Annual report 2005

Surveillance and control programmes for terrestrial and aquatic animals in Norway



















Editor Edgar Brun Scientific editors Hege Hellberg and Tormod Mørk Technical editor Hanne Mari Jordsmyr National Veterinary Institute

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# Introduction

The spread of disease from one country to another through animals carrying pathogenic microorganisms is well recognized. Historically, there are numerous records of invading armies introducing diseases like rinderpest and bovine pleuropneumonia into conquered territories, while diseases such as foot-and-mouth disease have been introduced to new countries and continents through trade. During the twentieth century, legislative measures with particular emphasis on border control became an important first line of defence against introducing diseases into countries. A number of these diseases are zoonoses.

For many countries, strict border control has been an important measure in maintaining a favourable animal health situation. However, societal and political changes during the last decades have made this concept less reliable. Several factors contribute to the spread of pathogens to new areas and to ecosystems with susceptible animals, including an increasing human population, and an increase in trade and wealth, which result in greater international movement of people, animals and animal products. An international legislative framework has been developed to regulate this. In Europe, a political union with the concept of free movement of individuals and goods as an ideological basis has been established. Globally, the concept of international free trade has expanded through new international agreements. This political and economical progress represents a zoosanitary challenge for authorities responsible for the health of humans and animals.

The agreement on the European Economic Area (EEA) established 1 July 1994 and its revision of 1 January 1999, introduced new regulations for trade in animals and animal products in Norway. Import restrictions based on routine border control and quarantine were modified. Legislation based on the concept of recognized freedom from a particular disease or additional guarantees given by the exporting country for animals or their products was an acceptable substitute for some diseases, while more protection was required for other diseases.

The agreement which established the World Trade Organisation (WTO) on 1 January 1995 has also removed barriers for international trade. The agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) introduced measures for protection of public, animal and plant health related to trade. The fundamental basis for the SPS Agreement is that trade regulations should be non-discriminatory and based on scientifically sound risk assessment.

In response to the international agreements, Norway adopted new legislation that included surveillance programmes as integrated components for some diseases. In addition, new programmes were introduced for documentation and control of other diseases.

# Surveillance programmes for documentation and control

# Programmes according to EU-directives and regulations

Bovine tuberculosis and brucellosis were eradicated in Norway 40 to 50 years ago and a freedom of disease status was approved on historical data. In order to maintain the free status a moderate surveillance programme was established in 2000. After the EEA-agreement in 1994, Norway achieved the status of freedom from Brucella melitensis in small ruminants based on historical data. In order to maintain this position, a surveillance and control programme was established in 2004. The status of enzootic bovine leukosis (EBL) has been documented and the few infected animals have been eliminated. On this basis, Norway has applied for free-status for enzootic bovine leukosis. In poultry, programmes for Newcastle disease, Mycoplasma and Salmonella were established according to EU-directives. Surveillance of bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep and goats is performed according to the requirements of the EU regulations. A comparable programme is the testing for residues of drugs and toxic substances in live animals and animal products of ruminants, pigs and poultry. In the autumn of 2005 the threat of global avian influenza increased substantially. A surveillance programme on avian influenza in wild birds was initiated as part of the preparedness for preventing introduction in commercial poultry flocks. Furthermore, plans for a surveillance programme in commercial flocks have been developed.

The programmes for aquatic animals are of increasing importance due to an expanding aquaculture industry. Their purpose is twofold, combining prevention of introduction of the diseases through import from infected premises or regions, and the documentation of a freestatus to benefit the export of aquaculture products. The surveillance for viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) was initially based on the recognition of free-status for these diseases on historical data. In 2004 the entire coastline of Norway was recognized as an approved zone with regard to *Bona-mia ostreae* and *Marteilia refringens*. The decision is based on the results of the surveillance and control programmes for bonamiosis and marteiliosis which were initiated in the autumn of 1995.

#### Programmes approved by the EFTA Surveillance Authority (ESA)

Some diseases are not regulated by common EEA rules. However, countries may apply for additional guarantees based on their documented status. In 1994, additional guarantees for infectious bovine rhinotracheitis (IBR) in cattle and Aujeszky's disease (AD) in pigs were granted to Norway.

The favourable *Salmonella* situation in Norway was recognized by the ESA in 1994. The additional guarantees were based on national surveillance and control programmes for cattle, pigs and poultry.

Ongoing programmes for terrestrial and aquatic animals in 2005 (the year of initiation in parentheses)

Animal category	Programmes according to EU-directives and regulations	Programmes approved by ESA	Other national surveillance and control programmes
Cattle	BSE (1998) Residual substances (1999) EBL (1994) Tuberculosis (2000) Brucellosis (2000)	IBR/IPV (1992) Salmonella (1995)	Paratuberculosis (1996) BVD (1992)
Swine	Residual substances (1999)	AD (1994) Salmonella (1995)	TGE (1995) PRRS (1995) Swine influenza (1997)
Small ruminants	Scrapie (1997) Brucellosis (2004)		Maedi (1997)
Poultry	Residual substances (1999) Newcastle disease Mycoplasma Salmonella (1995-breeding flocks)	Salmonella (1995-96)	ILT (1997) ART (1997) <i>Campylobacter</i> (2001) AI (2005)
Farmed deer	Tuberculosis (2000)		CWD (2005)
Llama			Paratuberculosis (2000)
Fish	VHS/IHN (1994)		Gyrodactylus salaris (2000)
Shellfish	Bonamia/Marteilia (1995)		

BSE=bovine spongiform encephalopathy, EBL=enzootic bovine leukosis, IBR=infectious bovine rhinotracheitis, IPV=infectious pustular vulvovaginitis, BVD=bovine virus diarrhoea, AD=Aujeszky's disease, TGE=transmissible gastroenteritis, PRRS=porcine reproductive and respiratory syndrome, ILT=infectious laryngotracheitis, ART=avian rhinotracheitis, Al=avian influensa, CWD=chronic wasting disease, VHS=viral haemorrhagic septicaemia, IHN=infectious haematopoietic necrosis.

# Other national surveillance and control programmes

Several diseases of great national significance have no legal basis in the EU legislation. Norwegian authorities and industries have for years used great efforts and resources to control and eradicate diseases such as bovine virus diarrhoea (BVD) in cattle, and maedi in small ruminants.

#### Responsibilities for the programmes

The surveillance and control programmes are included in the legislation for terrestrial and aquatic animal health and food in Norway, as decided by the Ministry of Agriculture and Food and the Ministry of Fisheries and Coastal Affairs. The Norwegian Food Safety Authority is responsible for implementation of all measures related to this legislation. The National Veterinary Institute ensures the scientific quality of the programmes with regard to epidemiological design, by testing and analysing with approved methods and by presenting, interpreting and reporting the results according to accepted standards.

The economic funding for the programmes in 2005 was provided by the Ministry of Agriculture and Food, and the Ministry of Fisheries and Coastal Affairs with some contribution from the industries.

Jakin hypell

Joakim Lystad Director general, Norwegian Food Safety Authority

Sampling is performed by or under the supervision of official inspectors in the Norwegian Food Safety Authority.

#### Impact of the programmes

The programmes serve several purposes for Norwegian authorities and for the agriculture and aquaculture industries. The scientific documentation shows that Norway complies with legal commitments in relation to international agreements. The programmes have contributed to decreasing the risk associated with trade of animals and animal products. Contagious diseases with great economic significance for the Norwegian livestock population have also been diagnosed through the programmes, enabling both their prompt eradication and the rapid introduction of preventive measures to counter further exposure.

Furthermore, several of the diseases included are zoonotic diseases and consequently the programmes constitute a scientific documentation with great significance for food safety. Finally, the documentation provided is important for industries exporting animals, breeding material and products originating from Norwegian terrestrial and aquatic animals.

The dury

Roar Gudding Director general, National Veternary Institute

# Main results from the surveillance and control programmes in 2005

National surveillance and control programmes have been performed for Aujeszky's disease and transmissible gastroenteritis since 1994, and for porcine respiratory and reproductive syndrome and swine influenza since 1995 and 1997, respectively. The results from the surveillance programmes for 2005 give additional documentation of freedom from specific virus infections in the Norwegian swine population. This status is currently unique in an international context.

From 2000 to 2005, more than 100,000 bovines have been investigated for BSE. All samples have been negative, clearly indicating that the Norwegian cattle population has not been infected with the BSE-agent. Scrapie was diagnosed in four sheep in 2005. In all instances the causative agent was scrapie-strain Nor98. Classical scrapie has not been detected by active surveillance during the last four years, despite the examination of more than 80,000 animals.

In December 2002, maedi was diagnosed in a central breeding flock. Follow-up investigations revealed 45 contact flocks with seropositive animals. A nationwide surveillance programme revealed seropositive animals in four out of 2,626 breeding flocks analyzed in 2003 to 2005. The affected herds were situated in three different counties, indicating maedi-visna virus to be present in different parts of Norway.

Following the launching of an action plan against *Campylobacter*, which has included a surveillance and control programme, the prevalence of flocks positive for *Campylobacter* has steadily decreased from 7.7 % in 2001 to 3.3 % in 2004. In 2005, 3.6 % of the flocks were positive for *Campylobacter* sp.

Antibodies against avian rhinotracheitis (ART) were for the first time detected in a commercial broiler breeder holding in 2003. A large layer breeder company tested positive on several occasions in 2004 and 2005. Clinical signs, however, were not observed. The use of stamping out measures was unable to control the spread of infection and, as of May 2005, chickens are no longer tested for the presence of antibodies against ART.

At the turn of the year 2005/2006, only one cattle herd was subjected to restrictions due to bovine virus diarrhoea. The number of restricted herds has decreased from 2,950 in 1994.

The Norwegian *Salmonella* programmes for live animals, eggs, and meat were launched in 1995 simultaneously with comparable programmes in Sweden and Finland. The results from 11 years of active surveillance document that the Norwegian cattle, swine, sheep, and poultry populations are only sporadically infected with *Salmonella* sp.

Infections with *Mycobacterium avium* subsp *paratuberculosis* seems to be endemic in goat herds in six counties comprising half the goat population in Norway. The prevalence of infected cattle herds and sheep flocks appears to be very low.

In late 2005, a new surveillance programme for aquatic animals was initiated; the surveillance and control programme for bacterial kidney disease (BKD). Due to the limited number of farms sampled, results from 2005 will not be reported separately, but included in the annual report for 2006. Norway has a disease-free status for viral haemor-rhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN), bonamiosis and marteiliosis. The results from 2005 support the free status for these infections in Norwegian populations of aquatic animals. *Gyrodactylus salaris* reappeared in two rivers in 2005. Both rivers were rotenone treated first time in 2001/2002.

