



Veterinærinstituttet
Norwegian Veterinary Institute

⋮ Annual Report

The surveillance programme for scrapie in Norway 2019



Comissioned by



The surveillance programme for scrapie in Norway 2019

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Authors

Ståle Sviland, Sylvie Lafond Benestad,
Petter Hopp

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Summary

In 2019, 18,693 sheep and 672 goats were examined for prion protein scrapie. Ten sheep from ten flocks were positive for scrapie Nor98. All the goats were negative for prion protein scrapie.

Introduction

Scrapie was first diagnosed in indigenous Norwegian sheep in 1981. Increasing numbers of scrapie-infected flocks were identified in the 1990s, culminating with 31 detected flocks in 1996 (Figure 1).

By the end of 2019, scrapie had been diagnosed in a total of 238 sheep flocks and one goat herd (1). Scrapie has been a notifiable disease in Norway since 1965, and control measures have involved destruction of all sheep in affected flocks and in close contact flocks until 2004. The Norwegian scrapie surveillance programme was launched in 1997 (2).

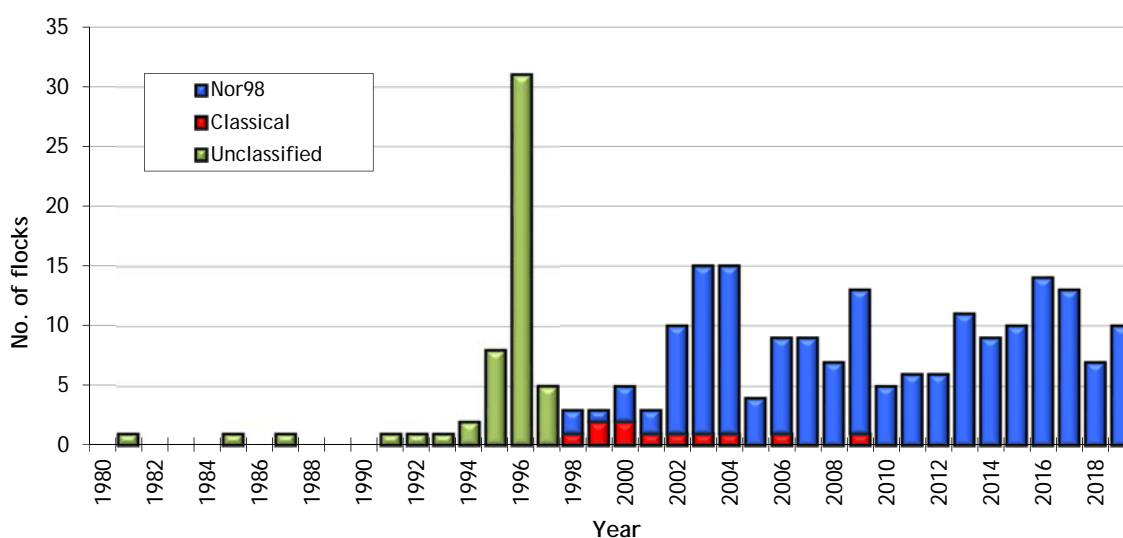


Figure 1. Annual number of sheep flocks and goat herds diagnosed with classical scrapie and Nor98 scrapie during the time period 1980-2019. Before 1998 the cases were not classified according to type of scrapie, but the majority of the scrapie cases are supposed to have been the classical type.

In 1998, a new type of scrapie, Nor98 scrapie, was identified in Norway. The diagnosis of Nor98 scrapie is verified by Western blot. Nor98 scrapie differs from classical scrapie in several aspects, including the Western blot profile, the distribution of protease resistant prion protein (PrP^{Sc}) in the brain, and absence of detectable PrP^{Sc} in lymphoid tissues (3). The main clinical sign observed in Nor98 scrapie cases has been ataxia. The PrP genotype distribution among Nor98 scrapie cases differs markedly from that of the cases with classical scrapie (4).

