Surveillance and control programmes for terrestrial and aquatic animals in Norway
National Veterinary Institute

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The surveillance and control programme for scrapie in Norway

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In 2009, Nor98 scrapie was diagnosed in 12 sheep coming from 12 different flocks. Classical scrapie was diagnosed in five sheep coming from the same flock.

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 2008, scrapie had been diagnosed in a total of 135 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 National 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.

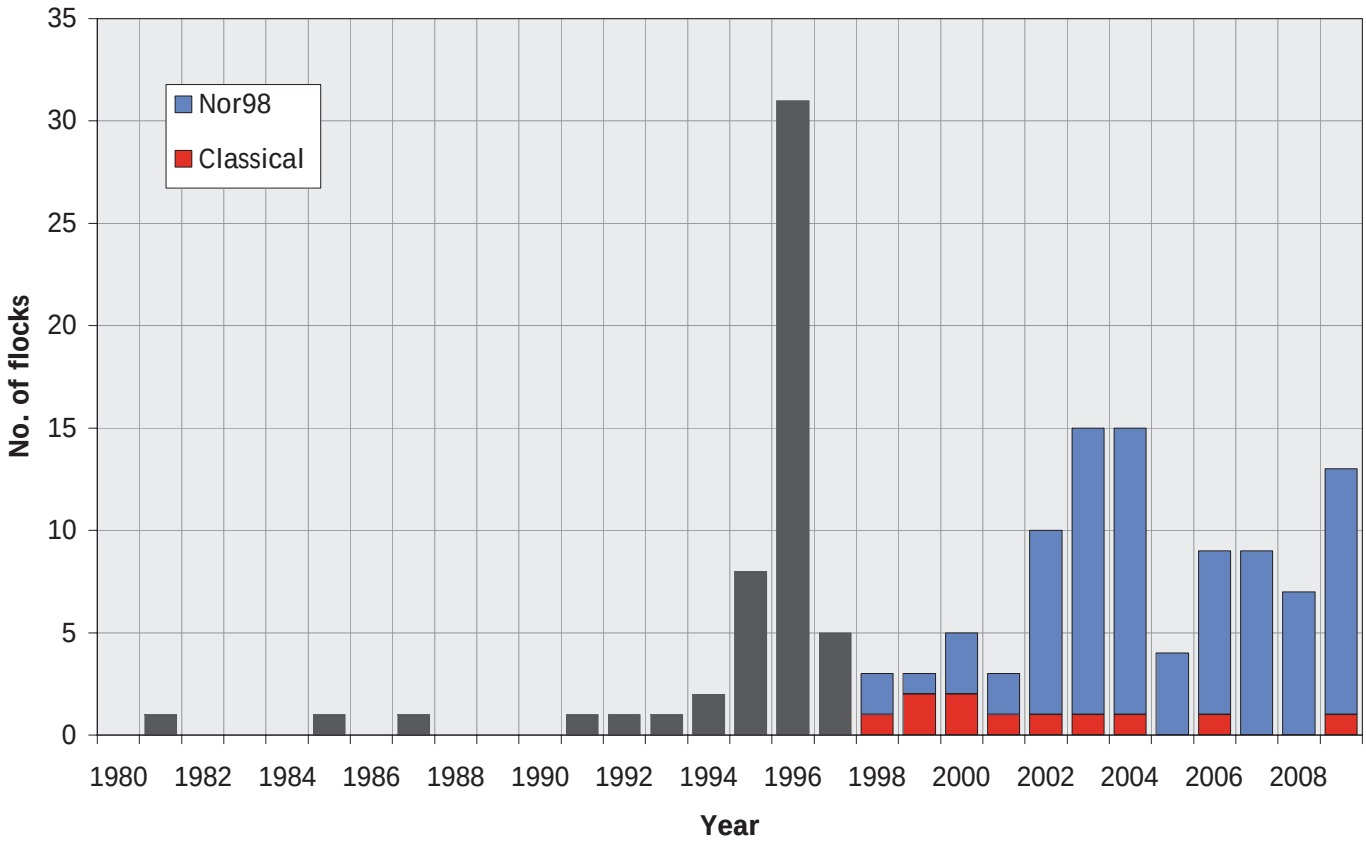

Figure 1. Annual number of sheep flocks and goat herds diagnosed with classical scrapie and Nor98 scrapie during the time period 1980-2009. Before 1998 the cases were not classified according to type of scrapie, but the majority of the scrapie cases are supposed to have been the classical type.