Species	Infection	Start	Extent of program
Cattle	IBR/IPV	1992	10 % of dairy cattle 10 % of beef cattle
	Brucella abortus	2000	In cases of abortio
	BVD	1992	20 % in most areas All herds in certain
	EBL	1994	10 % of dairy cattle 10 % of beef cattle
	Bovine tuberculosis	2000	Inspection of carca of suspected lesion
	BSE	1998	Investigation of cli
		2000	Testing of importe
		2001	Testing of fallen stoc
			Testing of animals
			Testing of random
Swine	AD	1994	All breeding herds and a selection of tested
	TGE	1994	
	PRRS	1995	
	Swine influenza	1997	
Poultry	Newcastle disease	1993	All chicken and tu
	ILT	1997	All chicken (broile
	ART	1997	All chicken(broiler flocks
	Campylobacter	2001	All broiler flocks
Small ruminants	Scrapie	1997	Testing of clinicall
		2002	Testing of fallen st
		1997	Random sampling
		1997	Testing of primary
	Maedi	1997	All breeding flocks 2003-2005
	Brucella melitensis	2004	All breeding flocks 2004-2005
Several species	Salmonellosis	1995	Cattle: 3,000 lymp Swine: 3,000 lymp from all breeding Poultry: faecal sar or >250 layers/bre
	Paratuberculosis	1996	Testing of clinicall Testing of all llama randomly selected
Fish	VHS/IHN	1994	Sampling of appro- turbot farms (all fa of a two-year peri
	Gyrodactylus salaris	2000	Sampling of appro salmon and rainbo salmon fingerlings 130 rivers
-	Bonamiosis	1995	Sampling of select
Oyster			twice annually

nmes in 2005	Number of samples examined in 2005	Positive samples in 2005	Previous positive results
e herds herds	1,919 bulk milk samples 4,766 blood samples from 484 herds	None None	1992: 1 positive herd
ns	24 foetuses from 21 herds 96 blood samples from 56 cows (30 herds)	None None	
1 areas	7,481 bulk milk samples 1,230 pooled blood samples	2004: restrictions lifted in	ed in 1097 herds and imposed on 413 herds 4 herds and imposed on 4 herds 4 herds and imposed on 2 herds
e herds herds	1,919 bulk milk samples 4,766 blood samples from 484 herds	None None	1995-1996: 7 positive herds 2002: 1 positive herd
asses at slaughter, submission ns for testing	Organs from 1 individual	None	1984: 1 positive herd 1986: 1 positive herd
nically suspect animals	1 sample	None	None
d animals and their progeny	10 samples	None	None
k and emergency slaughtered animals	10,711 samples	None	None
selected at ante mortem control	102 samples	None	None
ly selected slaughtered animals	10,484 samples	None	None
, all nucleus herds of the sow pools integrated and fattening herds are	4,644 samples from 468 herds	None	None
«	4,635 samples from 468 herds	None	None
«	4,637 samples from 468 herds	None	None
«	4,635 samples from 468 herds	None	1998: 1 positive herd (H3N2)
key breeder flocks	6,891 samples from 79 holdings	None	None
and layer) breeder flocks	3,690 samples from 75 holdings (127 flocks)	1 seropositive flock	None
and layer) and turkey breeder	1,437 samples from 31 holdings (41 flocks)	1 seropositive flock	2003: 2 positive flocks (1 holding) 2004: 2 positive flocks (1 holding)
	Samples from 3652 flocks	132 (3.6 %) positive flocks	2001: 7.7 % positive flocks 2002: 6.3 % positive flocks 2003: 4.9 % positive flocks 2004: 3.3 % positive flocks
y suspect animals	7 samples	1 positive individual	1997-2003: 21 positive individuals 2004: 3 positive individuals
ock	3,621 samples	1 positive individual	2002-2003: 11 positive individuals 2004: 4 positive individuals
of slaughtered animals	10,887 samples	2 positive individuals	2001-2003: 8 positive individuals 2004: 8 positive individuals
and secondary flocks	248 samples	None	2003: 1 positive flock 2004: 1 positive flock
of sheep once during the period	29,248 samples from 940 flocks	2 positive flocks	1998-2003: 3 positive flocks 2004: 1 positive flock
of sheep once during the period	28,406 samples from 935 flocks	None	None
h node samples h node samples, faecal samples nerds nples from all flocks of >50 broilers eders	2,209 lymph node samples 3,476 lymph node samples and 2,492 faecal samples from 148 herds 6,777 faecal samples from 1,374 holdings	2 positive samples 1 positive sample	1995-2002: Only a few positive samples each year 2003: 5 positive (2 cattle, 2 swine and 1 broiler) 2004: 3 positive samples (2 cattle, 1 swine)
/ suspect animals as older than 48 months and cattle, goat and sheep herds	Organ and faecal samples from 483 cattle, 1,420 goats, 245 sheep and 9 llamas	14 goat herds	1997: 4 cattle herds (imported animals) 1998-2003: 5 cattle herds, 13 goat herds and 2 sheep flocks, 2004: 4 goat herds
cimately half of all salmonid and arms should be tested in the course od)	13,460 samples from 417 sites	None	None
kimately half of all fresh water w trout farms. Sampling of Atlantic 'parr/smolts from approximately	2,503 fish from 81 salmonid farms (app. 1/3 of relevant farms) 3,833 fish from 120 rivers	No positive salmonid farms 2 positive rivers	1975-2004: 39 positive salmonid farms, last time 2002 (3 hatcheries) 1975-2004: 45 positive rivers
ed farms and wild populations	349 oysters from 7 sampling points	None	None
ed farms and wild populations	349 oysters from 7 sampling points	None	None



# The livestock and aquaculture populations in Norway

Annual report 2005





Petter Hopp Gry Grøneng

# The livestock population

Norway covers an area of 323,895 square km and has a population of about 4.7 million people of which about 0.8 million live in or in the vicinity of the capital Oslo. The livestock production is targeted for the national market. Table 1 gives an overview of the livestock population and the number of animals slaughtered in 2005.

Until 1994 there was a general ban on the import of live animals and animal products to Norway. Live animals could only be imported if derogation was given by the Veterinary Authorities. Consequently, there have been very few imports of live animals to Norway. Table 2 shows the number of live animals and animal products imported to Norway in 2004 and 2005.

As a consequence of the European Economic Area (EEA) agreement which was implemented in 1994, the trade of certain animals and animal products within the area was regulated through EU harmonised directives, and the general ban on import of these animals and products to Norway was lifted. There was a general increase in the interest to import live animals during that decade. The authorities encouraged beef production, and the need for suckling cows was met by import of live animals.

#### The cattle population

Approximately 15,900 dairy herds were registered in Norway in 2005 of which approximately 910 also kept suckling cows. The average number of dairy cows per herd was 16.7. The number of specialized beef herds with at least one suckling cow was about 5,100 with a mean number of 10.8 suckling cows per herd. Overall, the number of Norwegian dairy herds has decreased over the last 15 years (Figure 1).

From 1980 to 1986, approximately 560 cattle were imported. There were no imports from 1987 to 1990. The European Economic Agreement in 1994 allowed more imports of live cattle. Nevertheless, as seen in Figure 2, the number of imports has been limited and most imported animals came from Sweden and Denmark. Close to 100 % of the imports have been beef cattle. In 2005, no live cattle were imported to Norway (Table 2).

#### The swine population

The Norwegian swine population is relatively small and the production is destined for the domestic market. In 2005, about 1.5 million swine were slaughtered.

The population consists of approximately 61,400 breeding swine aged more than six months. A national breeding programme is organised by the industry. The approximately 180 approved elite and multiplier breeding herds house only 5 % of the live sows, while more than 95 % of the sows purchased on the national market are raised in these herds. About 50 % of the swine production is located in the counties of Hedmark, Oppland, Rogaland and Nord-Trøndelag. The numbers of live animals imported during the time period 1991 to 2005 are given in Figure 3.

In 2005, 49 live swine were imported to Norway.

Table 1. The livestock population in Norway and the number of slaughtered animals in 2005

	No. of				
Animal category	herds*	animals*	slaughtered animals*		
Cattle	21,500 <sup>1</sup>	930,100 <sup>1</sup>	331,800 <sup>2</sup>		
Dairy cows only**	14,700 <sup>1</sup>	242,300 <sup>1</sup>	-		
Suckling cow only**	3,900 <sup>1</sup>	46,900 <sup>1</sup>	-		
Combined production (cow)**	1,200 <sup>1</sup>	30,700 <sup>1</sup>	-		
Goat	1,300 <sup>1</sup>	72,700 <sup>1</sup>	19,200 <sup>2</sup>		
Dairy goat**	550 <sup>1</sup>	44,400 <sup>1</sup>	-		
Sheep	16,700 <sup>1</sup>	2,393,200 <sup>1</sup>	1,248,600 <sup>2</sup>		
Breeding sheep > 1 year**	16,500 <sup>1</sup>	927,400 <sup>1</sup>	-		
Swine	3,300 <sup>1</sup>	802,800 <sup>1</sup>	1,473,700 <sup>2</sup>		
Breeding animal > 6 months**	2,000 <sup>1</sup>	61,400 <sup>1</sup>	-		
Fattening pig for slaughter	2,900 <sup>1</sup>	432,500 <sup>1</sup>	-		
Poultry					
Egg laying hen (> 20 weeks of age)	2,400 <sup>1</sup>	3,318,500 <sup>1</sup>	2,197,700 <sup>2</sup>		
Flocks > 250 birds**	820 <sup>1</sup>	3,285,500	-		
Broiler	500 <sup>2</sup>	-	44,327,600 <sup>2</sup>		
Turkey, duck and goose for slaughter	170 <sup>1</sup>	328,200 <sup>1</sup>	1,040,300 <sup>2</sup>		
Flocks > 25 birds**	81 <sup>1</sup>	327,500	-		
Ostrich	12 <sup>1</sup>	120 <sup>1</sup>	-		

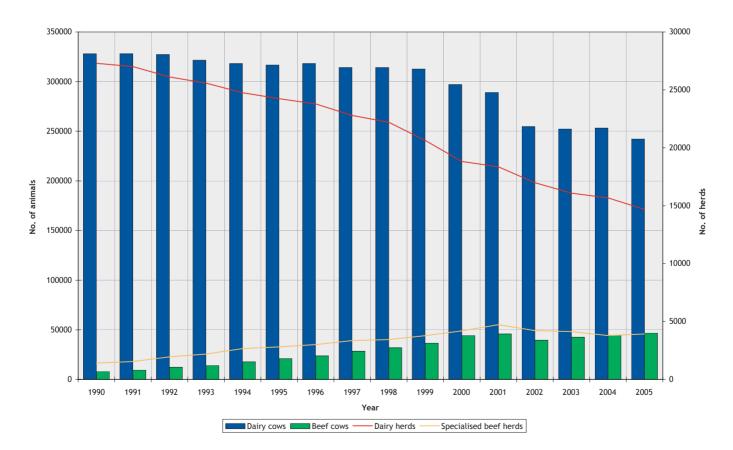
<sup>1</sup> Register of Production Subsidies as of 31 July, 2005, <sup>2</sup> Register of Slaughtered Animals.

\* Numbers >100 rounded to the nearest ten, numbers > 1000 rounded to the nearest hundred, \*\* Included in above total.

Table 2. Import of	of live animals and	animal products to	Norway in 2004 and 2005
Table Z. Import o	I live annuals and	annial products to	Noi way in 2004 and 2005

		20	04	20	05
Species	Imported product	No. of consignments	No. of animals or products	No. of consignments	No. of animals or products
Cattle	Live animals	-	_1	-	01
	Semen (doses)	-	40,000 <sup>1</sup>	с	<b>39,265</b> <sup>1</sup>
	Embryos	-	69 <sup>1</sup>	7	63 <sup>1</sup>
Swine	Live animals	-	_1	1	<b>49</b> <sup>1</sup>
	Semen (doses)	-	200 <sup>1</sup>	с	<b>394</b> <sup>1</sup>
Sheep	Live animals	2	11 <sup>1</sup>	2	<b>39</b> <sup>1</sup>
	Embryos	-	_1	2	339 <sup>1</sup>
	Semen (doses)	-	750 <sup>1</sup>	3	500 <sup>1</sup>
Goat	Live animals	2	26 <sup>1</sup>	2	531
	Semen (doses)	-	_1	1	100 <sup>1</sup>
Reindeer	Live animals for slaughter	2	350 <sup>1</sup>	1	2 <sup>2</sup>
Fur animal	Live animals	1	213 <sup>1</sup>	38	4,631 <sup>2</sup>
Poultry	Day-old chicks	16	157,357 <sup>1</sup>	18	133,155 <sup>1</sup>
	Fertilised eggs	-	-	51	2,313,130 <sup>1</sup>
Turkey	Day-old chicks	7	14,326 <sup>1</sup>	4	8,757 <sup>1</sup>
Duck and goose	Live birds	2	840 <sup>1</sup>	3	1,505 <sup>1</sup>
Halibut	Live fish	-	-2	-	0 <sup>2</sup>
Turbot	Live fish	2	600 <sup>2</sup>	7	181,820 <sup>2</sup>
Atlantic salmon	Live fish	2	429,480	-	<b>0</b> <sup>2</sup>

<sup>1</sup> Data from Norwegian Livestockindustry's Biosecurity Unit (KOORIMP), <sup>2</sup> Data from the Norwegian Food Safety Authority. c=Continuous import, no measurable number



**Figure 1.** The number of dairy and beef cows in holdings with specialized dairy and beef production during the time period 1990-2005 (Statistics Norway and Register of production subsidies (RPS) for 2005).