The Norwegian Food Safety Authority (NFSA) is responsible for carrying out the surveillance programme for scrapie. The samples from small ruminants with clinical signs consistent with scrapie and sheep at the abattoirs are collected by inspectors from the NFSA. The NFSA also carries out inspections of sheep flocks and goat herds. In 2019 the rendering company "Norsk Protein" started to sample brains from "Fallen Stock" on behalf of the NFSA. The employees involved, mainly truck drivers, have been trained in the sampling by attending courses arranged by the NFSA.

The Norwegian Veterinary Institute (NVI) is performing the laboratory examinations and the reporting of the results.

Aims

The aims of the surveillance programme are to identify scrapie infected sheep flocks and goat herds to support disease control and to estimate its prevalence in sheep and goats in the fallen stock and in the sheep population slaughtered for human consumption.

Materials and methods

In 2019, the surveillance programme was performed according to the European Union Regulations, Regulation (EC) No. 999/2001 Annex III, with amendments and included examination of the following categories of small ruminants:

- all small ruminants with clinical signs consistent with scrapie, irrespective of age.
- 10,000 sheep older than 18 months, which had died or been killed on the farm, but not slaughtered for human consumption (fallen stock).
- 10,000 randomly sampled healthy sheep older than 18 months slaughtered for human consumption.
- 500 goats older than 18 months which had died or been killed on the farm, but not slaughtered for human consumption (fallen stock).

Animals with clinical signs consistent with scrapie

When the sheep and goat farmers recognized sheep or goats with clinical signs consistent with scrapie, they were responsible for reporting the case to the NFSA.

If indicated, the animals were subject to either post mortem examination at a laboratory, or formalin-fixed and unfixed brain halves and medial retropharyngeal lymph nodes were submitted for laboratory examination. All the animals were examined at the NVI.

Surveillance of fallen stock

The sheep and goat farmers were responsible for reporting to the Norwegian Food Safety Authority small ruminants older than 18 months that died or were killed on the farm due to disease. Employees from "Norsk Protein" collected the samples which consisted of unfixed *medulla oblongata* and often also a small part of the cerebellum and midbrain obtained through the *foramen magnum* using a plastic spoon specially designed for the purpose. Alternatively, the samples consisted of formalin-fixed and unfixed brain halves and unfixed retropharyngeal lymph nodes. The samples were examined at the NVI.

Abattoir surveillance

Brain samples from apparently healthy sheep older than 18 months were collected by the Norwegian Food Safety Authority. The sheep samples were collected at 25 abattoirs, which process all the commercially slaughtered sheep in Norway.

To ensure an appropriate distribution of the samples, the inspectors at the local Norwegian Food Safety Authority were responsible for the sampling to be representative for each region and season, and the sample selection should be designed to avoid overrepresentation of any group as regards to the origin, species, age, breed, production type or to any other characteristic.

The brain samples consisted of *medulla oblongata*, and often also a small part of the cerebellum and midbrain, obtained through the *foramen magnum* using the specially designed metal spoon. The samples were examined at the NVI.

Laboratory examination procedures

A rapid test (TeSeE® SAP ELISA, Bio-Rad) was performed for all submitted samples on a pooled brain tissue sample of obex and cerebellum when both areas were available or on the obex when cerebellum is

missing. In clinical suspects, tissues from the midbrain, cerebrum and retropharyngeal lymph node were examined additionally by the rapid test. In case of inconclusive or positive result, a western blot analysis (TeSeE Western Blot, Bio-Rad) was used as confirmative test. Samples from clinical suspects were examined by western blot independently of the result in the rapid test. The differentiation between classical scrapie and Nor98 scrapie was based on the Western blot profile.

PrP genotyping

PrP genotyping was performed on all scrapie positive sheep. Genotyping of scrapie positive sheep was performed on unfixed brain samples at NVI. Genomic DNA was isolated using the DNeasy Tissue Kit (QIAGEN). Polymorphisms in the PrP gene were detected through automated sequencing of a PCR-generated product covering codons 99 to 209 of the PrP open reading frame (forward primer 5' AGGCTGGGGTCAAGGTGGTAGC; reverse primer 5' TGGTACTGGGTGATGCACATTTGC).

From 2004 to 2016 genotyping of approximately 600 brain samples from healthy slaughtered sheep were performed each year to get an estimate of the PrP genotypes in the normal Norwegian sheep population.

