

The surveillance programme for scrapie in Norway 2015



Surveillance programmes for terrestrial and aquatic animals in Norway

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The surveillance programme for scrapie in Norway 2015

Ståle Sviland, Sylvie Lafond Benestad, Attila Tarpai

In 2015, Nor98 scrapie was diagnosed in 10 sheep coming from 10 different flocks.

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 2015, scrapie had been diagnosed in a total of 196 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).

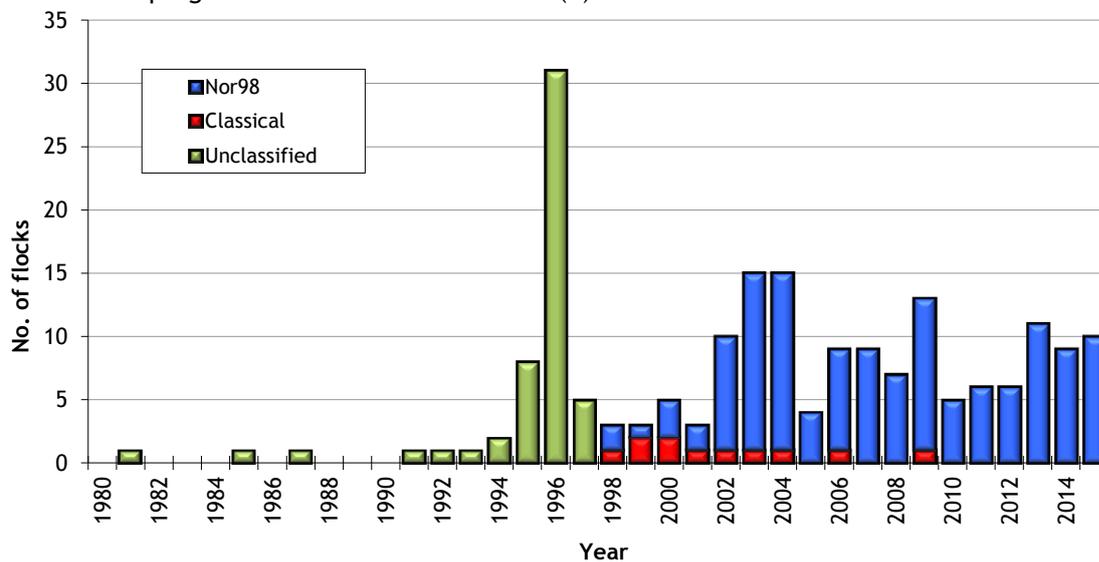


Figure 1. Annual number of sheep flocks and goat herds diagnosed with classical scrapie and Nor98 scrapie during the time period 1980-2015. Before 1998 the cases were not classified according to type of scrapie, but the majority of the scrapie cases are supposed to have been the classical type.

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 is responsible for carrying out the surveillance programme for scrapie. The samples are collected at the abattoirs or in the herds by inspectors from the Norwegian Food Safety Authority. The Norwegian Food Safety Authority also carries out inspections of sheep flocks and goat herds. The Norwegian Veterinary Institute 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 2015, 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 Norwegian Food Safety Authority.

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 Norwegian Veterinary Institute.

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. Inspectors collected the samples which consisted of retropharyngeal lymph nodes and unfixed *medulla oblongata* obtained through the *foramen magnum* using a metal 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 Norwegian Veterinary Institute in Oslo.

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 29 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 Norwegian Veterinary Institute's laboratory in Oslo.

Laboratory examination procedures

A rapid test (TeSeE Sheep and Goat[®] 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. To obtain an indication of PrP genotype distribution in the Norwegian sheep population every 18th sheep slaughtered and examined for PrP^{Sc} was PrP genotyped (Regulation (EC) No. 999/2001 Annex III, as amended by Regulation (EC) No 2245/2003).

Genotyping of scrapie positive sheep was performed on unfixed brain samples at the Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Science. 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).

Genotyping of unfixed brain samples from the abattoir was performed at the Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science. DNA was extracted using the DNeasy 96 Tissue Kit (QIAGEN). The samples were amplified with the described forward and reverse primers modified by 5' attachment of M13-21 and M13 rev tails allowing the use of commercially available fluorescence labelled primers, and sequenced using Big Dye Primer chemistry (Applied Biosystems). Polymorphisms were identified by manual inspection of the sequence electropherograms.

