

Report

Distribution of Prion protein alleles in Norwegian sheep breeds

Petter Hopp
Mette Valheim

To Norwegian Food Safety Authority
From National Veterinary Institute
Date 05.02.2005



Veterinærinstituttet
National Veterinary Institute

Introduction

This document compiles the results of a survey of the prion protein genotypes (*PrnP*) of the Norwegian breeds in accordance with EU Decision 2002/1003/EC (Anon., 2002a). The aim of the survey is to estimate the prevalence of the *PrnP*-alleles in each Norwegian sheep breed with special emphasis on the *PrnP*-allele: ARR, which is considered the most resistant allele to scrapie.

Participants and contributors

The Norwegian Food Safety Authority is responsible for the survey. The district offices of the Food Safety Authority have been responsible for the collection of the samples.

The Norwegian School of Veterinary Science is responsible for genotyping the samples. Ingrid Olsaker at the Norwegian School of Veterinary Science has also given valuable input to the report.

The National Veterinary Institute of Norway has organised the sampling and is responsible for writing the report. This document has been compiled by Petter Hopp and Mette Valheim.

Sheep breeds included in the survey

The Norwegian Sheep and Goat Association recognizes 15 sheep breeds in Norway (Dahl og Nævdal, 2002). These include indigenous breeds, imported breeds and breeds imported only for crossbreeding.

The "breed" Norwegian White Sheep is a population of crossbreed sheep. The introduction of the term in 2000 was a realization of reduced differences between some of the crossbreeds, especially Dala Sheep, Rygja Sheep, and Steigar Sheep due to centralized breeding for production traits. For this survey, it was decided to treat the Norwegian White Sheep, Dala Sheep, Rygja Sheep and Steigar Sheep as one "breed", hereafter designated Norwegian White Sheep. However, it was decided to sample 150 samples Norwegian White Sheep with 50 samples from the three regions: Eastern and Middle Norway (previously dominated by Dala Sheep), South and Western Norway (previously dominated by Rygja Sheep and Dala Sheep), and Northern Norway (previously dominated by Steigar Sheep and Dala Sheep). In addition, fifty samples were collected from Pure-bred Dala Sheep.

It was decided to exclude Grey Trønder Sheep from the survey as the breed consists of only two preserved flocks and 28 adult animals of these flocks had been tested previously (Høyheim m.fl., 2000). It was further decided to exclude Merino because only one single pure-bred flock is known of, and Oxford Down because there are no known pure-bred flocks. Finnsheep and East Friesian Milk Sheep were excluded as these breeds have only been used for crossbreeding with no known pure-bred animals in the population.

A list of the breeds surveyed is given in Table 1.

Selection of samples

Principles

Principles for the sampling were laid down in EU Decision 2002/1003/EC, annex II (Anon., 2002a).

- The sampling shall be carried out on sheep from flocks of high genetic merit
- At least 50 samples shall be collected from each breed
- Samples shall be chosen to be representative of the entire breed within the country

Rams used for artificial insemination (AI)

From each breed rams used for AI in 2003 were selected.

Table 1. Norwegian sheep breeds included in the survey with description of the breed type, population size, breeding system and number of collected samples. The total Norwegian sheep population is approximately 1,100,000 breeding sheep.

Breed	Breed type	Breeding population		No. genotyped animals	
		No. sheep	No. flocks	AI rams	Flock samples
Norwegian White Sheep	Population of Norwegian crossbreeds	850,000 *	EM: 820 †	20	30
			SW: 628 †	15	34
			NN: 208 †	4	50
Spæl Sheep	Norwegian breed, Northern Short-tailed type	200,000 *	265 †	10	36
Cheviot	Imported breed	20,000 *	76 †	3	48
Texel	Imported breed	20,000 *	34 ‡	5	46
Feral Sheep	Norwegian breed, Northern Short-tailed type	15-20,000	341 ‡	3	50
Norwegian Pelt Sheep	Norwegian breed, Northern Short-tailed type	10,000 *	18 †	2	50
Suffolk	Imported breed	< 10,000 *	42 ‡	2	48
Pure-bred Dala Sheep	Norwegian breed, crossbreed type	< 10,000	59 ‡	1	50
Blackface	Imported breed	< 10,000 *	25 ‡		51
Blæset Sheep	Norwegian breed, crossbreed type	500-1,000	106 ‡		49
Fuglestad Sheep	Norwegian breed, crossbreed type	500-1,000	84 ‡	1	50
Old Spæl Sheep	Norwegian breed, Northern Short-tailed type	ca. 500	101 ‡	1	55

EM = Eastern and Middle Norway, SW = Southern and Western Norway, NN = Northern Norway.

