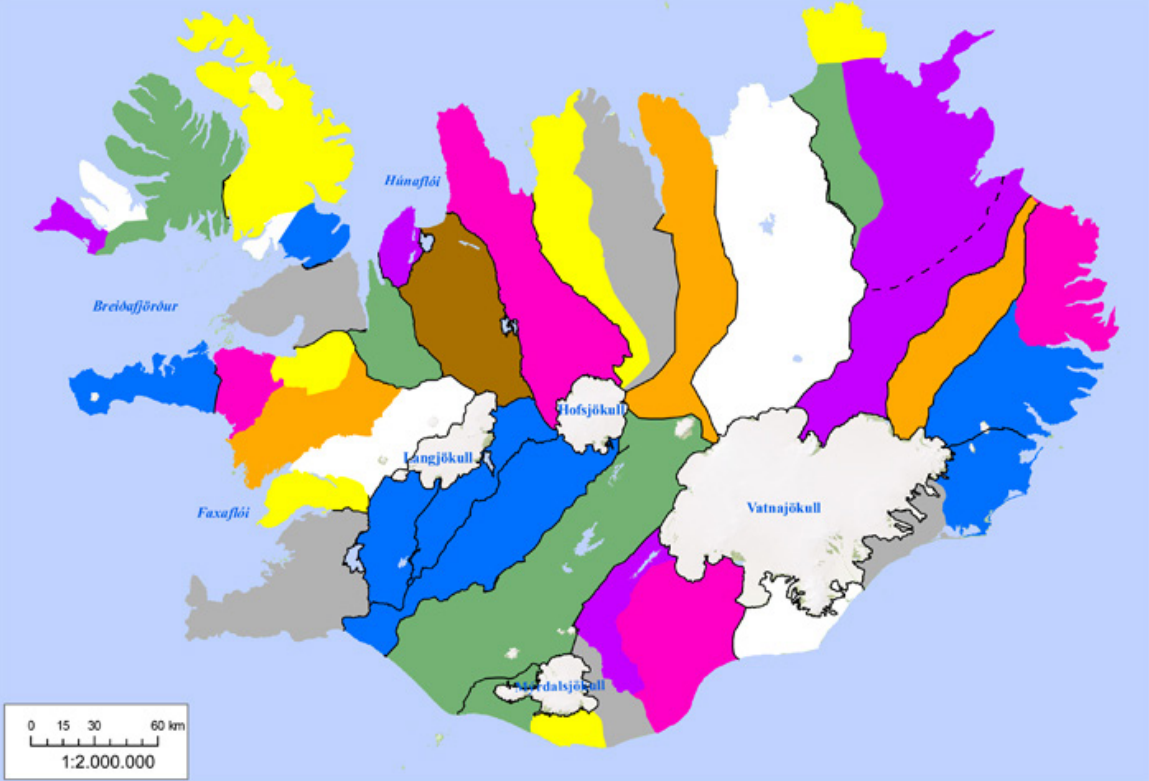


Figure 1. Movement restriction zones/Quarantine zones

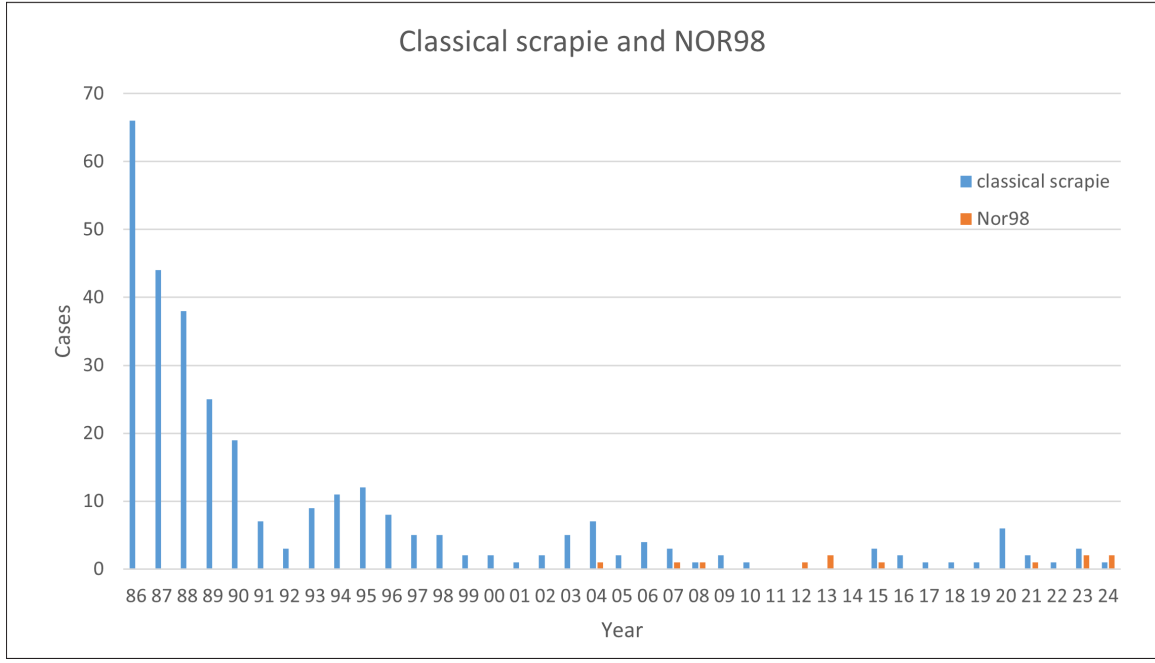


Figure 2. Number of scrapie cases 1986–2024