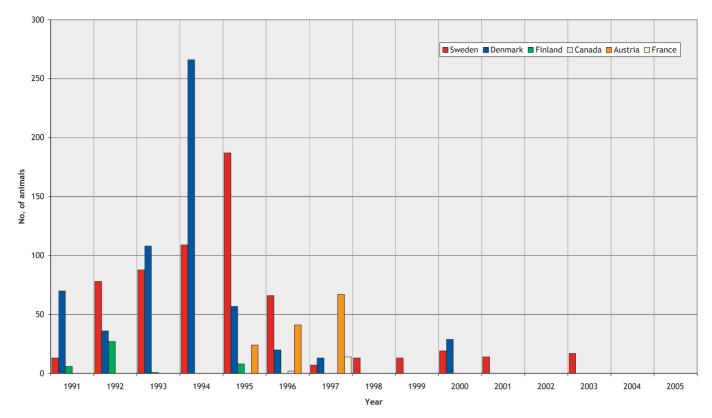


Figure 2. Imports of live cattle to Norway during the time period 1991-2005.

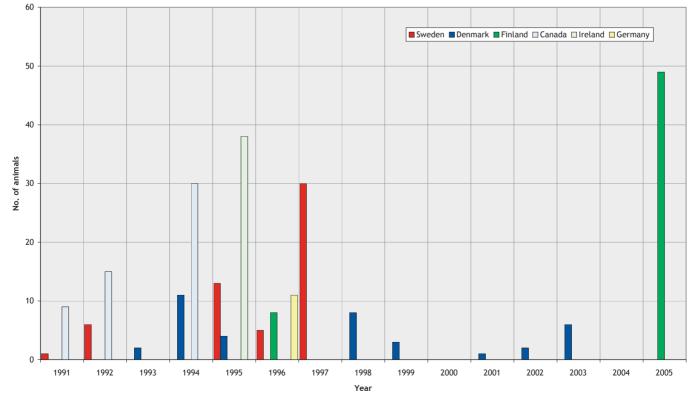


Figure 3. Import of live swine to Norway during the time period 1991-2005.

#### The sheep population

The Norwegian sheep population consists of approximately 927,400 sheep above one year of age. The sheep flocks are widely distributed over the country, with the biggest population found in the south-west. The sheep population consists of combined meat and wool producing breeds, with the dala, spæl, steigar and rygja sheep predominating. Each year about 1.25 million sheep are slaughtered and approved for human consumption. Only a few live animals have been imported since the 1970s. Thirty-nine live animals were imported in 2005.

#### The goat population

The Norwegian goat population is comprised of approximately 44,400 dairy goats and is principally composed of one Norwegian breed. The goat flocks are located in some mountainous regions in the southern part of the country, in the fjord districts of the western part, and in the counties of Nordland and Troms in northern Norway. The main product is milk used for cheese production. About 19,000 goats are slaughtered and approved for human consumption each year. Fifty-three live goats were imported in 2005.

#### The poultry population

The Norwegian poultry production is strictly regulated and the population has a hierarchical structure. Egg and broiler meat production are the most important branches, but the production and consumption of turkey is increasing slightly. Figure 4A shows the location and structure of the Norwegian layer population comprising two hatcheries, 18 pullet rearing farms and about 870 commercial layer farms. The layer population consists of two white layer strains (Lohmann white and Shaver white).

The commercial broiler production takes place in three hatcheries with two strains (Cobb and Ross), about 70 breeding farms with parent flocks and about 500 commercial broiler flocks. None of these farms are located in the northern part of Norway, as shown in Figure 4B.

The layer and broiler industry import day-old grand parent flocks mainly from Sweden.

#### The population of farmed fish and shellfish

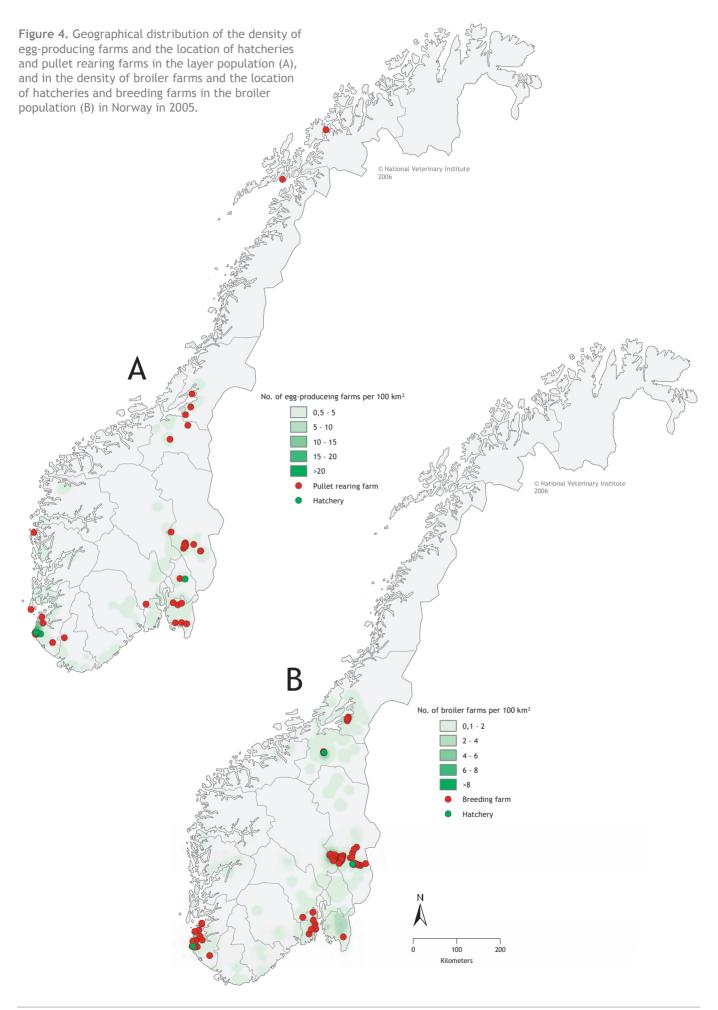
Aquaculture is an important industry for Norway and the value of exported Atlantic salmon and rainbow trout represents about 2 % of the total value of all exports. Atlantic salmon is the most important species in the fish and shellfish farming industry. The counties of Hordaland and Nordland are the major counties for seawater farms producing Atlantic salmon. The production volume of Atlantic salmon increased with 3 % from 2003 to 2004. A small reduction was observed in the volume of rainbow trout production in 2003 and 2004. Data for 2005 are not yet available (Table 3).

The import of live fish in 2005 consisted only of seven consignments of turbot (Table 2).

**Table 3.** Production volume of the most important species in Norwegian aquaculture during the time period 1992-2004<sup>1</sup>. Data for 2005 are not yet available

Year	Atlantic salmon (tons)	Rainbow trout (tons)	Cod (tons)	Arctic char (tons)	Halibut (tons)	Blue mussels (tons)	Scallops <sup>2</sup> (tons)	Oysters (tons)
1992	141,000		-		-		-	-
1993	170,000		-	-	-		-	-
1994	204,686	14,571	569	262	63	542	-	-
1995	261,522	14,704	284	273	134	388	-	-
1996	297,557	22,966	191	221	138	184	-	-
1997	332,581	33,295	304	350	113	502	-	-
1998	361,879	48,431	203	200	291	309	-	-
1999	425,154	48,692	157	498	451	662	67.1	40.6
2000	440,861	48,778	169	129	548	851	37.6	7.6
2001	436,103	71,764	864	318	377	920	22.3	2.5
2002	462,495	83,560	1,258	319	424	2,557	5.0	1.7
2003	509,544	68,931	2,185	272	426	1,829	1.2	1.6
2004	563,815	63,401	3,165	350	649	3,747	45.5	3.3

<sup>1</sup> Data from The Directorate of Fisheries, <sup>2</sup> From the wild population.



The surveillance and control programmes for *Salmonella* in live animals, eggs and meat in Norway

Annual report 2005





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# Introduction

The occurrence of *Salmonella* in Norwegian production animals and animal products is very low compared to most other countries, and has been so during the last decades.

The recorded incidence of human salmonellosis has increased in Norway during the last three decades. Since 1998, the annual incidence of human salmonellosis has remained between 1,400 and 1,900 (1). About 80 % of the patients with salmonellosis have acquired the infection abroad. Meat produced in Norway is not considered a source of indigenous human salmonellosis.

It is very important to maintain this favourable situation in Norway. In connection with the Norwegian negotiations for membership in the European Union, the Norwegian *Salmonella* control programme was established (2). The programme was launched in 1995, simultaneously with comparable programmes in Sweden and Finland (3, 4).

The Norwegian *Salmonella* control programmes for live animals, eggs and meat, consists of two main parts; surveillance and control. The surveillance covers live animals (pigs, cattle and poultry), fresh meat (pigs, cattle and sheep) and poultry meat (2). When *Salmonella* is isolated, action is taken to eliminate the infection, prevent transmission, and prevent contamination of food products. The programme is approved by the EU Commission (EFTA Surveillance Authority Decision No. 68/95/COL of 19.06.1995), allowing Norway to require additional guarantees regarding *Salmonella* when importing live animals, feed and food products of animal origin from the European Union.

The surveillance programmes for live animals, fresh meat and poultry meat are based on bacteriological examination for *Salmonella*. Isolation of *Salmonella*, irrespective of serovar, is notified to the Norwegian Food Safety Authority which maintains overall responsibility for the *Salmonella* surveillance and control programmes. The National Veterinary Institute coordinates the surveillance programmes, examines the faecal samples and publishes the results in monthly and annual reports. Private laboratories perform the examination of samples collected at slaughterhouses and cold stores.

## Aims

The aims of the programmes are to ensure that Norwegian food-producing animals and food products of animal origin are virtually free from *Salmonella*, to provide reliable documentation of the prevalence of *Salmonella* in the livestock populations and their products, and to prevent an increased occurrence of *Salmonella* in Norway.

# Materials and methods

The *Salmonella* surveillance and control programme for live animals includes examination of faecal samples from swine and poultry, and lymph node samples from cattle and swine (at least five ileo-caecal lymph nodes from each animal). The *Salmonella* surveillance and control programme for fresh meat and poultry meat includes examination of swab samples from cattle, swine and sheep carcasses, neck skin samples from poultry and samples of crushed meat from slaughterhouses and cold stores.

The number of samples examined in the different parts of the programmes is sufficient to detect at least one *Salmonella*-positive sample if the prevalence in the population is at least 0.1 %, with a confidence level of 95 %.

#### Sampling scheme for live animals

#### Swine

In Norway there are approximately 170 elite and multiplier breeding herds for swine. More than 95 % of marketed breeding animals are purchased from these herds. All elite and multiplier breeding herds are surveyed annually at herd level. Pooled faecal samples are collected from all pens (up to a maximum of 20) containing piglets aged two to six months. If there are less than three pens of piglets at this age, additional individual faecal samples are collected from all sows (up to a maximum of 59) (5).

The pig population is surveyed by sampling a representative proportion of all pigs slaughtered in Norway. A total of 3,000 lymph node samples from swine (both sows and slaughter pigs) are collected at the slaughterhouses. The sample size for each slaughterhouse ranges from 20 to 240 and is based upon the number of onsite slaughtered animals in relation to the national total. The sampling is distributed evenly throughout the year (6).

#### Cattle

The surveillance is based on sampling a representative proportion of all cattle slaughtered in Norway. A total of 3,000 lymph node samples from cattle are collected at the slaughterhouses. The sample size for each slaughter-house ranges from 20 to 100 and is based upon the number of onsite slaughtered animals in relation to the national total. The sampling is distributed evenly throughout the year (6).