Materials and methods

In 2009, 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 recognised sheep or goats with clinical signs consistent with scrapie, they were responsible for reporting the animal to the local Food Safety Authority. The Food Safety Authority evaluated the reported cases. 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 National 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 National 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 over-representation 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 National Veterinary Institute's laboratories in Oslo and Harstad.

Laboratory examination procedures

A rapid test (TeSeE Sheep & 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 or occasionally the cerebellum alone should only one of them be available. 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 a binominal distribution.

Results

Sheep

Nor98 scrapie was diagnosed in 12 sheep from 12 flocks. Four Nor98 scrapie cases were identified in fallen stock, one showed some clinical sign during ante mortem control, and seven cases were apparently healthy animals slaughtered for human consumption (Table 1). There was one detected case of classical scrapie identified in fallen stock, and four additional sheep were positive when examining the animals eradicated from the flock.

The individual age and breed were registered, and the prion protein genotype examined for all 13 scrapie cases (Table 2). Eleven sheep had PrP genotypes with at least one allele with polymorphisms at codon 141 (AF141RQ) or 154 (AHQ). One sheep had the PrP genotype ARR/ARR.

In total, 13,615 samples from sheep were received. Of these, 13 (0.1%) 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 0.1% (0.03-0.25%), (95% confidence interval [CI]) (Figure 2), and the prevalence of Nor98 scrapie in sheep slaughtered for human consumption was estimated to 0.08% (0.03-0.16%), (95% CI) (Figure 3).

For 332 (2.4%) samples (321 healthy slaughtered and 11 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 13,283 samples were collected from carcasses originating in 5,433 different sheep flocks. The mean number of animals tested per flock was 2.6 (range 1-24), flocks eradicated due to scrapie are excluded). From 1,662 flocks more than two samples were tested. The samples were obtained throughout the year, with approximately 32% of the samples collected in September and October, which is the main slaughtering season for sheep in Norway.

PrP genotyping was performed on 527 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).

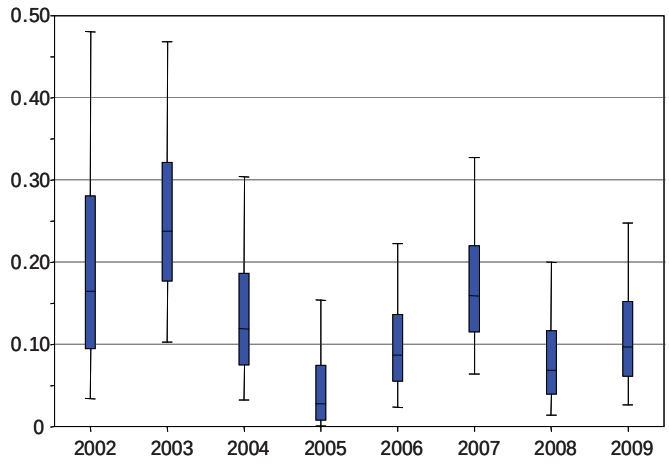

Figure 2. Box and whiskers plot of the prevalence of Nor98 scrapie in fallen stock during 2002-2009. The boxes represent the 25% to 75% quartiles and the whiskers represent the 2.5% and 97.5% exact binomial confidence intervals.

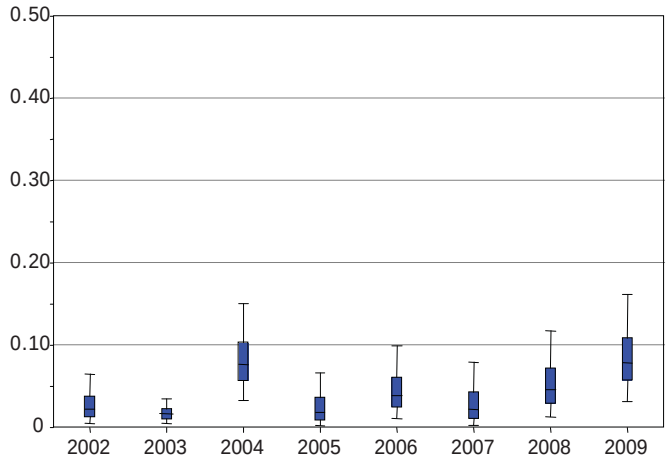

Figure 3. Box and whiskers plot of the prevalence of Nor98 scrapie in slaughtered animals during 2002-2009. The boxes represent the 25% to 75% quartiles, and the whiskers represent the 2.5% and 97.5% exact binomial confidence intervals.

