Surveillance and control programmes for terrestrial and aquatic animals in Norway
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The surveillance and control programme for scrapie in Norway 2011

Sviland Ståle, Benestad Lafond Sylvie, Eikenæs Olav, Norström Madelaine

In 2011, Nor98 scrapie was diagnosed in 6 sheep coming from 6 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 2009, scrapie had been diagnosed in a total of 148 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 and control programme was launched in 1997 (2).

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 previous cases with classical scrapie (4).

The Norwegian Food Safety Authority is responsible for carrying out the surveillance and control 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, all of which should be inspected every second or third year. The Norwegian Veterinary Institute is performing the laboratory examinations and the reporting of the results.

Aims

The aims of the surveillance and control 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 2011, 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)

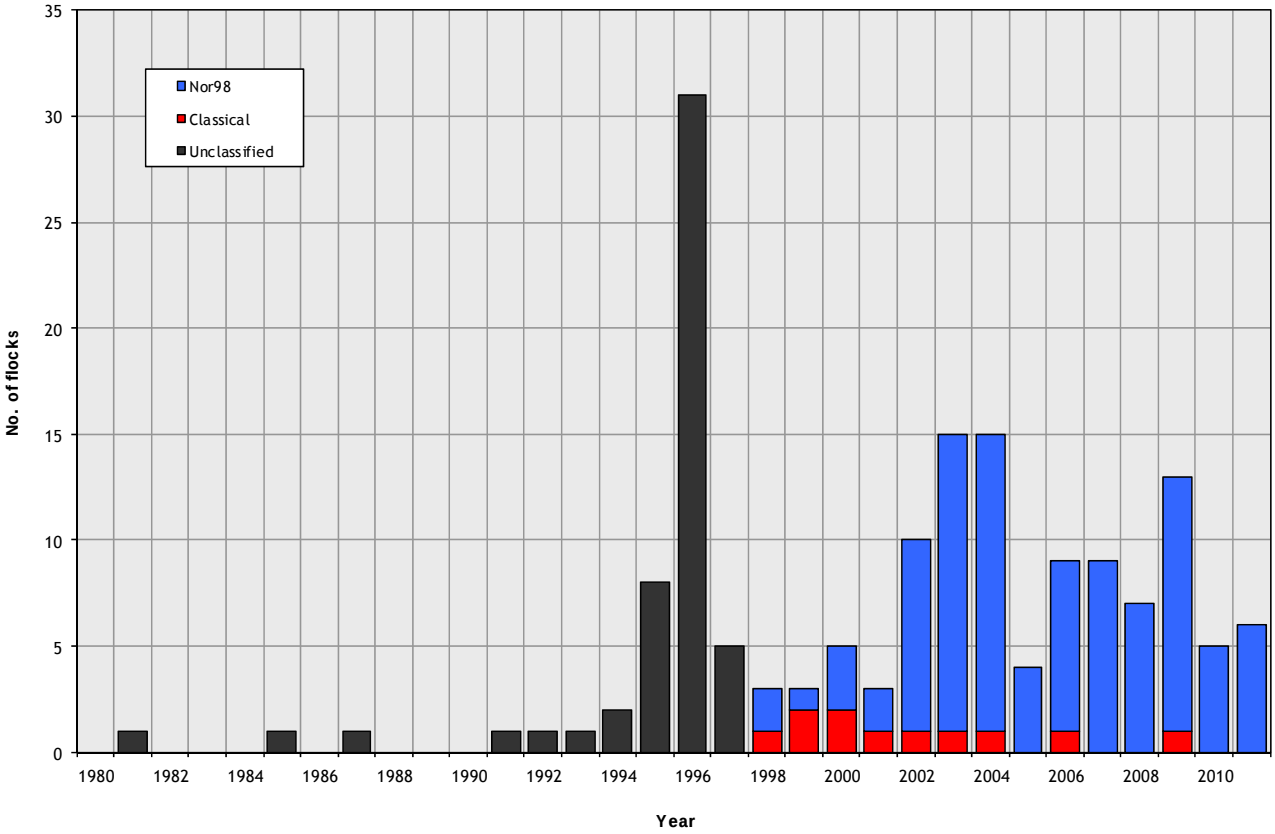

Figure 1. Annual number of sheep flocks and goat herds diagnosed with classical scrapie and Nor98 scrapie during the time period 1980-2011. Before 1998 the cases were not classified according to type of scrapie, but the majority of the scrapie cases are supposed to have been the classical type.

Animals with clinical signs consistent with scrapie

When the sheep and goat farmers recognised sheep or goats with clinical signs consistent with scrapie, they were responsible for reporting the animal 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 small ruminants older than 18 months that died or were killed on the farm due to disease. Inspectors from the Norwegian Food Safety Authority 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 and goats older than 18 months were collected by the Norwegian Food Safety Authority. The sheep samples were collected at 34 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 laboratories in Oslo and Harstad.

