# Annual Report · 2015

The surveillance programme for bovine spongiform encephalopathy (BSE) in Norway 2015





# Surveillance programmes for terrestrial and aquatic animals in Norway

Annual report 2015

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# The surveillance programme for bovine spongiform encephalopathy (BSE) in Norway 2015

Ståle Sviland, Sylvie Lafond Benestad, Helga Rachel Høgåsen, Attila Tarpai

In 2015 there was one atypical positive case of BSE. The remaining 6,780 samples, originating from 4,323 herds, were tested negative for BSE.

### Introduction

The BSE surveillance programme was initially based on passive surveillance (1998-2000), with active surveillance introduced in May 2000. In the period 1998-2000 the samples were investigated by histopathological examination. From 2001 onwards the samples were examined by an Enzyme-Linked ImmunoSorbent Assay (ELISA) method for detection of resistant prion protein (PrP<sup>Sc</sup>).

In addition, clinically suspected animals were also investigated by Western blot investigation for the detection of Pr<sup>Sc</sup> and/or histopathological/immunohistochemical examination according to the protocol of the Office International des Epizooties (OIE) (1). The number of samples collected in each category in the period 1998 - 2015 is presented in Table 1. Except for the positive atypical case in 2015, BSE has never been detected in any of the examined animals.

 Table 1. Number of collected samples for BSE examination by the Norwegian surveillance programme according to categories from 1998-2015.

Year	Clinically suspected animals	Fallen stock	Emergency slaughtered animals	Ante-mortem animals	Imported slaughtered animals	Healthy slaughtered animals*	Total
1998-2000	78	0	0	0	19	0	97
2001	14	1 352	7 073	2 612	88	2 400	13 539
2002	2	1 482	7 246	3 562	39	9 907	22 238
2003	2	1 936	7 334	4 107	39	10 727	24 145
2004	3	2 145	9 217	1 355	24	10 443	23 187
2005	1	2 318	8 462	102	10	10 486	21 379
2006	0	2 364	8 177	36	4	10 455	21 036
2007	0	2 213	7 304	48	9	10 000	19 574
2008	0	2 391	8 358	16	5	9 373	20 143
2009	1	2 435	8 320	27	3	9 451	20 237
2010	0	2 788	7 438	11	1	127	10 365
2011	1	3 078	7 241	23	1	7 878	18 222
2012	1	2 936	6 841	7	0	8 744	18 529
2013	1	3 239	7 887	9	4	9 421	20 561
2014	1	1 946	4 270	12	1	264	6 494
2015	0	1 793	5 088	48	1	0	6 930**
Total	105	34 416	110 256	11 975	248	109 676	266 676

\* Healthy slaughtered animals were excluded from the surveillance programme in 2010, 2014 and in 2015.

\* Including 149 samples unsuitable for examination.

#### Aim

The aim of the surveillance programme is to document that the Norwegian cattle population is free from classical BSE.

# Materials and methods

#### **Programme outline**

For 2015 the surveillance programme included examination of the following categories:

- clinically suspected cattle irrespective of age
- all cattle older than 48 months of age, which have died or been culled, but not slaughtered for human consumption (fallen stock)
- all emergency slaughtered cattle older than 48 months
- all cattle older than 48 months, with abnormal findings at ante-mortem examination, rejected for human consumption, or which died at the abattoir or during transport (referred to as ante-mortem animals)
- all slaughtered cattle with unknown age or origin irrespective of age
- all slaughtered imported cattle from any country irrespective of age

#### Implementation

The farmers were requested to report all cases of clinically suspected cattle irrespective of age, fallen stock older than 48 months and when delivering imported cattle to slaughter to the Norwegian Food Safety Authority. The brain or head from clinically suspected cattle or a spoon sample from the medulla oblongata from fallen stock were submitted and analysed at the Norwegian Veterinary Institute, Oslo. Inspectors from the Norwegian Food Safety Authority collected the spoon samples of the medulla oblongata from the other categories at the abattoirs and sent them within 24 hours in a cool insulated container to the Norwegian Veterinary Institute in Oslo.

#### **Clinically suspected animals**

The usual protocol followed for the clinically suspected animals is that the whole brain is divided midsagittally into equal halves. One half is formalin-fixed and processed according to a standard routine protocol, embedded in paraffin, sectioned at 2 to 4 µm and stained with haematoxylin eosin (HE). Immunohistochemical staining for detection of PrP<sup>Sc</sup> is performed on selected sections using a monoclonal anti-PrP antibody (SAF 84, courtesy of J. Grassi, CEA, France). From the non-fixed half, tissue from the obex area is analysed by ELISA for detection of PrP<sup>Sc</sup> (TeSeE®, Bio-Rad) and by Western blot (TeSeE<sup>®</sup> WESTERN BLOT, Bio-Rad) for detection of PrP<sup>Sc</sup> as described by the manufacturer.

#### Risk population and routine slaughtered animals

Non-fixed brain tissue from the obex area was analysed by ELISA for detection of  $PrP^{Sc}$  (TeSeE®, Bio-Rad) as described by the manufacturer. In cases with positive or inconclusive test results, the remaining half obex was fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained with Hematoxylin and eosin (H&E stain) HE. Subsequently, the sections were analysed by immunohistochemical detection of  $PrP^{Sc}$  using the same protocol as for specimens from clinical suspects.

Brain samples were evaluated as unsuitable for examination when they were severely autolysed, the dorsal part of the obex area was partially missing, the obex was not present, or the medullar anatomy was not recognisable.