* Estimated by: "No. of breeding sheep" = "Total population" x "Proportion of sheep registered in the Sheep Recording System".

† Breeding flocks being members of ram circles.

‡ Breeding flocks being members of the respective breeding associations.

Selection of flocks

For breeds where the breeding was organised into ram circles, the sheep flocks participating in the ram circles were obtained from the Norwegian Sheep and Goat Association. For each breed, a random selection of flocks among the ram circle members was performed.

For the remaining breeds, lists of memberships were obtained from the relevant breeding associations. For each breed, a random selection of flocks among the breeding association members was performed. Flocks considered important for the production of breeding sheep by the breeding associations, were first selected for the survey.

The number of selected flocks per breed was chosen to be sufficient to examine 50 animals per breed, AI rams included, given that five animals in each flock were genotyped.

Selection of sheep within flocks

Five animals per flock were selected for *PrnP*-typing. The sampler was instructed to choose animals typical for the breed, breeding rams should be selected before ewes and, none of the sampled animals (within a flock) should have common grand-sires.

The samples were collected by the local office of The Norwegian Food Safety Authority.

PrnP-typing

Genotyping was performed on EDTA blood samples at the Norwegian School of Veterinary Science, Department of Basic Sciences & Aquatic Medicine. A 393 bp fragment of the coding region for exon three of the PrP gene, encompassing codons 92 to 222, was amplified by PCR with primers F294 (5'-agg ctg ggg tca agg tgg tag c-3') and R642 (5'-TGG TAC TGG GTG ATG CAC ATT TGC-3') modified by 5' attachment of M13-21 and M13 rev tails respectively, and analysed by sequencing with Big Dye Primer chemistry (Applied Biosystems). The PCR and sequencing reactions were repeated for samples yielding sequences of inferior quality or unexpected rare genotype combinations. Two persons were reading the codons independently followed by comparison of the results.

The sequences were read at codons 136, 154 and 171 and translated into the alleles ARR, ARQ, ARH, AHQ and VRQ. When the sequence result showed amino acid changes in more than one codon compared to the ARQ sequence, the allele combination resulting from only one mutation in each allele compared to ARQ was chosen.

Results

The frequency of the *PrnP*-alleles is given in Table 2.

Table 2. Prevalence of *PrnP*-alleles in Norwegian sheep breeds. The alleles are characterized by the amino acids in codon 136, 154 and 171. Differences in any other codons are included in ARQ.

Breed	No.	ARR	ARQ	VRQ	AHQ	ARH
Norwegian White Sheep EM	51	46.0	38.0	1.0	11.0	4.0
Norwegian White Sheep SW	49	50.0	22.4	7.1	6.1	14.3
Norwegian White Sheep NN	54	34.3	39.8	9.3	8.3	8.3
Spæl Sheep	46	14.1	64.1	2.2	19.6	-
Cheviot	51	31.4	32.4	14.7	20.6	1.0
Texel	51	29.4	35.3	6.9	8.8	19.6
Norwegian Pelt Sheep	52	-	68.3	16.3	15.4	-
Suffolk	50	19.0	66.0	12.0	2.0	1.0
Feral Sheep	53	22.6	53.8	7.5	16.0	-
Pure-bred Dala Sheep	50	49.0	28.4	9.8	8.8	3.9
Blæset Sheep	49	24.5	31.6	36.7	7.1	-
Fuglestad Sheep	51	30.4	32.4	8.8	26.5	2.0
Blackface	51	28.4	17.6	-	53.9	-
Old Spæl Sheep	56	9.8	69.6	4.5	16.1	-

EM = Eastern and Middle Norway, SW = South Western Norway, NN = Northern Norway

Interpretation of the results

The sheep flocks, from which the samples were collected, were a random sample of sheep flocks of high genetic merit within each breed. We therefore consider the sample to be representative for the sheep flocks of high genetic merit. These flocks will deliver breeding material to the whole breed. We do not expect the different prion protein haplotypes to be closely connected to other important traits of the animal. We therefore expect these flocks to be representative for the whole breed.