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. Differentiation between classical scrapie and BSE in sheep was performed by using differential western blot (Discriminatory Western Blot, Bio-Rad).

Histopathological and immunohistochemical examination were usually performed supplementary when scrapie was confirmed.

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 16th 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 6 sheep from 6 flocks. One Nor98 scrapie case was identified in fallen stock, five 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 six scrapie cases (Table 2). Four sheep had PrP genotypes with at least one allele with polymorphisms at codon 141 (AF141RQ) or 154 (AHQ), whereas two sheep had the PrP genotype ARR/ARR.

In total, 13,486 samples from sheep were received. Of these, 13 (0.09%) 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 0.02% (0.0-0.12%), (95% confidence interval [CI]) (Figure 2), and the prevalence of Nor98 scrapie in sheep slaughtered for human consumption was estimated to 0.06% (0.0-0.13%), (95% CI) (Figure 3).

For 135 (1.0%) samples (111 healthy slaughtered, 22fallen stock and one from the ante mortem control), 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 13,352 samples were collected from carcasses originating in 5,596 different sheep flocks. The mean number of animals tested per flock was 2.3 (range 1-29), flocks eradicated due to scrapie are excluded. From 1,746 flocks more than two samples were tested. The samples were obtained throughout the year, with approximately 26% of the samples collected in September and October, which is the main slaughtering season for sheep in Norway.

PrP genotyping was performed on 639 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 2011.

In total, 390 samples from goats were received. In six of these the flock of origin was not reported. None 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 168 different herds. The mean number of animals tested per herd was two (range 1-13). From 51 herds more than two samples were tested.

Table 1. Brain samples from sheep and goats submitted for examination for scrapie in 2011

Reason for submission to the laboratory	No. of samples	No. of rejected samples	Negative	Positive
Sheep				
Animals with clinical signs consistent with scrapie	7	0	7	0
Fallen stock	4546 *	7	4538 *	1
Healthy slaughtered animals	8692 *	6	8681 *	5
Animals killed under scrapie eradication	241	0	241	0
Total sheep	**13,486**	**13**	**13,467**	**6**
Goats				
Animals with clinical signs consistent with scrapie	0	0	0	0
Fallen stock	381	4	377	0
Healthy slaughtered animals	9	0	9	0
Animals killed under scrapie eradication	0	0	0	0
Total goats	**390**	**0**	**390**	**0**

*33 samples (9 healthy slaughtered and 24 fallen stock) from unspecified small ruminants tested negative. These samples are included in the figures given for sheep.

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 2011

Case nr	Year of birth	Reason for submission to laboratory examination [1)]	Breed [2)]	Prion Protein Genotype	Scrapie type
1	2004	Healthy slaughtered animals	Norwegian white sheep	AFRQ/AHQ	Nor98
2	2006	Fallen stock	Spæl sheep/Dala sheep	ARR/ARR	Nor98
3	2005	Healthy slaughtered animals	Norwegian white sheep*	AHQ/ARR	Nor98
4	2008	Healthy slaughtered animals	Norwegian white sheep	AFRQ/AFRQ	Nor98
5	2004	Healthy slaughtered animals	Norwegian white sheep	ARR/ARR	Nor98
6	2005	Healthy slaughtered animals	Norwegian white sheep	AFRQ/ARQ	Nor98

1) The 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).
2) Crossbred long-tailed breeds: Rygja sheep, Steigar sheep, Dala sheep, Norwegian white sheep; indigenous short-tailed breed: Spæl sheep. *with some Texel

Table 3. PrP genotypes in the healthy slaughtered population in 2011 grouped in accordance with the British National Scrapie Plan (NSP)

Genotype category	Number	Percent
NSP1, genetically most resistant, ARR/ARR	76	11.9
NSP2, genetically resistant, ARR/ARQ, ARR/ARH, ARR/AHQ, VRR/ARQ	273	42.7
NSP3, genetically low level resistant, ARQ/ARQ	80	12.5
NSP3, genetically low level resistant, AHQ/AHQ, ARH/ARH, ARH/ARQ, AHQ/ARH, AHQ/ARQ	99	15.5
NSP4, genetically susceptible, ARR/VRQ	31	4.9
NSP5, genetically highly susceptible, ARQ/VRQ, ARH/VRQ, AHQ/VRQ, VRQ/VRQ	80	12.5
Total	**639**	**100**