Prevalence

The classical scrapie and Nor98 scrapie prevalences in the fallen stock and abattoir populations were estimated assuming an exact binominal distribution.

Results

Sheep

Nor98 scrapie was diagnosed in ten sheep from ten flocks. Three Nor98 scrapie case was identified in fallen stock, seven cases were apparently healthy animals slaughtered for human consumption (Table 1).

The individual age were registered, and the prion protein genotype examined for all scrapie cases (Table 2). Four sheep had PrP genotypes with at least one allele with polymorphisms at codon 141 (AF₁₄₁RQ).

In total, 18,707 samples from sheep were received. Of these, 14 samples were unsuitable for examination. The numbers of animals examined within each category are presented in Table 1. The prevalence of Nor98 scrapie in the fallen stock of sheep was estimated to 0.03% (0.01-0.095%), (95% confidence interval [CI]), and the prevalence of Nor98 scrapie in sheep slaughtered for human consumption was estimated to 0.07% (0.03-0.15%), (95% CI) (Figure 2).

For 178 (1.1%) samples (75 healthy slaughtered and 103 fallen stock), the flock of origin was not reported. In the event of a positive sample from slaughtered animals, the flock identity could be traced using the carcass number. The remaining 18,529 samples were collected from carcasses originating in 6,198 different sheep flocks. The mean number of animals tested per flock was 3.0 (range 1-69), sheep slaughtered for human consumption in flocks eradicated due to scrapie are excluded. From 792 flocks, more than five animals were tested.

Goat

Scrapie was not detected in any goat in 2019.

In total, 673 samples from goats were received. For ten of these, the flock of origin was not reported (fallen stock). The numbers of animals examined within each category are presented in Table 1.

The collected samples originated from 208 different herds. The mean number of animals tested per herd was 3.2 (range 1-25). From 30 herds, more than five animals were tested.

Table 1. Brain samples from sheep and goats submitted for examination for scrapie in 2019.

Reason for submission to the laboratory	Number of samples			
	Total	Positive	Negative	Rejected
Sheep - total	18,707	10	18,683	14
Animals with clinical signs consistent with scrapie	13	0	13	0
Fallen stock	9,209	3	9,192	14
Healthy slaughtered animals	9,349	7	9,342	0
Animals killed under scrapie eradication	136	0	136	0
Imported animals	0	0	0	0
Goats - total	673	0	672	1
Animals with clinical signs consistent with scrapie	0	0	0	0
Fallen stock	626	0	625	1
Healthy slaughtered animals	47	0	47	0
Animals killed under scrapie eradication	0	0	0	0

Table 2. Year of birth, reason for submission to laboratory examination, breed, prion protein genotype and type of scrapie of the scrapie cases detected in 2019.

Case no	Year of birth	Reason for submission to laboratory examination ¹	Prion Protein Genotype	Scrapie type
1	2010	Healthy slaughtered animals	AF141RQ/AHQ	Nor98
2		Healthy slaughtered animals	AF141RQ/AF141RQ	Nor98
3	2013	Fallen stock	AHQ/ARR	Nor98
4		Fallen stock	AHQ/ARQ	Nor98
5	2015	Fallen stock	AF141RQ/AF141RQ	Nor98
6	2010	Healthy slaughtered animals	AHQ/ARQ	Nor98
7	2016	Healthy slaughtered animals	AHQ/AHQ	Nor98
8	2015	Healthy slaughtered animals	AF141RQ/ARR	Nor98
9	2010	Healthy slaughtered animals	ARR/ARR	Nor98
10	2010	Healthy slaughtered animals	AHQ/AHQ	Nor98

¹The categories are: Healthy slaughtered animals, Animals killed under scrapie eradication measures, Suspect clinical signs consistent with scrapie including animals showing clinical signs at ante-mortem inspection, fallen stock (monitoring of fallen stock including animals examined because of other diseases than scrapie).