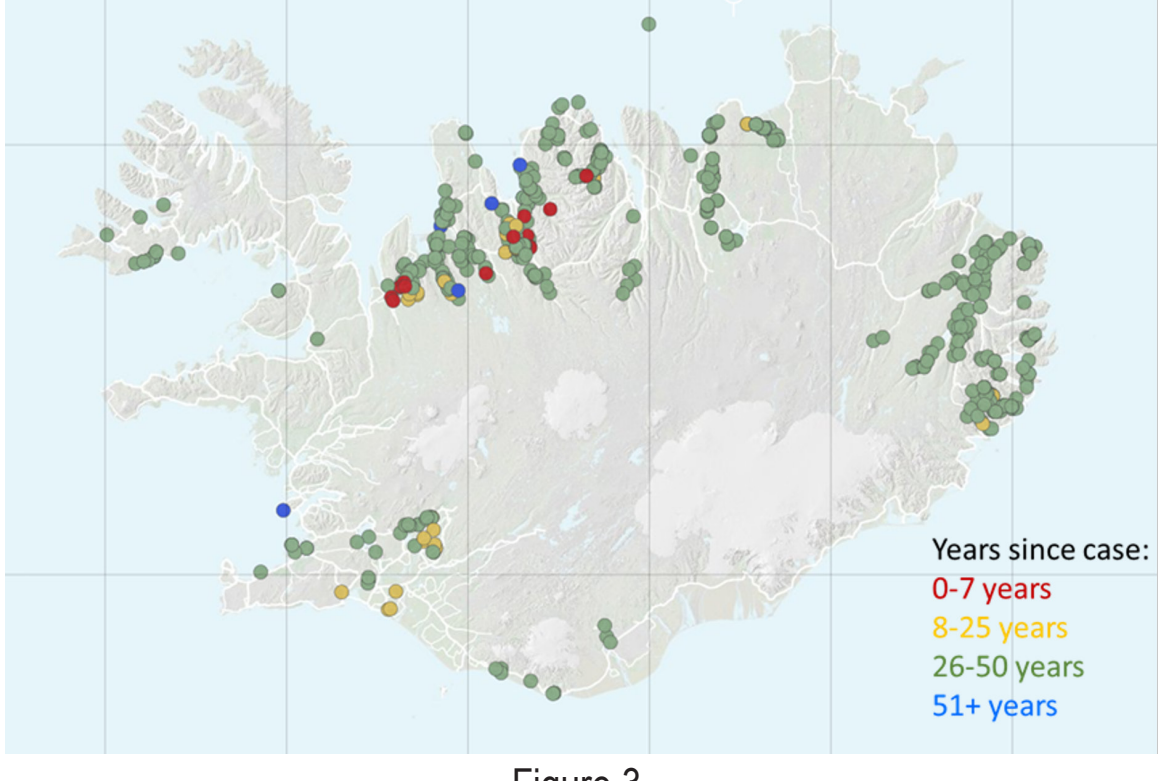


Figure 3. Distribution of classical scrapie cases
Map: Icelandic Food and Veterinary Authority (<https://landdyggingar.mast.is/>)