#### Poultry

All breeding flocks and commercial production flocks, except layer flocks with less than 250 birds, are included in the surveillance programme. All breeder flocks are certified and the sampling scheme is in accordance with the old Zoonosis Directive (Council Directive 92/117/ EEC) (Table 1). All broiler flocks and flocks of turkeys, ducks and geese other than breeders are sampled one to three weeks Table 1. Sampling of poultry breeders (simplified) in the Salmonella surveillance and control programme in 2005

Category of pou	ltry	Time of sampling	Sample material
Grandparents	Day old	At arrival	Organs or meconium
	Rearing	1-2 weeks, 4 weeks, 9-11 weeks and 13-14 weeks	Faecal samples
	Egg production* - from the house - in the hatchery	Monthly Every 2nd week of production	Faecal samples Organs or meconium
Parents	Day old	Day 1	Organs or meconium
	Rearing	4 weeks and 2 weeks before start of production	Faecal samples
	Egg production* - in the hatchery	Every 2nd week of production	Organs or meconium

\* Hatcheries with a production <1,000 eggs per year are sampled at the poultry house every two weeks.

before slaughter (faecal samples), while layer flocks are sampled twice during the rearing period and once or twice during the egg laying period (2).

#### Clinical cases – all animal species

Animals with clinical symptoms consistent with salmonellosis should be sampled for bacteriological diagnosis. In addition, all sanitary slaughtered animals are tested for the presence of *Salmonella*. Any *Salmonella* isolated from animals, irrespectively of serovar, is notifiable in Norway.

# Sampling scheme for fresh meat and poultry meat

#### Swab samples from carcasses

The testing of slaughtered pigs, cattle and sheep for *Salmonella* is done by swabbing carcass surfaces. For each animal species, a total of 3,000 swab samples should be collected at slaughter. For each slaughterhouse, the sample size ranges from 20 to 100 and from 20 to 240 for cattle and swine, respectively. The number of swab samples of cattle and swine from each slaughterhouse equals the number of lymph node samples. The number of swab samples from sheep ranges from 20 to 160 per slaughterhouse. The sampling is distributed evenly throughout the year. The sampling is done before the carcasses are refrigerated, near the end of the slaughter line. Approximately 1,400 cm<sup>2</sup> of each carcass is swabbed (somewhat less for sheep) (6).

#### Neck skin samples

Neck skins from broilers and layers, turkeys, ducks and geese are tested for *Salmonella*. At each slaughterhouse, a minimum of five neck skins samples are collected per day and at least one sample must be taken from each flock slaughtered on a single day.

#### Food products

The surveillance and control programme for cutting plants and cold stores are based upon samples of crushed meat taken from the equipment or from trimmings. Each sample consists of 25 grams. Each production line is sampled separately. The sampling is done randomly during operation. The number of samples taken in cutting plants and cold stores is given by the production capacity of the plant, and ranges from one sample per week to two per year (6).

Pre-packed fresh meat intended for cold stores does not have to be examined if originating from cutting plants which are included in the programme. Fresh packed or repacked meat should be sampled.

#### Laboratory methods

All lymph nodes from one animal are divided into two equal parts. One half is used for testing and the other part is stored at 4 °C until the results of the bacteriological examination is ready. The lymph node from at most five animals are pooled and homogenized before bacteriological examination. Swab samples are pooled in groups of five before testing. Each neck-skin sample is divided into two equal parts. One part is pooled with four to eleven other samples. The other half of neck skin samples are stored separately at 4 until the results of the bacteriological examination are ready. If the pooled samples are examined separately.

Microbiological examination of the samples is carried out according to the Nordic Committee on Food Analysis Method No. 71, slightly amended to make the method applicable to the various kinds of materials. This is a qualitative bacteriological method based on selective enrichment and cultivation. All positive samples are confirmed and serotyped by a reference laboratory.

## Results

#### Live animals

#### Swine

A total of 2,492 faecal samples from 148 elite and multiplier breeding herds (including AI centres and testing stations) were examined in 2005 (Table 2). *Salmonella* was not detected in any of the samples. A total of 3,476 lymph node samples from slaughtered pigs were examined. Approximately 32 % of the samples were taken from sows and 68 % from slaughter pigs. None of the samples was positive for *Salmonella* (Table 3) giving an estimated *Salmonella* prevalence of 0 % (95 % confidence interval: 0 % - 0.1 %) at the individual carcass level.

#### Cattle

In 2005, a total of 2,209 lymph node samples from cattle were examined (Table 3). Two samples were positive for *Salmonella* Typhimurium (Table 3) giving an estimated *Salmonella* prevalence of 0.12 % (95 % confidence interval: 0.03 % - 0.33 %) at the individual carcass level.

#### Poultry

A total of 6,777 faecal samples from 1,374 different holdings were examined (Table 4). *Salmonella* Montevideo was detected in one of the samples.

#### Fresh meat and fresh poultry meat

# Swab samples from cattle, sheep and swine carcasses

A total of 7,925 swab samples from 39 slaughterhouses were examined in 2005 (Table 5). *Salmonella enterica* subsp. *diarizonae* was detected in three samples taken from sheep at two different slaughterhouses.

#### Neck skin samples from poultry

A total of 6,056 neck skin samples from poultry were examined in 2005. The samples came from all the seven poultry slaughterhouses in Norway. Nearly 85 % of the samples came from broilers, 10 % from layers and 7 % from other species (turkeys and ducks). *Salmonella* Senftenberg was detected in one sample from layers.

# Cutting plants and cold-stores for fresh meat and poultry meat

A total of 1,770 samples of crushed meat from 84 different plants were examined. *Salmonella* was not detected in any of the samples.

Table 2. Sampling in elite and multiplier breeding swine herds in the Salmonella surveillance and control programme in 2005

Herd category	No. of herds sampled (total*)	No. of samples examined	No. of positive samples	Salmonella serovar
Elite breeding herds	57 (65)	946	0	
Multiplier herds	88 (103)	1,466	0	
A.I. centres and testing stations	3 (4)	80	0	

\* Total number of herds is estimated as elite and multiplier breeding herds per 1 January 2005 excluding herds which ended breeding activity during 2005 before being tested.

Table 3. Number of individual lymph node samples from cattle and swine examined in the Salmonella surveillance and controlprogramme in 2005

Species	No. of slaughterhouses sampled (total*)	No. of samples examined	No. of positive samples	Salmonella serovar
Cattle	35 (40)	2,209	2	S. Typhimurium (4,5,12:i:1,2)
Slaughter pigs	24 (32)	2,376	0	
Sows	17 (32)	1,100	0	

\* Slaughterhouses where the number of slaughtered animals of a species is less than 100 according to the Slaughter Statistics for 2005 are not included in the sampling scheme.

Table 4. Samples from poultry in the Salmonella surveillance and control programme in 2005

Poultry breeding flocks	No. of samples tested	No. of holdings tested	No. of positive holdings	Salmonella serovar
Grandparents				
Layers	20	4	0	
Broilers	4	1	0	
Parents				
Layers and broilers	755	65	0	
Turkeys	49	3	0	
Ducks	22	2	0	
Geese	0	0	0	
Total – Breeders	755	74	0	
Other commercial poultry				
Pullets	208	20	0	
Layers	1,346	732	0	
Meat production - Broilers	3,883	549	1	S. Montevideo
- Turkeys	310	68	0	
- Ducks	40	5	0	
- Geese	4	2	0	
Unknown	136	22	0	
Total — Non breeder holdings	5,927	1,344	1	
Total	6,777	1,374	1	

Table 5. Number of swab samples from carcasses of cattle, swine and sheep and neck skin samples from poultry examined in the *Salmonella* surveillance and control programme in 2005

Species	No. of slaughterhouses sampled (total*)	No. of samples examined	No. of positive samples	Salmonella serovar
Cattle	33 (40)	2,076	0	
Swine	28 (32)	3,157	0	
Sheep	26 (35)	2,692	3	S. enterica subsp. diarizonae (61:-:1,5,7) S. enterica subsp. diarizonae (61:-:1,5)
Poultry	7 (7)	6,056	1	S. Senftenberg

\* Slaughterhouses where the number of slaughtered animals of a species is less than 100 according to the Slaughter Statistics for 2005 are not included.

# Discussion

The results from the *Salmonella* surveillance programme in 2005 document that the Norwegian cattle, swine, sheep and poultry populations are only sporadically infected with *Salmonella*. This is in accordance with previous findings (7-9). The estimated prevalence is below 0.2 % in the examined populations for any of the years the surveillance programme for live animals has run. The number of positive samples has never exceeded ten in total per year. S. Typhimurium has been isolated most frequently from swine, cattle and poultry, while *S. enterica* subsp. *diarizonae* is found most frequently from sheep. *S.* Enteritidis has never been found in the surveillance programme. Between 15 % and 25 % of the recorded human cases of salmonellosis are domestic in origin showing that domestic food products of animal origin represent a minor risk with regard to *Salmonella* infection in humans. In 2002 it was shown that two clones of *Salmonella* Typhimurium in the wild fauna (wild birds and hedgehogs) represented a risk for human infection (10). Such wild animal reservoirs may also be considered a risk for farm animals. As no increase in prevalence of *Salmonella* has been demonstrated in the programme, it may be assumed that farm animal populations have been and still are well protected from these reservoirs.

The number of swab and lymph node samples examined per species should have been 3,000 per year. The required sample size was reached for the swine population, but not for the cattle and sheep populations. A follow up of the personnel taking and reporting the samples is needed. Nevertheless, the programme was able to document a very low *Salmonella* prevalence in the examined populations.

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Residues of prohibited substances in live animals and of veterinary drugs, prohibited substances and environmental contaminants in animal products in Norway





Annual report 2005

Dag Grønningen

## Introduction

Surveying of residues in animal products has been carried out in Norway since 1985, initially in samples from bovine and porcine products. Since 1988, the Norwegian Food Control Authority has been in charge of the programme. In 1993 the programme was expanded to include sheep, poultry and reindeer products in accordance with EU Directive 86/469. It was further expanded in 1999 to include live animals and milk, eggs, honey, and fish. The number of samples and substances tested in the programme was at the same time substantially increased in accordance with EU Directive 96/23. The programmes for surveillance of residues in live animals and fish were taken over by the Norwegian Animal Health Authority and the Directorate of Fisheries, respectively.

The programmes for surveillance of residues in live animals, fish, and animal products were taken over by the Norwegian Food Safety Authority from 1 January 2004 (1,2,3). The Norwegian Food Safety Authority represents a merger of the Norwegian Animal Health Authority, the Norwegian Agricultural Inspection Service, the Norwegian Food Control Authority, the Directorate of Fisheries' seafood inspectorate, and local governmental food control authorities.

## Aims

The aim of the present programme is to ensure food safety by monitoring the occurrence of residues of veterinary medicines, prohibited substances and environmental contaminants in animal products and foods. The programme also provides data to satisfy export documentation requirements from the EU, USA and Switzerland.

The results of fish and products thereof are reported by National Institute of Nutrition and Seafood Research.

# Regulations

To prevent consumption of animal products that contain potentially harmful residues, the Residue Control Regulation (RCR) was introduced in 2000 (4). This aims to prevent production, import and sale of products containing residues of prohibited substances, contaminants and veterinary drugs above Maximum Residue Limits (MRL). The legislation implements EU Directive 96/23 and requires control measures for any activity in agricultural and animal production (5).

The RCR determines MRLs for veterinary drugs. The use of veterinary drugs without MRLs in production animals is prohibited. In 2002 the EU introduced the phrase Minimum Required Performance Limit (MRPL) through Commission Decision 2002/657/EC (6). It is intended to harmonise the analytical performance of methods for substances for which no MRLs have been established or are prohibited.

## Materials and methods

#### Group of substances

EU regulations define the species and groups of substances to be included in the programme (Appendix).

Samples of live animals (e.g. bovines, pigs, and poultry) are monitored for the presence of prohibited substances (Group A) only.