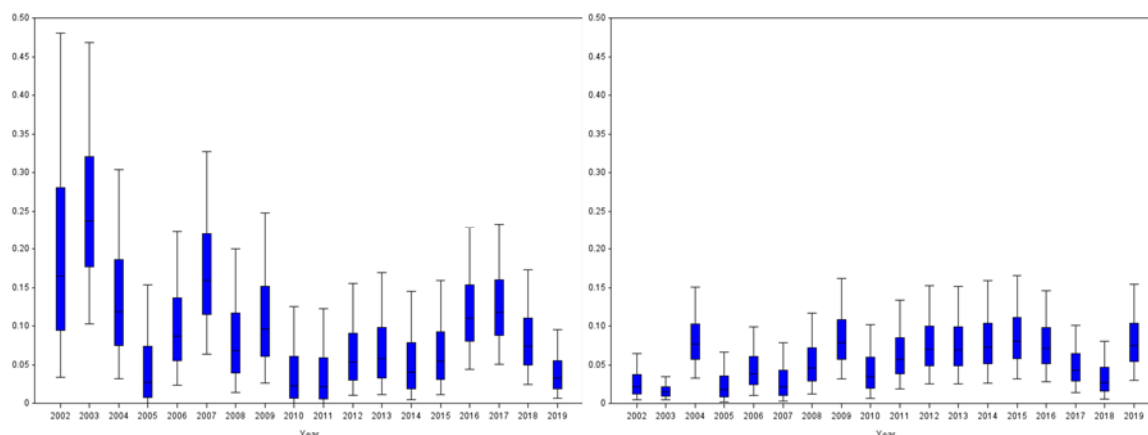


Figure 2. Box and whiskers plot of the prevalence of Nor98 scrapie from 2002 to 2019 in fallen stock (left) and slaughtered animals (right). The boxes represent the 25% to 75% quartiles and the whiskers the 2.5% and 97.5% exact binomial confidence intervals.

Discussion

Nor98 scrapie was diagnosed in ten sheep, originating from ten different flocks. The age and genotypes of these sheep, and the results of the immunohistochemical examinations, were in accordance with the previous experience of Nor98 scrapie (5). Nine cases had at least one of the alleles AF₁₄₁RQ or AHQ which previously had been found to be associated with Nor98 scrapie (4).

The sheep were between three and ten years old, which are in agreement with the result from previous years with the mean age being seven years (Table 2).

The Nor98 scrapie cases detected in 2019 were located in seven different counties. In all of them, the disease had previously been diagnosed. Nor98 scrapie cases have been found in most parts of Norway, in 16 of 19 counties. In contrast, the classical form of scrapie, has been detected only in the western part of Norway (3 counties) and in Nordland County.

The prevalence estimates of Nor98 scrapie in fallen stock and in sheep slaughtered for human consumption have varied during 2002–2019; however most estimates have been within the confidence intervals (Figure 2) (1). The results from the surveillance programmes indicate that the prevalence of Nor98 scrapie in the sheep population has not changed since the start of the programme.

Scrapie was not detected in goats in 2019. The first and only Nor98 scrapie case in goats in Norway was diagnosed in 2006 and originated from a county with a large goat population. Both classical and atypical scrapie in goats has been diagnosed in several countries in Europe (5).

Acknowledgment

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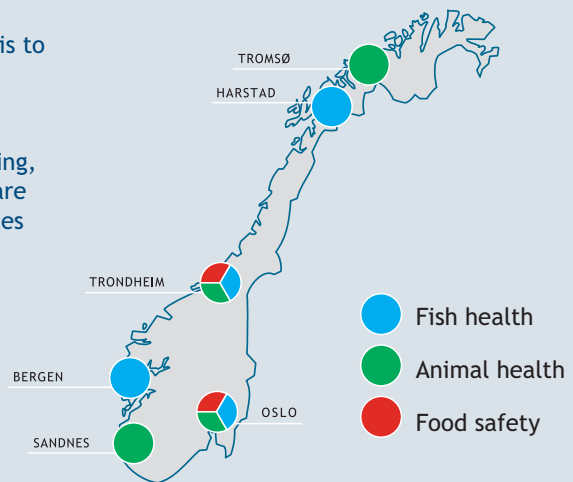
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Veterinærinstituttet
Norwegian Veterinary Institute