All 18 flocks	ARQ/ARQ	ARQ/VRQ	VRQ/VRQ	T137/ARQ	T137/VRQ	T137N138	T137/AHQ	N138/ARQ	N138/VRQ	N138/AHQ	N138C151	N138N138	C151/ARQ	C151/AHQ	C151/VRQ	C151C151	AHQ/ARQ	AHQ/VRQ	AHQ/AHQ	ARR/VRQ
4978 neg	3212	401	17	7	1	1	2	312	10	25	13	27	73	10	10	1	376	53	14	1
406 pos	221	152	20	0	0	0	0	6	5	0	0	0	0	0	0	0	1	0	0	0
4978 sum	3433	553	37	7	1	1	2	318	21	25	13	27	73	10	11	1	377	53	14	1
All 18 flocks, percentage	ARQ/ARQ	ARQ/VRQ	VRQ/VRQ	T137/ARQ	T137/VRQ	T137N138	T137/AHQ	N138/ARQ	N138/VRQ	N138/AHQ	N138C151	N138N138	C151/ARQ	C151/AHQ	C151/VRQ	C151C151	AHQ/ARQ	AHQ/VRQ	AHQ/AHQ	ARR/VRQ
neg	70.3%	8.6%	0.4%	0.2%	0.0%	0.0%	0.0%	8.8%	0.3%	0.5%	0.3%	0.6%	1.6%	0.2%	0.2%	0.0%	8.2%	1.2%	0.3%	0.0%
pos	54.4%	37.4%	4.9%	0.0%	0.0%	0.0%	0.0%	1.5%	1.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.2%	0.0%	0.0%	0.0%
sum	69.0%	11.1%	0.7%	0.1%	0.0%	0.0%	0.0%	6.4%	0.4%	0.5%	0.3%	0.5%	1.5%	0.2%	0.2%	0.0%	7.6%	1.1%	0.3%	0.0%
% of positives within this genotype	6.4%	27.5%	54.1%	0.0%	0.0%	0.0%	0.0%	1.9%	23.8%	0.0%	0.0%	0.0%	0.0%	0.0%	9.1%	0.0%	0.3%	0.0%	0.0%	0.0%
Odds Ratio in relation to ARQ/ARQ (wild type)	1	5.51	17.10	0.00	0.00	0.00	0.00	0.28	4.54	0.00	0.00	0.00	0.00	0.00	1.45	0.00	0.04	0.00	0.00	0.00
Pearson's Chi (every genotype and ARQ/ARQ): p =	<0.0001	<0.0001	0.4987	0.7982	0.7982	0.7175	0.0011	0.1899	0.0019	0.3444	0.1731	0.0259	0.4099	0.6841	0.7931	<0.0001	0.0593	0.3265	0.7931	0.0001
Fisher's exact (every genotype and ARQ/ARQ): p =	<0.0001	<0.0001	1.0000	1.0000	1.0000	1.0000	0.0013	0.0051	0.3993	1.0000	0.4027	0.0215	1.0000	0.5029	1.0000	<0.0001	0.0481	1.0000	1.0000	0.0001
while: p >= 0.05																				