Each country may select the specific substances to be monitored. In Norway this is based on data from the Norwegian Medical Agency, as well as advice from the Norwegian School of Veterinary Science, Aker University Hospital and the National Veterinary Institute.

#### Sampling plan

The sampling plan for the various animal species and products is determined on the basis of earlier production (Table 1). The plan is designed to ensure an even sampling throughout the year and throughout the country. Information on each sample is registered in a protocol at the time of sampling and sent to the central registration unit.

#### Modification of the sampling plan

Table 1. The number of animals slaughtered and

The Norwegian Food Safety Authority reduced the sampling plan during the period of economic reasons.

production figures for animal products in Norway in 2003 Categories Production Bovine 333,424 \* Porcine 1,328,943 \* Sheep 1,229,189 \* Equine 2,141 \* Reindeer 1,715 tons Wild game 92,300 animals Poultry 48,629 tons 1,538 mill litre Milk Eggs 50,356 tons 940 tons Honey

\* Total number of approved carcasses.

#### Laboratory analysis

Samples are analysed within three months of sampling. Values exceeding MRLs and any prohibited substances detected are reported immediately.

All analyses are carried out by national reference laboratories. The Norwegian laboratories are accredited by the Norwegian Accreditation and thereby meet the requirements of the standard ISO/IEC 17025. Substances A1, A3, A4, A5 and B2d are analysed at the Hormone Laboratory, Aker University Hospital. Substances A2 are analysed at Ghent University, Belgium. Substances A6, B1, B2b, B2e, and B2f are analysed at the Laboratory for Veterinary Drug Residue Analysis in Food, the Norwegian School of Veterinary Science (NVH). Substances B2a and c are analysed at the Laboratory for Analysis of Veterinary Drugs, NVH. Substances B3a and b are analysed at the Laboratory of Environmental Toxicology, NVH, and the Plant Protection Center, Ås. Substances B3c and d are analysed at the Section of Chemistry, National Veterinary Institute.

## **Results and comments**

#### General

It was planned to collect 3,163 samples in 2005. Totally 2,850 samples from animals and primary animal products were collected. 93 samples (3.3%) were classified as non-compliant.

The report (in Norwegian) delivered to the Norwegian Food Safety Authority contains a more detailed description of the substance being analysed, the laboratory methods, and the results (7).

#### Live animals

Table 2 presents an overview of the number of samples tested in 2005 with respect to the sampling plan, and grouped according to substances.

Chloramphenicol was detected in one sample of turkey. The laboratory measured the concentration equals 0.2  $\mu$ g/kg. This is a prohibited substance and EU has established a MRPL for this substance at 0.3  $\mu$ g/kg. Norway considers this as a non-compliant sample.

#### Animal products

Table 3 presents an overview of the number of animal products sampled in 2005.

#### **Coccidiostats**

A trace amount of narasin was detected in eggs. Administration of this substance to egg producing hens is prohibited. Narasin does not have an established MRL nor MRPL. Norway considers this as a non-compliant sample/result.

#### Heavy metals

Residues of cadmium exceeding MRLs were detected in 29 samples of bovine, 35 samples of porcine, 14 samples of ovine, and 13 samples of poultry.

In 2005 cadmium was distributed through contaminated feed to nearly every farmed species in Norway. Norwegian Food Safety Authority collected samples to get a picture of the situation and implement appropriate actions to reduce the effect of the incident.

Chemical elements accumulate in organs throughout life as a result of environmental pollution, particularly in free ranging animals (farmed and wild game, sheep).

	Bovi	ines	Pi	igs	Poul	ltry
Substances	Sampled	Planned	Sampled	Planned	Sampled	Planned
A1 Stilbenes	74	82	9	10	3	4
A2 Thyrostatics	25	43	10	10	3	4
A3 Steroids	87	83	5	10	4	4
A4 Resorcyclic acid lactones	82	83	8	10	3	4
A5 Beta-agonists	80	83	9	10	3	4
A6 Annex IV substances*	40	43	3	10	10 (1 n-c)	10
Total A	388	417	44	60	26	30

Table 2. The number of live animals tested vs. planned in 2005

\* A6: Annex IV: chloramphenicol; nitrofuranes; dimetridazole, metronidazole, n-c: non-compliant.

	bovines	Ines	Σ	rigs	sneep	ер	Horses	ses	Poultry	Icry	Keindeer	eer	Milk	Y	Eggs	S	Honey	ey	Wildg	Wildgame
Substances	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.
A1 Stilbenes	68		34		17				10		2									
A2 Thyrostatics	22		7		6															
A3 Steroids	67		35		21				2		2									
A4 Resorcyclic acid lactones	68		37		15				10		2									
A5 Beta-agonists	60		30		25				10		9									
A6 Annex IV substances*	50		16		15				60		9		10		5		5			
Total A	335	0	159	0	102	0			95	0	18	0	10	0	ß	0	ß	0		
B1 Tiamulin (pigs); penicillin (milk)			20										49							
B1 Quinolones	20		20		20															
B1 Sulfonamides	20		20		20				60		20		54		40					
<b>B1</b> Tetracyclines	2								60		20		37		40		5			
Total B1	45	0	60	0	40	0			120	0	40	0	140	0	80	0	2	0		
B2a Anthelmintics	50		40		50				10		7		24							
B2b Anticoccidials	10		10		18				106						52	-				
B2c Carbamates and pyrethroides	20		10		35				10		10				15					
B2d Sedatives	25		15		15															
B2e NSAIDs	20		10		10		15		10											
B2f Glucorticoids	10		10				10						16				10*			
Total B2	135	0	95	0	128	0	25	0	136	0	17	0	40	0	67	-	10	0		
B3a Organochlorine compounds	20		20		15				10		10		19		20					
B3b Organophos- phorous compounds	20		20		15				5				21							
<b>B3c</b> Chemical elements	69	29	41	35	41	14			14	13	20								69	
B3d Mycotoxins			10		ß				m				13							
Total B3	109	29	91	35	76	4			32	13	30	0	53	0	20	0			69	0
Total B	289	29	246	35	244	14	25	0	288	13	87	0	233	0	167	-	15	0	69	0
Total A+B	674	29	405	35	346	14	25	0	383	12	105	c	243	0	172	-	20	C	69	0

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In 2005 cadmium was distributed through contaminated feed to nearly every farmed species in Norway. Norwegian Food Safety Authority collected samples to get a picture of the situation and implement appropriate actions to reduce the effect of the incident.

Chemical elements accumulate in organs throughout life as a result of environmental pollution, particularly in free ranging animals (farmed and wild game, sheep).

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# Appendix

# Group A – Substances having anabolic effect and unauthorized substances

- 1. Stilbenes, stilbene derivatives, salts and esters
- 2. Thyrostatics
- 3. Steroids
- 4. Resorcyclic acid lactones
- 5. Beta-agonists
- 6. Annex IV substances. (incl. chloramphenicol, nitrofuranes, dimetridazole and metronidazol)

#### Group B – Veterinary drugs and contaminants

- 1. Antibacterial substances, (incl. sulphonamides, fluoroquinolones)
- 2. Other veterinary drugs
  - a. Anthelmintics
  - b. Anticoccidials
  - c. Carbamates and pyrethroids
  - d. Sedatives
  - e. NSAIDs
  - f. Other pharmacologically active substances
- 3. Environmental contaminants and other substances
  - a. Organochlorine compounds, incl PCBs
  - b. Organophosphorus compounds
  - c. Chemical elements
  - d. Mycotoxins



# The surveillance and control programmes for paratuberculosis in Norway

Annual report 2005





Ola Nyberg Berit Djønne

Paratuberculosis was first diagnosed in cattle and goats in Norway in 1907 and 1934, respectively (1, 2). *Mycobacterium avium* subsp. *paratuberculosis* infection is a notifiable disease (List B) in ruminants in Norway, and the disease in cattle is controlled by government restrictions. Confirmation of infection most often results in the culling of the herd. Affected herd owners are compensated by the government, which also covers the expenses involved in testing. In goat flocks, government restrictions combined with vaccination are used to control paratuberculosis. From 1967 to 2001, a live attenuated vaccine was used (3), whereas from October 2001 vaccination has been performed using an inactivated vaccine (4).

A national surveillance and control programme for paratuberculosis was established in 1996 (5, 6, 7). Descriptions of occurrence of the disease in Norway, control measures taken up to 1995, and results from the surveillance and control programmes from 1996 to 2002, can be found in the annual reports for 2001 (6) and 2002 (7).

#### Aim

The aim of the surveillance programme for paratuberculosis in 2005 was to estimate the prevalence of the infection in the Norwegian population of vaccinated goats. In addition, cattle, goats from unvaccinated flocks, sheep and llamas in limited numbers were screened for infection with *M. a. paratuberculosis*.

# Materials and methods

Four animal species were included in the surveillance and control programme for paratuberculosis in 2005; cattle, llamas, goats and sheep. Faecal samples from these species were collected on the farms, while organ samples were collected at slaughterhouses.

#### Active surveillance

#### Cattle

The group of herds from which the animals were selected for testing consisted of all cattle herds delivering milk to dairies in the sampling period and all beef cattle herds receiving state support according to records of July 2004. Seventy-five randomly selected herds and twenty-five combined herds with cattle and goats were chosen for sampling. Faecal samples were collected from the five oldest cows in each herd.

#### Llamas

The llama was introduced as a new species to Norway in 1997-98. A few animals have been imported, mostly from Sweden but also from South America, over the last six to

seven years. All llamas are included in the programme, and faecal samples from animals over four years of age are collected each year. In addition, organ samples are collected from llamas at slaughter and from animals that die when over four years of age.

#### Goats

One hundred and thirty vaccinated and twenty unvaccinated flocks were selected for sampling. Faecal samples were taken from the 10 oldest goats, or from sick goats. The unvaccinated flocks were randomly selected. Included in the vaccinated flocks were all flocks with known infection and flocks with both goats and sheep or goats and cattle.

#### Sheep

Twenty flocks from the areas where goat kids are vaccinated were randomly selected for sampling by faecal samples from the ten oldest sheep, or from sick sheep.

#### Herds with restrictions

Samples collected from infected cattle herds, from infected flocks of small ruminants, or from contact herds are also included in the surveillance programme.

#### Passive clinical surveillance

Clinical surveillance has been a part of the programme since 2000. For cattle, special emphasis is placed on the collection of samples from animals with reduced milk production, loss of weight, diarrhoea lasting more than 14 days, and animals that are over four years old. Not all of these criteria need to be met.

#### Sampled herds and animals

A total of 469 faecal samples and 15 organ samples were collected from cattle, while 1,415 faecal samples and 26 organ samples were collected from goats. A total of 243 faecal samples and two organ samples were collected from sheep, and eight faecal samples and one organ sample were collected from llamas (Table 1).

#### Histopathological examination

Samples from jejunum, ileum, ileocecal valve, and mesenteric lymph nodes were examined histopathologically. The tissue was fixed in 10 % neutral-buffered formalin, processed by routine methods and stained with haematoxylin and eosin (HE) and the Ziehl-Neelsen (ZN) method for acid-fast bacteria.

#### Bacteriological examination

The samples were decontaminated with 4 % sodium hydroxide and 5 % oxalic acid with 0.1 % malachite green (8), and inoculated onto selective and non-selective Dubos medium with mycobactin (2  $\mu$ g/ml) and pyruvate (4 mg/ml) (9). Incubation time was 16 weeks.

Mycobactin dependency, acid-fastness by Ziehl-Neelsen staining, and presence of the insertion segment IS900 by a PCR technique (10) were used to identify the isolates.

# Results

#### Histopathological examination

Formalin-fixed tissue samples from 15 cattle from two different herds were examined with no positive results (Table 2).

A total of 24 goats from four different flocks were examined (Table 3). The goats came from two infected flocks and two suspected flocks. Granulomatous lesions and acid fast bacteria were found in the intestines and lymph nodes of three goats (Table 3).

Two sheep from contact flocks were examined with negative results (Table 4).