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 10 sheep from 10 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 and breed were registered, and the prion protein genotype examined for all scrapie cases (Table 2). Five sheep had PrP genotypes with at least one allele with polymorphisms at codon 141 (AF₁₄₁RQ).

In total, 14,320 samples from sheep were received. Of these, one sample 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 0.05% (0.01-0.16%), (95% confidence interval [CI]) (Figure 2), and the prevalence of Nor98 scrapie in sheep slaughtered for human consumption was estimated to 0.08% (0.03-0.17%), (95% CI) (Figure 3).

For 132 (0,9%) samples (74 healthy slaughtered and 58 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 14,188 samples were collected from carcasses originating in 5,645 different sheep flocks. The mean number of animals tested per flock was 2.5 (range 1-34), flocks eradicated due to scrapie are excluded. From 1932 flocks more than two samples were tested. The samples were obtained throughout the year, with approximately 25% of the samples collected in January and February, while 20% of the samples are received in September and October which is the main slaughtering season for sheep in Norway.

PrP genotyping was performed on 626 sheep randomly sampled from the healthy slaughtered population examined in Harstad. The PrP genotypes are grouped in accordance with the British National Scrapie Plan (NSP) (Table 3).

Goat

Scrapie was not detected in any goat in 2015.

In total, 483 samples from goats were received. For -four of these, the flock of origin was not reported (fallen stock). One of these were unsuitable for examination. The numbers of animals examined within each category are presented in Table 1.

The samples were collected from carcasses originating from 166 different herds. The mean number of animals tested per herd was 2.9 (range 1-20). From 54 herds more than two samples were tested.

Table 1. Brain samples from sheep and goats submitted for examination for scrapie in 2015.

Reason for submission to the laboratory	No. of samples	No. of rejected samples	Negative	Positive
Sheep				
Animals with clinical signs consistent with scrapie	6	0	6	0
Fallen stock	5 501	1	5 497	3
Healthy slaughtered animals	8 672	0	8 665	7
Animals killed under scrapie eradication	141	0	141	0
Imported animals	0	0	0	0
Total sheep	14 320	1	14 309	10
Goats				
Animals with clinical signs consistent with scrapie	0	0	0	0
Fallen stock	468	1	467	0
Healthy slaughtered animals	14	0	14	0
Animals killed under scrapie eradication	0	0	0	0
Total goats	482	1	481	0

*Includes 49 samples (33 healthy slaughtered and 16 fallen stock) from unspecified small ruminants tested negative.

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 2015.

Case no	Year of birth	Reason for submission to laboratory examination ¹	Breed	Prion Protein Genotype	Scrapie type
1	2007	Healthy slaughtered animals	Norwegian white sheep	AFRQ/ARR	Nor98
2	2010	Healthy slaughtered animals	Norwegian white sheep	ARQ/VRQ	Nor98
3	2008	Fallen stock	Cheviot	AFRQ/AHQ	Nor98
4	2006	Healthy slaughtered animals	Norwegian white sheep	AFRQ/AFRQ	Nor98
5	2001	Fallen stock	Unknown	ARQ/ARR	Nor98
6	2010	Fallen stock	Unknown	AFRQ/AFRQ	Nor98
7	Unknown	Healthy slaughtered animals	Unknown	AFRQ/AHQ	Nor98
8	2009	Healthy slaughtered animals	Norwegian white sheep	AHQ/ARQ	Nor98
9	2005	Healthy slaughtered animals	Norwegian white sheep	ARQ/ARR	Nor98
10	2007	Healthy slaughtered animals	Unknown	ARQ/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).

Table 3. PrP genotypes in the healthy slaughtered population in 2015 grouped in accordance with the British National Scrapie Plan (NSP).

Genotype category	Number	Percent
NSP1, genetically most resistant, ARR/ARR	87	13.9
NSP2, genetically resistant, ARR/ARQ, ARR/ARH, ARR/AHQ, VRR/ARQ	230	36.7
NSP3, genetically low level resistant, ARQ/ARQ	103	16.5
NSP3, genetically low level resistant, AHQ/AHQ, ARH/ARH, ARH/ARQ, AHQ/ARH, AHQ/ARQ	96	15.3
NSP4, genetically susceptible, ARR/VRQ	47	7.5
NSP5, genetically highly susceptible, ARQ/VRQ, ARH/VRQ, AHQ/VRQ, VRQ/VRQ	63	10.1
Total	626	100