Table 1. Results from case-control study

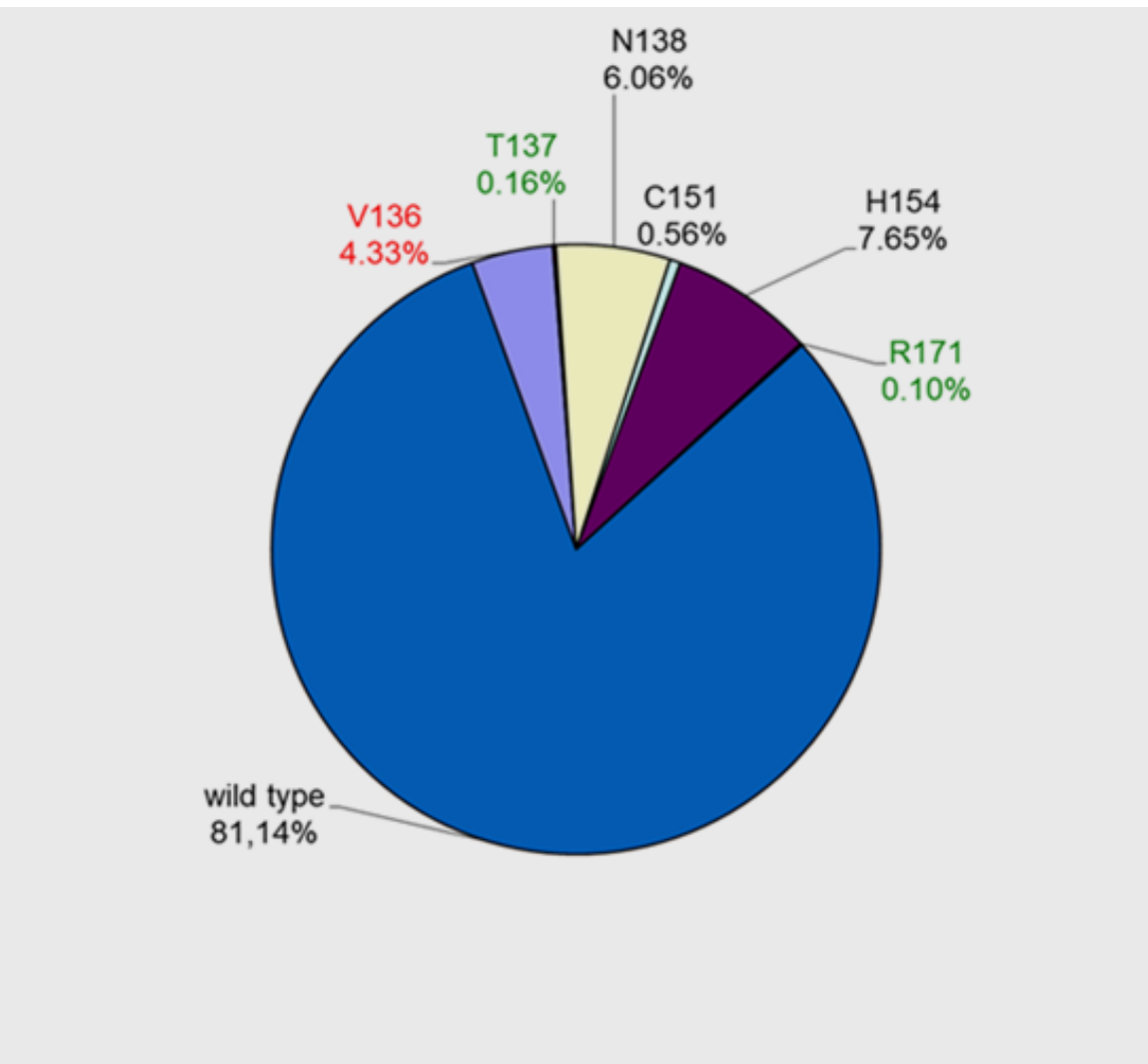


Figure 5. Variants of the prion protein in Icelandic sheep. % of alleles

Case-control study – summary of results

Total number of 20 different PRNP genotypes were detected, when polymorphism at six codons (136, 137, 138, 151, 154, 171) was considered. Significant higher scrapie susceptibility compared to wild type (ARQ/ARQ); ARQ/VRQ, VRQ/VRQ, AN₁₃₈RQ/VRQ. Significant lower scrapie susceptibility compared to wild type (ARQ/ARQ); AC₁₅₁RQ/ARQ, AHQ/ARQ, AHQ/VRQ, AN₁₃₈RQ/ARQ. No scrapie-positive sheep carrying AT₁₃₇RQ was identified, however, the frequency of genotypes with AT₁₃₇RQ in scrapie-affected flocks was too low for statistical validation.

Additional information:
AC₁₅₁RQ: very rare, one pos case detected; AC₁₅₁RQ /VRQ.
AHQ: two pos cases detected.
AN₁₃₈RQ: 18 pos cases detected (10 in this study); found in lymph nodes in 17 AN₁₃₈RQ animals in one scrapie flock.