One llama was examined with negative results (Table 5).

#### Bacteriological examination

A total of 484 cattle in 90 herds were examined for paratuberculosis by bacteriological methods (Table 2). *M. a. paratuberculosis* was not found.

A total of 1,418 dairy goats from 146 flocks were examined for paratuberculosis by bacteriological methods (Table 3). *M. a. paratuberculosis* was isolated from 31 goats in 14 flocks. Nine of these flocks had tested previously positive earlier, while five flocks had not. The kids in these flocks were vaccinated against paratuberculosis since 1992-1993. A total of 273 sheep from 25 flocks were examined for paratuberculosis by bacteriological methods (Table 4). *M. a. paratuberculosis* was not isolated from any of the samples.

A total of nine llamas from four herds were examined for paratuberculosis by bacteriological methods (Table 5). *M. a. paratuberculosis* was not isolated.

# Discussion

Since the surveillance programme for paratuberculosis started in 1996, infection with *Mycobacterium avium* subsp *paratuberculosis* has been detected in nine cattle herds, two sheep flocks and in 24 goat flocks. The infection is endemic among goats in six out of 18 counties in Norway. All the cases among cattle and sheep can be attributed to one of two reasons; either brought into the country with imported cattle (seven cattle herds, one sheep flock) or contact with infected goats (two cattle herds, one sheep flock). Importation of live cattle nearly stopped after 1996 and has been replaced by importation of semen and embryos. But importation of sheep and goats is increasing and thus represents, together with the presence of infected goat flocks, a risk for spread of the infection to other ruminants.

The total number of milking goats in Norway is 45,000 in 550 flocks. In the six counties with endemic paratuberculosis, there are 250 flocks. Thirty-eight flocks (15 %) have been recorded as infected with *M. avium* subsp *paratuberculosis* in this area, and have been given restrictions by the veterinary authorities. The infection was recorded in five new flocks this year. It is probable that even more flocks are infected because vaccination hides the symptoms. The surveillance programme for 2005 therefore gave priority to samples from vaccinated goat flocks while cattle and sheep were sampled less. By following this priority over a few years, our prevalence estimate could possibly come closer

		Faecal samples no. of animals	Intestinal samples no. of animals	Total no. of animals	Total no. of herds
Cattle	Dairy and beef cattle	444	0	444	88
	Suspected or imported cases	1	1	1	1
	Control of infected herds and contact herds	24	14	38	1
Goat	Vaccinated	1,122	0	1,122	112
	Unvaccinated	270	0	270	27
	Suspected cases	0	2	2	2
	Control of infected flocks and contact flocks	23	26	26	5
Sheep	Random sample	204	0	204	21
	Control of infected flocks and contact flocks	39	2	41	3
Llama		8	1	9	4

Table 1. Number of samples collected for examination for Mycobacterium avium subsp. paratuberculosis in 2005

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#### Table 2. Results of histopathological and bacteriological examination of cattle in 2005

Bacteriology		Histopathology				
Type of samples	No. of samples	No. of herds	No. of pos. samples	No. of samples	No. of herds	No. of pos. samples
Faeces	469	88	0			0
Intestinal samples	15	2	0	15	2	0

#### Table 3. Results of histopathological and bacteriological examination of goats in 2005

	Bacteriology			Histopathology		
Type of samples	No. of samples	No. of herds	No. of pos. samples	No. of samples	No. of herds	No. of pos. samples
Faeces	1,415	144	31			31
Intestinal samples	3	3	1	26	4	3

#### Table 4. Results of histopathological and bacteriological examination of sheep in 2005

	Bacteriology			Histopathology		
Type of samples	No. of samples	No. of herds	No. of pos. samples	No. of samples	No. of herds	No. of pos. samples
Faeces	271	23	0			0
Intestinal samples	2	2	0	2	2	0

Table 5. Results of histopathological and bacteriological examination of llamas in 2005

	Bacteriology			Histopathology		
Type of samples	No. of samples	No. of herds	No. of pos. samples	No. of samples	No. of herds	No. of pos. samples
Faeces	8	3	0			
Intestinal samples	1	1	0	1	1	0

to the true prevalence in the endemic area. This could be very useful in the near future, because the dairy organisation (TINE) and The Goat Health Services have started an eradication programme for three widespread infectious diseases in goats. The programme started in 2001 and concentrated the first years on caprine arthritis encephalitis and caseous lymphadenitis. From 2005 they included herds with goats suffering from paratuberculosis as well.

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

Annual report 2005

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#### Surveillance for Bovine Spongiform Encephalopathy (BSE)

BSE became a notifiable disease in Norway 1 February 1991, and the first surveillance and control programme for BSE was launched 1 August 1998. The Norwegian Animal Health Authority (from 2004: the Norwegian Food Safety Authority) was responsible for the implementation of the programme, while the National Veterinary Institute was responsible for laboratory analyses and reporting. The 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 enzymed-linked immunosorbent assay (ELISA) method for detection of resistant prion protein (PrPsc) (Platelia® BSE ELISA, Bio-Rad was replaced by TeSeE® Test Bio-Rad in June 2003). Clinically suspected animals were in addition investigated by histopathological examination according to the Office International des Epizooties (OIE) protocol (1, 2). The number of samples examined in each category in the period 1998-2004 is presented in Table 1. BSE has never been detected in any of the examined animals.

Table 1. Examination for BSE in cattle sampled by theNorwegian surveillance programme according to categoriesfrom 1998-2004

Reason for submission to the laboratory	1998- 2000	2001	2002	2003	2004
Clinically suspected	78	14	2	2	3
Fallen stock		1,352	1,482	1,872	2,145
Emergency slaughtered		7,073	7,246	7,322	9,217
Ante-mortem animals		2,612	3,562	4,102	1,355
Imported slaughtered animals	19*	88	39	39	24
Healthy slaughtered animals		2,400	9,907	10,726	10,443
Total	97	13,539	22,238	24,063	23,187

\* All the samples were examined in 2000.

# Aim

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

#### Surveillance programme

#### Programme outline

For 2005 the surveillance programme was in accordance with the Commission Regulations (EC) No 999/2001, No 1188/2003 and No 1915/2003. The programme included examination of the following categories:

- clinically suspected animals irrespective of age
- all animals older than 24 months of age, which have died or been culled, but not slaughtered for human consumption (fallen stock)
- all emergency slaughtered animals older than 24 months
- all animals older than 24 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 animals with unknown age or origin irrespective of age
- all imported cattle from any country irrespective of age and the over 24 month old progeny of imported female cattle
- 10,000 randomly selected healthy routinely slaughtered animals older than 30 months

#### Implementation

The farmers were responsible for reporting all cases of clinically suspected animals irrespective of age, fallen stock older than 24 months and when delivering an imported animal or progeny of an imported female animal to slaughter, to the Norwegian Food Safety Authority. The Norwegian Food Safety Authority forwarded the brain or the head from clinically suspected cattle and fresh material from the *medulla oblongata* sampled from fallen stock to the Norwegian Food Safety Authority collected the 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 National Veterinary Institute in Sandnes, Trondheim or Harstad.

# Laboratory methods

#### **Clinically suspected animals**

The whole brain was divided midsagittally in two equal halves. One half was formalin-fixed and processed according to a standard routine protocol, embedded in paraffin, sectioned at 5  $\mu$ m and stained with haematoxylin eosin (HE). Immunohistochemical staining for PrP<sup>sc</sup> was 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 was prepared for ELISA to detect  $PrP^{sc}$  (TeSeE®, Bio-Rad) as described by the manufacturer.

Table 2. Examination for BSE in cattle sampled by the Norwegian surveillance programme according to category in 2005

Reason for submission to the laboratory	No. of samples	No. of rejected samples	Negative	Positive
Clinically suspected animals	1	0	1	0
Fallen stock	2,318	58	2,260	0
Emergency slaughter	8,462	11	8,451	0
Ante-mortem animals	102	0	102	0
Imported animals	10	0	10	0
Healthy slaughtered animals	10,486	2	10,484	0
Total	21,379	71	21,308	0

#### Risk population and routine slaughtered animals

Non-fixed brain tissue from the *obex* area was prepared for ELISA to detect PrP<sup>Sc</sup> (TeSeE®, Bio-Rad) as described by the manufacturer. In cases with positive or inconclusive test results, the remaining half *obex* will be fixed in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 5  $\mu$ m, and stained with HE. Subsequently, the specimen will be processed for immunohistochemical detection of PrP<sup>Sc</sup> using the same protocol as for specimens from clinical suspects.

Brain samples were rejected for examination if the specimen was severely autolysed, the dorsal part of the *obex* area was cut obliquely, the *obex* was not present, or the medullar anatomy was not recognisable.

# **Results and discussion**

The National Veterinary Institute received samples from 21,379 cattle. Of these, 71 (0.3 %) samples were unsuitable for examination. The categories and number of animals examined are presented in Table 2.

For 1.3 % of the samples the herd of origin was not reported, but in case of a positive test result, the herd identity can be traced via the carcass number. The remaining 21,092 samples originated from 11,301 herds (9,806 dairy cattle herds and 1,495 beef cattle herds). The mean number of examined animals per herd was 1.9.

# Clinically suspected animals (passive surveillance)

Only one animal has been investigated as a clinical suspect. Improved methods for clinical examination to distinguish between real suspect BSE cases and cases with central nervous disease of other causes has resulted in few clinical suspect 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)

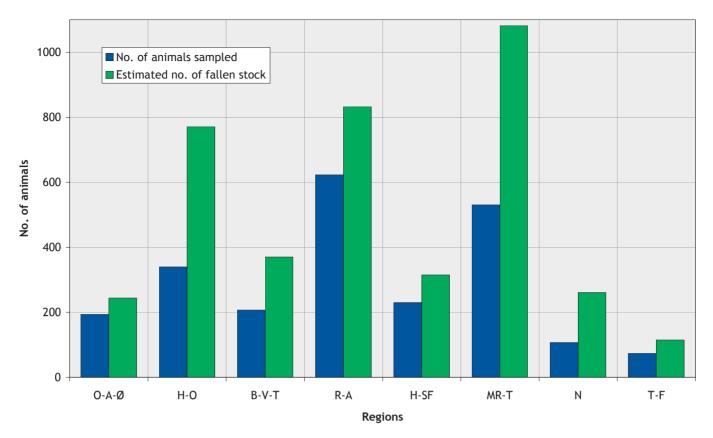
The number of examined cattle from emergency slaughtered animals in 2005 has decreased. Similarly, the number of examined cattle from the ante mortem category has decreased significantly compared to corresponding categories for 2004 (Table 1). Fallen stock older than 24 months comprises approximately 0.97 % of the adult population (Husdyrregisteret per 31.12.2005). The majority of samples from fallen stock were collected on farm.

The difference between the examined number and the number of fallen stock may be partly explained by the fact that many cattle herds are located in remote areas where sampling is time consuming and cumbersome. In addition, a proportion of the cattle is grazing on mountain and forest pastures where sampling of dead animals is difficult. Furthermore, another reason may be the lack of information to the farmers relating to their duty to report all cases of fallen stock older than 24 months to the Norwegian Food Safety Authority.

The number of samples examined in each region is compared to the number of fallen stock older than 24 months in each region (Husdyrregisteret per 31.12.2005). In all regions the number of animals sampled was low compared to the expected number to be sampled (Figure 1). In particular, in the regions Hedmark and Oppland, Møre og Romsdal and Trøndelag and Nordland approximately 50 % of the fallen stock population were investigated.

Norwegian cows are slaughtered at a low age, mean age is approximately 50 months for dairy cows and 67 months for suckling cows (suckling cows constitute only 15 % of the cattle population older than 24 months) (National Production Recording Scheme 2004, Norwegian Beef Herd recording System 2004).

The low age at culling implies that 37.0% of the samples from dairy cattle and 43.6% of the samples from beef cattle in the fallen stock population originated from cattle younger than 4 years. The age distribution of cattle sampled as fallen stock is shown in Table 3.