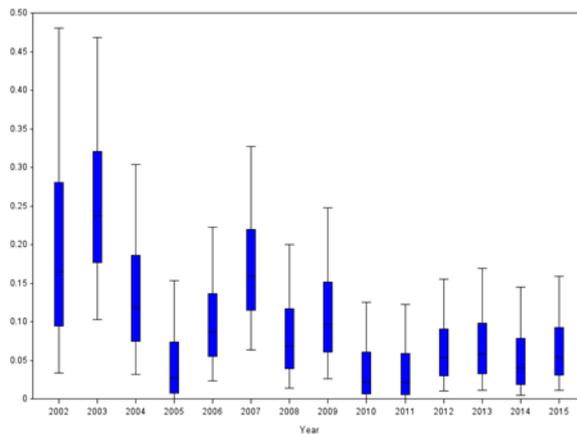


Figure 2. Box and whiskers plot of the prevalence of Nor98 scrapie in fallen stock during 2002-2015. The boxes represent the 25% to 75% quartiles and the whiskers 2.5% and 97.5% exact binomial confidence intervals.

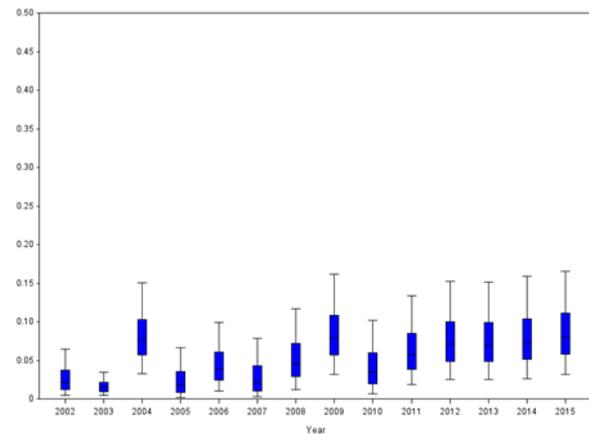


Figure 3. Box and whiskers plot of the prevalence of Nor98 scrapie in slaughtered animals during 2002-2015. The boxes represent the 25% to 75% quartiles and the whiskers 2.5% and 97.5% exact binomial confidence intervals.

Discussion

Nor98 scrapie was diagnosed in 10 sheep, each case originating in different flocks. The ages and genotypes of these sheep, and the results of the immunohistochemical examinations, were in accordance with the previous experience of Nor98 scrapie (5). Seven cases had at least one of the alleles AF₁₄₁RQ or AHQ which previously had been found to be associated with Nor98 scrapie (4).

Following the EU Regulation (EC) No. 999/2001 Annex VII, as amended by Regulation (EC) No 253/2006, of July 2007, states that genotyping might be performed on a proportion of the animals in the flock positive for Nor98 scrapie. No animal has to be removed from the flock on the basis of PrP genotype. The sheep were between five and fourteen 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 2015 were located in six different counties; in all of them the disease had previously been diagnosed. Nor98 scrapie cases have been found in most parts of Norway, in 14 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 (Figure 4).

The prevalence estimates of Nor98 scrapie in fallen stock and in sheep slaughtered for human consumption have varied during 2002-2015; however most estimates have been within the confidence intervals (Figure 2 and Figure 3) (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.

The difference between the number of examined sheep from fallen stock (5,501) and the calculated number according to EU regulation No 2245/2003 (10,000), may partly be due the fact that about 60% of the fallen stock population die while on remote mountain and forest pastures. In spite of this, the numbers of animals examined in the sheep fallen stock and slaughtered populations are sufficient to estimate the prevalences of Nor98 scrapie in these populations.

Scrapie was not detected in goats in 2015. The first and only scrapie case in naturally infected 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).

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The Norwegian Veterinary Institute (NVI) is a nationwide biomedical research institute and Norway's leading centre of expertise regarding biosafety in aquatic and terrestrial animals. The aim of the Institute is to become Norway's contingency centre of preparedness for One Health.

The primary mission of the NVI is to give research-based independent advisory support to ministries and governing authorities. Preparedness, diagnostics, surveillance, reference functions, risk assessments, and advisory and educational functions are the most important areas of operation. The Institute has its main laboratory in Oslo, with regional laboratories in Sandnes, Bergen, Trondheim, Harstad and Tromsø, with about 330 employees in total.

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The Norwegian Food Safety Authority (NFSA) is a governmental body whose aim is to ensure through regulations and controls that food and drinking water are as safe and healthy as possible for consumers and to promote plant, fish and animal health and ethical farming of fish and animals.

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