Flag in sheep database	Haplotype	Position	Gene allele frequency – Adult sheep 2023–2024.
R171	ARR	Resistant (V)	1,5%
T137	AT ₁₃₇ RQ		0,5%
C151	AC ₁₅₁ RQ	Likely resistant (MV)	0,6%
H154	AHQ		9,3%
N138	AN ₁₃₈ RQ	Less Susceptible (N)	5,2%
	ARQ	Susceptible (N) Wild type	80,4%
V136	VRQ	High susceptible (N)	2,6%

Table 2. Categorization of PRNP haplotypes

Breeding for resistance

The presence of ARR and AT₁₃₇RQ offer the possibility of a breeding program for scrapie resistance in the Icelandic sheep breed. ARR has been found on three farms and AT₁₃₇RQ on a total of 17 farms. 15% of the sheep population has been genotyped. The scrapie endemic area in the North is targeted first for breeding for resistance, where the emphasis is on ARR. Potentially protective alleles have been categorized: AT₁₃₇RQ, AC₁₅₁RQ, AHQ (H154).

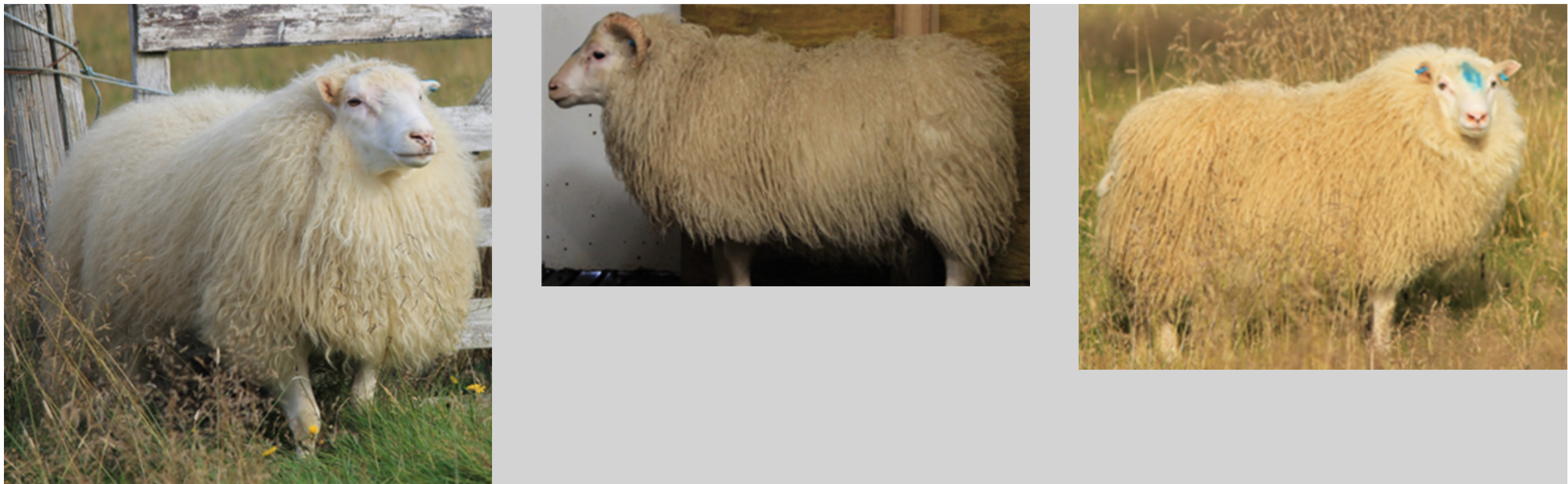


Figure 6. Gimsteinn, Gullmoli and Hornsteinn—the first ARR breeding rams of the Icelandic sheep breed, *Ovis brachyura borealis* pall



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Conclusion

A breeding program using ARR is already in use and based on the results of this case-control study, additional selection on three more alleles, AT₁₃₇RQ, AC₁₅₁RQ and AHQ, is recommended, both to assist in keeping the diversity of the sheep population and possibly provide protection against unforeseen changes in the scrapie infectious agent in the future.