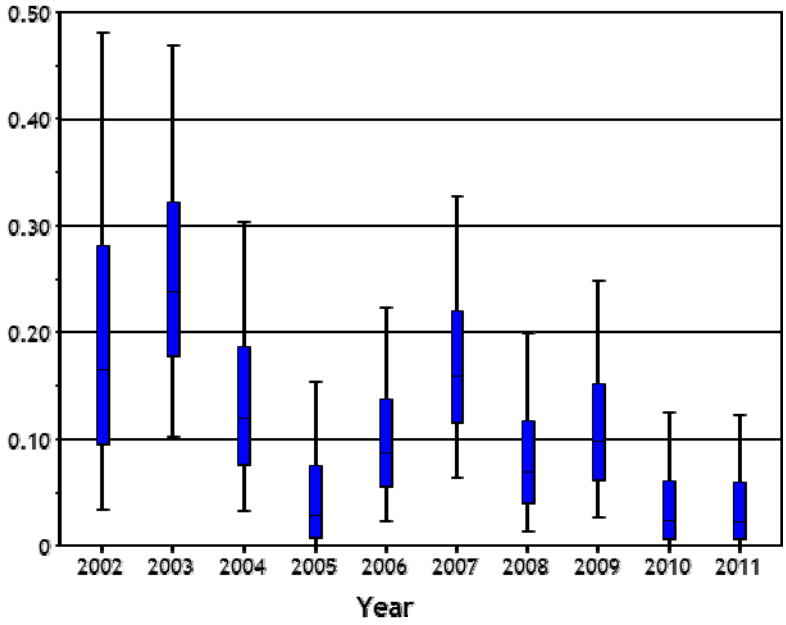

Figure 2. Box and whiskers plot of the prevalence of Nor98 scrapie in fallen stock during 2002-2011. The boxes represent the 25% to 75% quartiles and the whiskers represent the 2.5% and 97.5% exact binomial confidence intervals.

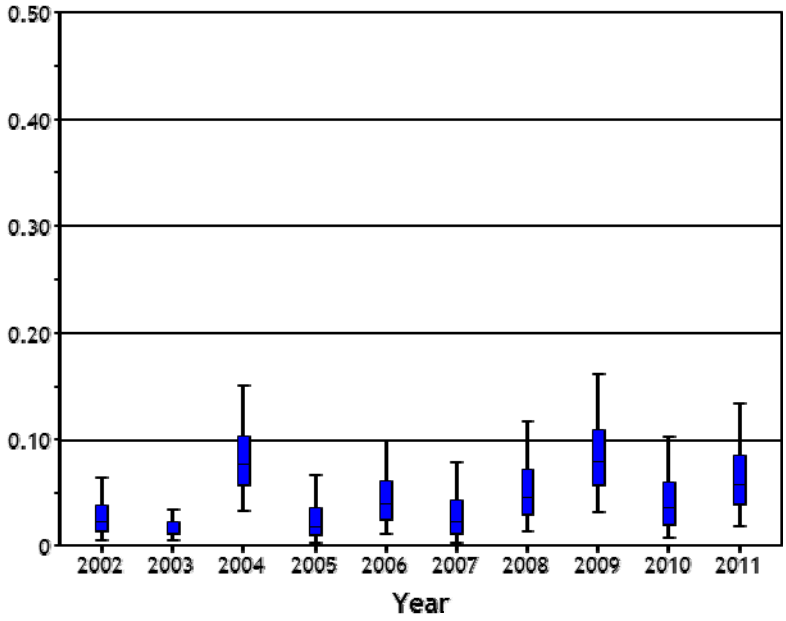

Figure 3. Box and whiskers plot of the prevalence of Nor98 scrapie in slaughtered animals during 2002-2011. The boxes represent the 25% to 75% quartiles, and the whiskers represent the 2.5% and 97.5% exact binomial confidence intervals.

Discussion

Nor98 scrapie was diagnosed in six 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). Two cases had the allele combination ARR/ARR which is known to be resistant against classical scrapie. The other four cases had at least one of the alleles $AF_{141}RQ$ 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 three and a half and seven and a half years old, which are in agreement with the result from previous years with the mean age being six years (Table 2).

The Nor98 scrapie cases detected in 2011 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 2002–2011; 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 (4,538) 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 2011. 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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References

1. Sviland S, Benestad SL, Eikenæs O, Norström M. The surveillance and control programmes for scrapie in Norway: annual report 2010. In: Hellberg H, Sviland S (editors). Surveillance and control programmes for terrestrial and aquatic animals in Norway: annual report 2010. Oslo: Veterinærinstituttet.

2. Alvseike KR, Melkild I, Thorud K. Scrapie control at the national level: the Norwegian example. In: Hörnlimann B, Riesner D, Kretzschmar H (editors). Prions in humans and animals. Berlin: de Gruyter; 2006. p. 648-53.

3. Benestad SL, Sarradin P, Thu B, Schönheit J, Tranulis MA, Bratberg B. Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. Vet Rec. 2003; 153: 202-8.

4. Moum T, Olsaker I, Hopp P, Moldal T, Valheim M, Moum T, Benestad SL. Polymorphisms at codons 141 and 154 in the ovine prion protein gene are associated with scrapie Nor98 cases. J Gen Virol. 2005; 86: 231-5.

5. Benestad SL, Arsac JN, Goldmann W, Nøremark M. Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. Vet Res. 2008; 39: 19.