**Figure 1.** Number of fallen stock (with reported identity) sampled in each surveillance region in 2005, compared with estimated number of dead animals, expected to be 0.97 % of the cattle populations older than 24 months. (Husdyrregistret per 31.12.2005).

Region abbreviations: O-A-Ø = Oslo, Akershus and Østfold, H-O = Hedmark and Oppland, B-V-T = Buskerud, Vestfold and Telemark, R-A = Rogaland and Agder, H-SF = Hordaland and Sogn og Fjordane, MR = Møre og Romsdal, T = Trøndelag, N = Nordland, T-F = Trøms and Finnmark.

	Relative number of	Relative number of tested animals					
Age groups (months)	Fallen stock (%)	Emergency slaughter (%)	Ante mortem animals (%)	Healthy slaugh- tered animals (%)	Total (%)		
< 24	1.8	4.4	1.0	1.6	2.7		
24-29	11.5	13.7	12.7	8.1	10.7		
30-35	10.4	7.7	7.8	11.2	9.7		
36-47	18.7	16.5	15.7	23.5	20.2		
48-59	17.3	16.3	14.7	20.2	18.3		
60-71	16.7	17.8	19.6	15.3	16.5		
72-83	11.3	11.0	15.7	10.1	10.6		
84-95	6.1	6.5	3.9	5.1	5.8		
96-107	3.4	3.0	5.9	2.7	2.9		
108-119	1.5	1.7	0.0	1.2	1.4		
120-131	0.5	0.7	2.0	0.7	0.7		
132-143	0.3	0.3	0.0	0.1	0.2		
144-155	0.2	0.2	0.0	0.1	0.2		
≥ 156	0.2	0.3	0.0	0.1	0.2		
Total no. of animals	2,318	8,462	102	10,486	21,368		

#### Table 3. Age distribution of cattle tested for BSE-agent in 2005

There were 2,057 samples (9.5 %) 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.

Results from the BSE-monitoring programme in EU 2004 show that only 7 (0.82 %) of 850 verified cases of BSE were younger than 48 months, and 0.03 positive cases were detected per 10,000 tests in cattle 36-47 months, in contrast to 0.93 in cattle 72-83 months (3). These results indicate that BSE-monitoring of animals younger than 48 months is of low value.

The geographical distribution of the cattle population and the animals tested are presented in Figure 2. The figure indicates that there is a variation between regions and areas in the following up of the BSE-surveillance programme.

# Conclusion

As mentioned in first BSE Surveillance report in 2001 (4), the Norwegian cattle population has probably never been infected with 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. The compiled results from the surveillance and control programme for BSE in the years 2001 to 2005 (5) with more than 104,000 negative samples, clearly support this view.

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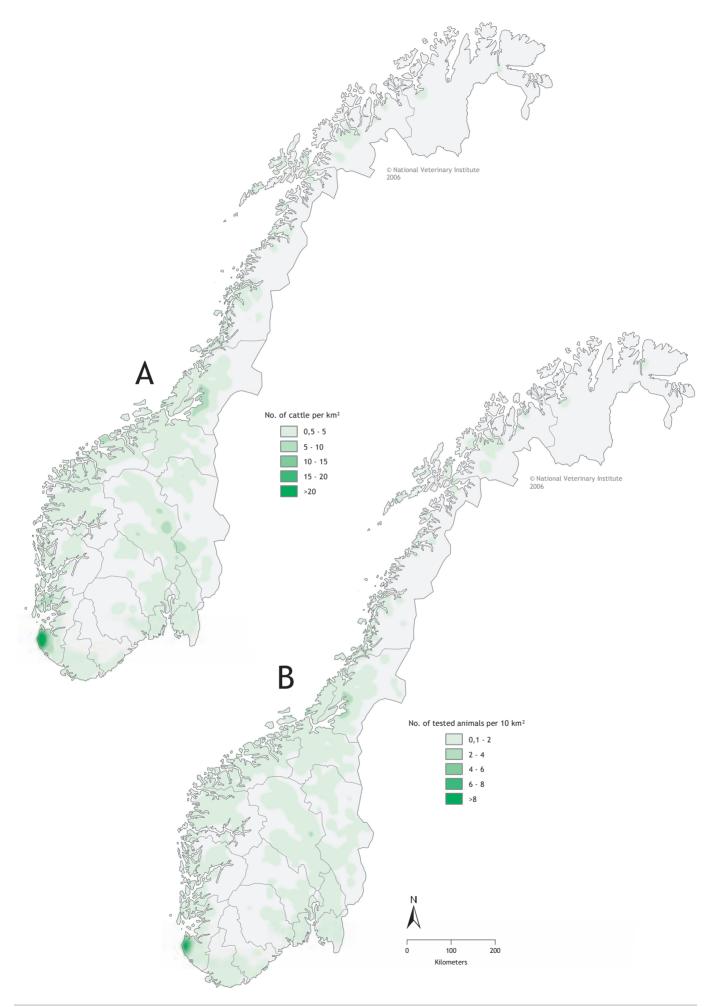
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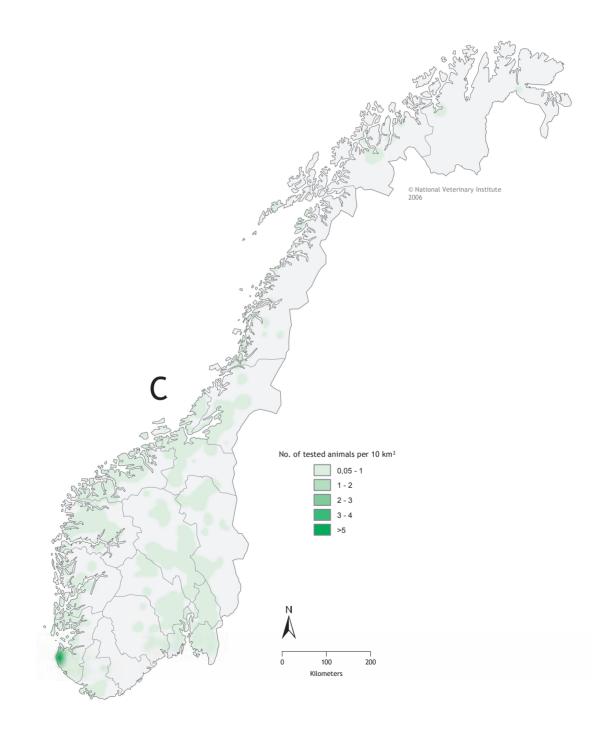
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**Figure 2.** Geographical distribution of the cattle population density (cattle > 24 months) (A), the density of emergency slaughtered animals (B) and the density of fallen stock tested (C) in the surveillance and control programme for BSE in 2005.





The surveillance and control programme for infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) in Norway

Annual report 2005

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In the early 1960s, two outbreaks of infectious pustular vulvovaginitis were diagnosed in cattle in Norway. No cases of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) were reported after these two cases until 1993, when several animals in one single herd were found to be serologically positive after primary testing of bulk milk collected in 1992. Clinical signs of IBR/IPV were never recorded on the farm. All animals on the farm were slaughtered. Attempts to isolate the virus from organ samples gave negative results. Sixteen contact herds and all dairy herds in the same region were serologically negative (1, 2). Likewise, 40 red deer that were shot in the neighbourhood during the hunting season the same year were serologically negative. After this incident, IBR/IPV virus infection has not been demonstrated in Norway. All breeding bull candidates are serologically tested before entering the breeding centres, and all breeding bulls are subject to a compulsory test each year.

EFTA Surveillance Authority (ESA) has recognised Norway as free from IBR since 1994. Decisions concerning the additional guarantees relating to IBR/IPV for bovines destined for Norway are described in ESA Decision 74/94/COL. Maintenance of the ESA Decisions accepting the IBR-free status of Norway requires annual reports of the surveillance of the disease.

The Norwegian Food Safety Authority is responsible for carrying out the surveillance and control programme for IBR/IPV. The National Veterinary Institute is in charge of planning the programme, collecting the bulk milk samples from the dairies and performing the tests. Blood samples from beef herds are collected by inspectors from the Norwegian Food Safety Authority.

#### Aims

The aim of the surveillance and control programme for IBR/ IPV is to document freedom from the infection in Norway according to the demands in ESA Decision 74/94/COL with amendments, and to contribute to the maintenance of this favourable situation.

# Material and methods

The surveillance of cattle for IBR/IPV in 2005 included both dairy and beef herds. Bulk milk samples from the dairy herds were provided by the dairies. From the beef herds, individual blood samples were collected on the farms from cattle older than 24 months.

The total group of dairy herds from which the selection of herds was made, consisted of all herds of cattle delivering milk to the dairies in the sampling period. In 2005, bulk milk samples from 1,919 randomly sampled dairy herds were tested. The group of beef herds to be sampled was based on a register of all beef herds receiving governmental support according to recordings of July 2004. A total of 4,766 individual blood samples from 484 beef herds were analysed in pools with a maximum of 86 samples in each. The sampled herds represented approximately 12.1 % of the Norwegian cattle herds.

The number of herds in the surveillance and control programme for IBR/IPV in 2005 is given in Table 1. The geographic distribution of the total number and the number of tested dairy and beef herds are shown in Figures 1 and 2.

All samples were tested for antibodies against bovine herpes virus 1 (BHV-1) using a blocking ELISA (3) at the National Veterinary Institute in Oslo.

Table 1. Total number of dairy herds and beef herds within the frame of the Norwegian surveillance and control programme for IBR/IPV in 2005

Herd category	Total no. of cattle herds*	No. of herds tested	% tested of the total no. of herds
Dairy herds	15,900	1,919	12.1
Beef herds	3,900	484	12.4
Total	19,800	2,403	12.1

\* Based on data from the Register of production subsidies as of July 31 2004.

## Results

All 1,919 bulk milk samples and 4,766 blood samples tested in 2005 were negative for antibodies against BHV-1. Table 2 shows the results of the testing during the period from 1993 to 2005.

# Discussion

Norway has been granted additional guarantees from ESA since 1994. Such guarantees depend on a continuous surveillance of the Norwegian cattle population based on serological examination. The surveillance and control programme for IBR/IPV has been evaluated using Monte Carlo simulation models (4). The Danish ELISA test is calculated to have a sensitivity of 82.9 % when used for bulk milk testing in Denmark (3). However, the sensitivity improves when the same test is used in Norway because the herds are smaller. The number of milking cows in an average Norwegian herd is 17, compared to approximately 100 in Denmark. The sensitivity is better when testing serum samples. Norwegian investigations have shown that the test has a specificity of 100 % (4).

The results of the continuous testing since 1992/93 strongly indicate that the Norwegian cattle population is free from IBR/IPV-infection (2, 4, 5). The surveillance and control programme, combined with the additional guarantees and testing procedures for imported cattle, are valuable means to prevent new introduction of the infection.