The animals collected within each flock should be unrelated, i.e. not have common grand-sires. This is in agreement with guidelines for sampling for genetic diversity of domestic animals (Anon., 1995) and we therefore consider this guideline as sufficient to ensure that the samples within flocks are unrelated.

The sample size of a minimum of 50 animals (equalling of 100 alleles) within each breed was given by the EU decision 2002/1003/EC (Anon., 2002a). The sample size is relatively small and do imply som uncertainty of the estimate. When the breeds are grouped according to the ARR-allele frequency using 10% and 25% for categorization, these limits are within the 95% confidence limits of the ARR-allele frequency estimate for some breeds (for example Spæl Sheep 14.6% [7.7% - 23%]). The Norwegian Pelt Sheep for which no ARR-allele was detected (0% [0% - 3.5%]), the upper confidence limit does not include 10%. Table 3 gives the 95% confidence limits for the ARR-allele frequency assuming a sample size of 100 alleles.

Table 3. The exact 95% confidence limits for the ARR-allele frequency assuming a sample size of 100 alleles.

ARR-allele frequency	Lower confidence limit	Upper confidence limit
30%	21.2%	40.0%
25%	16.9%	34.7%
20%	12.7%	29.2%
15%	8.7%	23.5%
10%	4.9%	17.6%
0%	0%	3.6%

Population size and ARR-allele frequency in relation to the EU Decisions

The EU Decision 2003/100/EC Annex I, part 3 (Anon., 2003) states that a derogation from the breeding programme may be granted for breeds which display an absence, or a level of less than 10%, of the ARR-allele. Further, a derogation from point 2 (c) and (d) of part 2 may be granted for breeds with an ARR-allele frequency of less than 25% or breeds which are in danger of being lost to farming, i.e. a population of less than 10,000 breeding sheep as defined in EU Regulation 445/2002/EC, Annex I (Anon., 2002b).

The population size is estimated from information from breeding associations or by extrapolating the percentage of Sheep Recording System to the whole sheep population. It has been difficult to obtain accurat values of the population sizes. Therefore, the population size should be checked further before any consequences for a potential breeding programme is taken. Table 4 gives the breeds grouped into categories based upon the ARR-allele frequency and the estimated population size.

Table 4. The Norwegian sheep breeds grouped in accordance with their ARR-allele frequency and the estimated population size.

Estimated population size (breeding sheep)	ARR-allele frequency			
	25 - 100%	10 - <25%	0 - <10%	Unknown
> 10 000	<ul style="list-style-type: none"> Norwegian White Sheep Cheviot Texel 	<ul style="list-style-type: none"> Spæl Sheep Feral Sheep 	<ul style="list-style-type: none"> Norwegian Pelt Sheep 	
< 10 000	<ul style="list-style-type: none"> Pure-bred Dala Sheep Fuglestad Sheep Blackface 	<ul style="list-style-type: none"> Suffolk Blæset Sheep 	<ul style="list-style-type: none"> Old Spæl Sheep 	<ul style="list-style-type: none"> Grey Trønder Sheep Merino Oxford Down

Referanser

- Anon., 1995. Global project for the maintenance of domestic animal genetic diversity (MoDAD). Draft project formulation report. Food and Agriculture Organization of the United Nations, Rome, 146 s.
- Anon., 2002a. Commission decision of 18 December 2002 laying down minimum requirements for a survey of prion protein genotypes of sheep breeds (Text with EEA relevance) (notified under document number C(2002) 5102). Off. J. Eur. Communities, 45: (L 349) 105-107.
- Anon., 2002b. Commission regulation (EC) No 445/2202 of February 2002 laying down detailed rules for the application of council regulation (EC) No 1257/1999 on support for rural development from the European agricultural Guidance and Guarantee fund (EAGGF). Off. J. Eur. Communities, 45: (L 74) 1-34.
- Anon., 2003. Commission decision of 13 February 2003 laying down minimum requirements for the establishment of breeding programmes for resistance to transmissible spongiform encephalopathies in sheep. Off. J. Eur. Communities, 46: (L 41) 41-45.
- Dahl, S., Nævdal, I., 2002. Saue- og geiteraser i Norge. Sau og Geit, 55: (2) 70-74.
- Høyheim, B., Espenes, A., Skretting, G., Olsaker, I., Tranulis, M., 2000. Genetiske forhold ved scrapie hos sau (in Norwegian, English summary). Nor. Vet. Tidsskr. 112: 350-358.