Table 1. Brain samples from sheep and goats submitted for examination for scrapie in 2009

Reason for submission to the laboratory	No. of samples	No. of rejected samples	Negative	Positive
Sheep				
Animals with clinical signs consistent with scrapie	2	0	1	1
Fallen stock	4,139*	7	4,127*	5
Healthy slaughtered animals	8,916*	6	8,903*	7
Animals killed under scrapie eradication	558	0	554	4
Total sheep	**13,615**	**13**	**13,585**	17
Goats				
Animals with clinical signs consistent with scrapie	1	0	1	0
Fallen stock	343	0	343	0
Healthy slaughtered animals	16	0	16	0
Animals killed under scrapie eradication	1	0	1	0
Total goats	**361**	**0**	**361**	**0**

* 67 samples (65 healthy slaughtered and two 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 2009

Case no.	Year of birth	Reason for submission to laboratory examination [1)]	Breed [2)]	Prion Protein Genotype	Scrapie type
1	2005	Fallen stock	Norwegian white sheep	AF141RQ/AF141RQ	Nor98
2	2001	Fallen stock	Norwegian white sheep	AF141RQ/ARQ	Nor98
3	2002	Fallen stock	Norwegian white sheep	AF141RQ/AF141RQ	Nor98
4	2002	Fallen stock	Texel	ARH/VRQ	Classic
5	2003	Healthy slaughtered animals	Norwegian white sheep	AF141RQ/ARQ	Nor98
6	2002	Healthy slaughtered animals	Norwegian white sheep	AF141RQ/ARR	Nor98
7	2002	Healthy slaughtered animals	Spæl sheep	ARR/ARR	Nor98
8	2002	Healthy slaughtered animals	Norwegian white sheep	AHQ/ARR	Nor98
9	2002	Healthy slaughtered animals	Dala sheep	AF141RQ/ARR	Nor98
10	2005	Healthy slaughtered animals	Norwegian white sheep	AHQ/AF141RQ	Nor98
11	2006	Healthy slaughtered animals	Old spæl sheep	AHQ/AHQ	Nor98
12	2005	Healthy slaughtered animals	Norwegian white sheep	AHQ/AF141RQ	Nor98
13	2005	Fallen stock	Norwegian white sheep	AF141RQ/AF141RQ	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.

Table 3. PrP genotypes in the healthy slaughtered population in 2009 grouped in accordance with the British National Scrapie Plan (NSP)

Genotype category	Number	Percent
NSP1, genetically most resistant, ARR/ARR	55	10.5
NSP2, genetically resistant, ARR/ARQ, ARR/ARH, ARR/AHQ, VRR/ARQ	205	38.9
NSP3, genetically low level resistant, ARQ/ARQ	88	16.7
NSP3, genetically low level resistant, AHQ/AHQ, ARH/ARH, ARH/ARQ, AHQ/ARH, AHQ/ARQ	98	18.6
NSP4, genetically susceptible, ARR/VRQ	22	4.2
NSP5, genetically highly susceptible, ARQ/VRQ, ARH/VRQ, AHQ/VRQ, VRQ/VRQ	59	11.2
Total	**527**	**100**

Goat

Scrapie was not detected in any goat in 2009.

In total, 361 samples from goats were received. None of these were unsuitable for examination. The numbers of animals examined within each category are presented in Table 1.

For one (0.28%) sample (fallen stock), the flock of origin was not reported. The remaining 360 samples were collected from carcasses originating in 181 different herds. The mean number of animals tested per herd was two (range 1-10). From 44 herds more than two samples were tested.

Discussion

Nor98 scrapie was diagnosed in twelve 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). Most cases had at least one of the alleles $AF_{141}RQ$ or AHQ which previously have been found to be associated with Nor98 scrapie (4). There were one Nor98 scrapie case that was carrying the ARR/ARR genotype considered to be strongly resistant (NSP1) towards classical scrapie.

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 absence of additional Nor98 scrapie cases in the flocks this year as well as previous years, suggests that Nor98 scrapie is, if contagious at all, less contagious than classical scrapie. This is supported by a case-control study on Nor98 scrapie in Norwegian sheep flocks, where animal-to-animal contact or movement of sheep between sheep flocks were not found as risk factors for Nor98 scrapie (6).

The sheep were between three and eight 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 2009 were located in nine 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 2002–2008; 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.

Classical scrapie was diagnosed in 2009 in one case of fallen stock. When the classical form of scrapie was detected, the whole flock was culled. Additionally four sheep were found positive when examined after the destruction of the flock. As far as more than 135,000 sheep were examined since 2002, the prevalence of this type of scrapie is considered to be very low.

The difference between the number of examined sheep from fallen stock (4,139) 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 2009. 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).