# **Results and discussion**

In 2015 one 15 years old cattle of the breed Scottish Highland was diagnosed with atypical BSE (H type). The animal was killed due to an injury, and an obligatory sample for BSE-testing was taken. Atypical BSE occurs spontaneously in an expected incidence of 1 case per million cattle, and differs from classical BSE which is caused by intake of infected meat and bone meal. Atypical BSE is diagnosed in animals over eight years of age, and the proportion of cattle above that age in Norway is less than 3 % (table 3). The probability of detecting an atypical BSE-case in the Norwegian cattle population is small.

The Norwegian Veterinary Institute received samples from 6,930 cattle. Of these, 149 (2.2%) samples were unsuitable for examination (136 from fallen stock and 13 from emergency slaughter).

For 65 samples (0.9%) the herd of origin was not reported. However, it is important to note that in case of a positive test result from such a herd, the identity could be traced via the carcass number. The remaining 6,865 samples originated from 3,569 dairy cattle herds and 889 beef cattle herds. The mean number of examined animals per herd was 1.54.

#### Clinically suspected animals (passive surveillance)

In 2015, none animal was investigated as clinical suspect due to abnormal behaviour and tremor. Improved methods for clinical examination to distinguish between real suspected BSE cases and cases with central nervous disease of other causes has probably resulted in few clinical suspected cases in later years. It is likely that animals with diseases related to the central nervous system have been examined either as fallen stock, emergency slaughtered animals or ante-mortem animals, and thus included in these categories.

#### Surveillance of slaughtered animals and fallen stock (active surveillance)

In 2013 The EU-commission decided to stop BSE testing in healthy slaughtered cattle in 25 EU-member states (decision 2013/76/EU). From 2014 Norway joined the EU25 and stopped the testing of healthy slaughtered animals.

The age limit for including cattle in the categories "Fallen stock", "Emergency slaughter" and "Ante mortem" has been changed to 48 months. This amendment has resulted in a reduced number of sampled animals in the categories fallen stock and emergency slaughter in 2014 and onwards compared to 2013.

The geographical distributions of the cattle population and the animals of different categories tested, are presented in Table 2. There is a relatively good correlation between the collection of samples for fallen stock and emergency slaughtered animals from different regions and the distribution of the cattle population in the regions. A corresponding comparison related to the age distribution of sampled animals is shown in Table 3. The proportion of sampled animals above 48 months of age is much larger than the corresponding proportion in the population, due to the lower age limits in this programme (Table 3).

**Table 2.** Regional distribution of Norwegian cattle and the cattle sampled for PrP<sup>Sc</sup> in 2015. There were 65 samples (0.9%) from cattle with unknown region. These samples are assumed to be distributed following the regional distribution of the cattle from known region within each target group.

Region	Total population (%)	Fallen stock (%)	Emergency slaughter (%)	Ante mortem animals (%)	Clinically suspected animals (%)	Total (%)
Region Stor-Oslo	5.0	7.9	3.9	2.1	0.0	4.9
Region Øst	25.9	16.1	25.7	6.3	0.0	23.1
Region Sør og Vest	31.3	24.4	26.9	39.6	0.0	26.4
Region Midt	28.1	40.4	34.7	37.5	0.0	36.2
Region Nord	9.7	11.2	8.8	14.6	0.0	9.4
Total number	899 535	1 793	5 088	48	0	6 930

\* Including 1 imported cattle.

**Table 3.** Age distribution of Norwegian cattle and the cattle tested for PrP<sup>Sc</sup> in 2015. There were 193 samples (2.8%) from cattle with unknown age. The age of these cattle are assumed to be distributed like the age distribution of the cattle with known age within each target group.

Age groups (months)	Total population (%)	Fallen stock (%)	Emergency slaughter (%)	Ante mortem animals (%)	Clinically suspected animals (%)	Total (%)
< 24	57.3	0.2	0.4	6.8	0.0	0.4
24-29	7.3	0.8	0.4	2.3	0.0	0.5
30-35	6.2	1.1	0.6	2.3	0.0	0.7
36-47	10.0	2.6	2.2	6.8	0.0	2.3
48-59	7.0	29.6	27.4	31.8	0.0	28.0
60-71	4.5	25.5	26.6	9.1	0.0	26.2
72-83	2.8	17.6	19.1	20.5	0.0	18.7
84-95	1.8	10.7	10.8	6.8	0.0	10.8
96-107	1.1	5.2	5.8	9.1	0.0	5.7
108-119	0.7	2.2	2.9	0.0	0.0	2.7
120-131	0.5	1.6	1.8	0.0	0.0	1.7
132-143	0.3	1.0	0.7	0.0	0.0	0.8
144-155	0.2	0.4	0.6	2.3	0.0	0.6
≥ 156	0.3	1.5	0.7	2.3	0.0	0.9
Total number	899 535	1 657	5 075	48	0	6 781*

\* Excluded samples unsuitable for examination. Including 1 imported cattle.

# Conclusion

In 2015 we detected the first atypical case of BSE in Norway. It is still considered highly probable that the Norwegian cattle population never has been infected with classical BSE-agent due to few imports to Norway of cattle and products potentially infected with the BSE-agent, limited use of meat and bone meal in concentrates intended for ruminants, and the use of high temperature and pressure in the domestic production of meat and bone meal (2). This is supported by a quantitative risk assessment for BSE in Norway (3) and by the compiled results from the surveillance programme for BSE in the years 2001 to 2015.

# Acknowledgment

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The primary mission of the NVI is to give researchbased 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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