Acknowledgment

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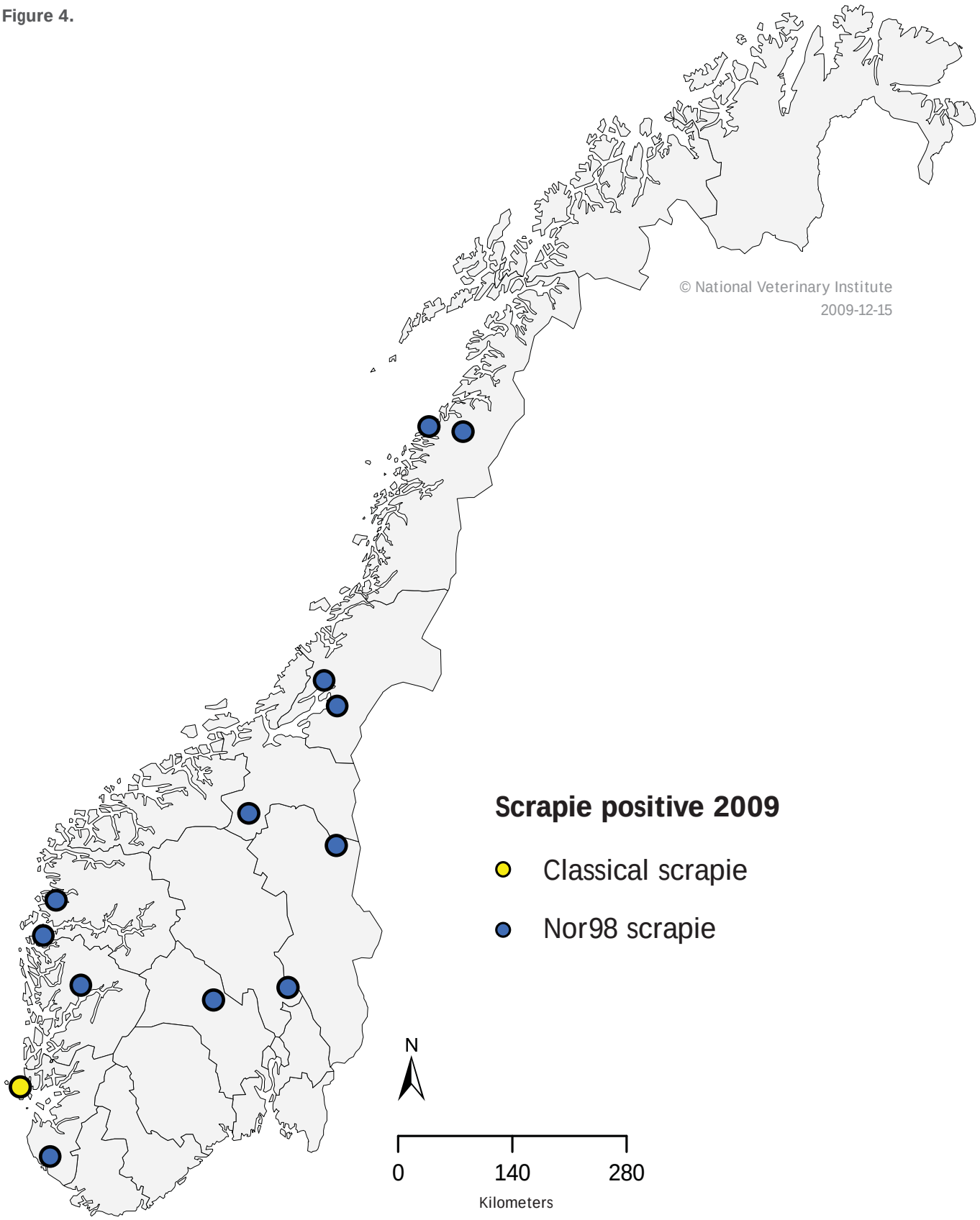
Figure 4.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2009-12-15
Scrapie positive 2009
Classical scrapie
Nor98 scrapie
N
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140
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Kilometers

References

1. Sviland S, Hopp P, Benestad SL, Tarpai A, Eikenæs O, Mork J, Moldal T. The surveillance and control programmes for scrapie in Norway: annual report 2008. In: Brun E, Jordsmyr HM, Sviland S, Hellberg H (editors). Surveillance and control programmes for terrestrial and aquatic animals in Norway: annual report 2008. 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.

6. Hopp P, Omer MK, Heier BT. A case-control study of scrapie Nor98 in Norwegian sheep flocks. J Gen Virol. 2006; 87: 3729-36.

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