**Table 2.** Samples in the surveillance and control programmefor IBR/IPV in the Norwegian bovine population during theperiod 1993-2005

	Dairy herds	Beef	herds	
Year	No. of bulk milk samples tested	No. of beef herds sampled	No. of individuals tested	No. of positive samples
1993	26,642	0	0	1
1994	24,832	1,430	5,954	0
1995	25,131	1,532	9,354	0
1996	2,863	303	1,523	0
1997	2,654	2,214	16,741	0
1998	2,816	2,191	17,095	0
1999	2,930	2,382	18,274	0
2000	1,590	340	2,892	0
2001	2,564	434	3,453	0
2002	2,308	462	3,693	0
2003	1,845	449	3,901	0
2004	1,573	402	3,364	0
2005	1,919	484	4,766	0

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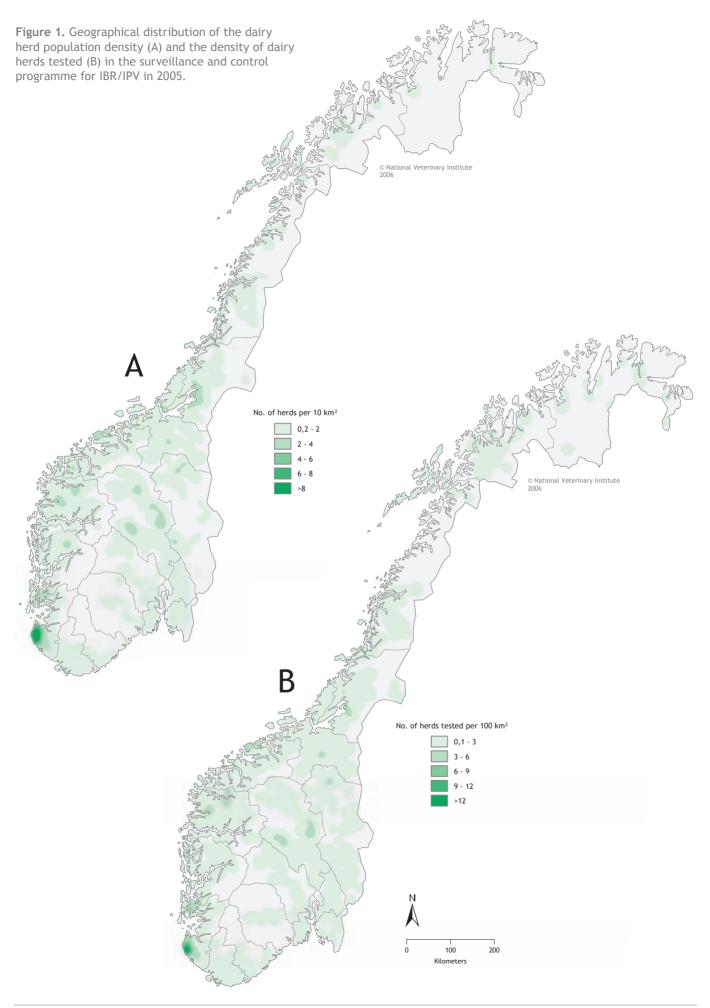
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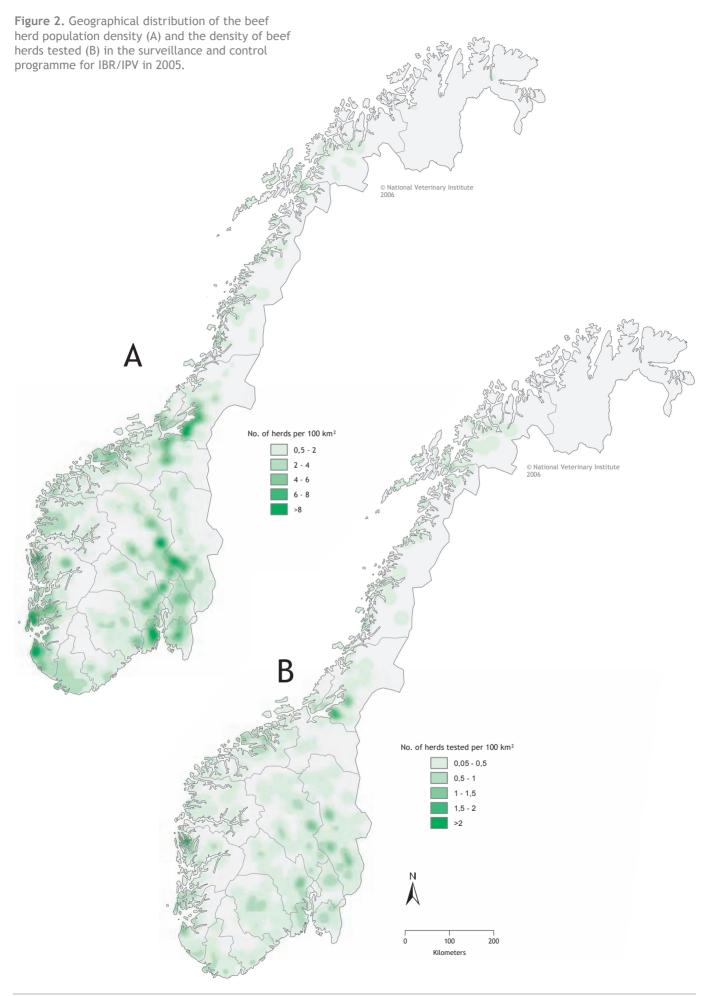
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# The surveillance and control programme for enzootic bovine leukosis (EBL) in Norway

Annual report 2005

Annette Hegermann Kampen Jorun Tharaldsen Ola Nyberg Peder Andreas Jansen

Enzootic bovine leukosis (EBL) had never been reported in Norway, neither clinically nor serologically, until the start of the surveillance and control programme in 1995. In 1976-77, blood samples from 3,885 cattle were examined with both haematological methods and serological methods for antibodies against bovine leukaemia virus (BLV) (1). In 1991, 1,575 bulk milk samples tested with an ELISA test resulted in no positive findings. From 1979, approximately 290 young bulls entering the breeding centres have been tested annually, first by an immunodiffusion test and then, from 1990, by an ELISA test.

From the material collected in 1994-95, antibodies against BLV were detected in eight dairy herds. In 1996, one positive dairy herd was found (2) (Figure 1A). Restrictions were immediately imposed on positive herds and control measures included culling of antibody-positive reagents. All the animals were retested over the next years. In one herd, all the animals were culled because more than 80 % of the adult animals were positive.

No new herds tested positive during the period 1997-2001 (3). In 2002, one bulk milk sample from a dairy herd gave a positive result for antibodies against BLV (Figure 1A). It was a small herd consisting of only nine dairy cows. Further investigations showed that only one cow was antibody positive. The cow, which was healthy and had no clinical symptoms, was slaughtered and the following pathological investigations gave no indication of leukosis. Further testing of individual blood samples of all cattle older than 24 months in the affected herd and six contact herds was negative. The conclusion was that the positive antibody test probably was due to a false positive serological reaction. The follow-up study was terminated in 2003 with no positive findings (4, 5).

The Norwegian Food Safety Authority is responsible for carrying out the surveillance and control programme for EBL. The National Veterinary Institute is in charge of planning the programme, collecting the bulk milk samples from the dairies, and performing the tests. Official inspectors from the Norwegian Food Safety Authority collected the blood samples from the beef herds.

#### Aims

The aim of the surveillance and control programme for EBL is to document freedom from the infection in Norway and to contribute to the maintenance of this favourable situation. Further, an application for EBL free status according to the EEC agreement (Council Directive 64/432/EEC of 26.06.64 as amended) has been submitted to the EU.

# Materials and methods

The surveillance and control programme included both dairy and beef herds. Bulk milk samples from the dairy herds were collected from the dairies. From the beef herds, individual blood samples were collected on the farms from cattle older than 24 months.

The group of dairy herds sampled was selected from all herds of cattle delivering milk to the dairies during the sampling period. In 2005, bulk milk samples from 1,919 randomly sampled dairy herds were tested for antibodies against BLV. The group of beef herds to be sampled was based on a register of all beef herds receiving governmental support according to recordings of July 2004. A total of 4,766 individual blood samples from 484 beef herds were analysed in pools, with a maximum of 86 samples in each. The sampled herds represented approximately 12.1 % of the Norwegian cattle herds (Table 1).

The geographic distribution of the total number of herds and the tested number of dairy and beef herds are given in Figure 1B and Figure 2A and 2B.

Bulk milk samples and blood samples (pooled serum) were examined by an indirect ELISA (SVANOVA®) (6). For verification and for follow-up of suspect cases, LACTELISA BLV Ab and SERELISA BLV Ab from SYNBIOTICS were used.

# Results

All bulk milk samples and blood samples tested in 2005 were negative for antibodies against BLV.

A historic survey of the surveillance of antibodies against BLV in the Norwegian population is given in Table 2, and the location of the antibody-positive herds found in 1995-96 is shown in Figure 1A.

Table 1. Total number of dairy herds and beef herds withinthe frame of the Norwegian surveillance and controlprogramme for EBL in 2005

Herd category	Total no. of cattle herds*	No. of herds tested	% tested of the total no. of herds
Dairy herds	15,900	1,919	12.1
Beef herds	3,900	484	12.4
Total	19,800	2,403	12.1

 $^{\ast}$  Based on data from the Register of production subsidies as of July 31 2004.

Table 2. Antibodies against BLV in the Norwegian bovinepopulation during the period 1995-2005

	Dairy herds	Beef	herds	
Year	No. of bulk milk samples analysed	No. of beef herds sampled	No. of individuals analysed	No. of positive samples
1995	25,131	1,532	9,354	8 (bulk milk)
1996	25,278	303	1,523	1 (bulk milk)
1997	26,903	2,214	16,741	0
1998	23,581	2,191	17,095	0
1999	19,933	2,382	18,274	0
2000	1,590	340	2,892	0
2001	2,564	434	3,453	0
2002	2,308	462	3,693	1 (bulk milk)
2003	1,845	449	3,901	0
2004	1,573	402	3,364	0
2005	1,919	484	4,766	0

# Discussion

The requirement from the EU for granting an EBL free-status is that the prevalence must be lower than 0.2 %, which represents 40 herds out of a total number of 19,800 herds.

EBL had never been reported until the surveillance and control programme detected nine positive herds in 1995-96. These herds are now free from EBL. From 1995 to 1999, all cattle herds were tested annually. Since 2000, 10 % of the herds have been tested each year.

The results of the continuous surveillance since 1995 indicate that the Norwegian cattle population is free from EBL according to the EU requirements (3, 4, 5, 7). Together with the possible isolation period of six months and the testing protocol for imported animals, the surveillance and control programme for EBL should be a valuable means to discover introduction of new infection.

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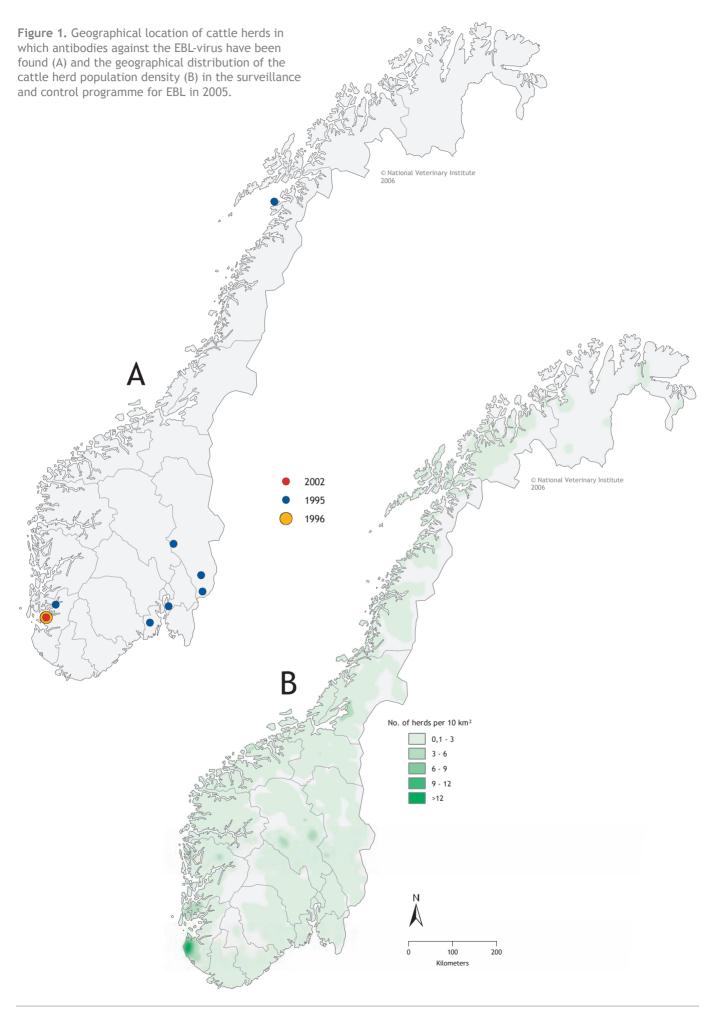
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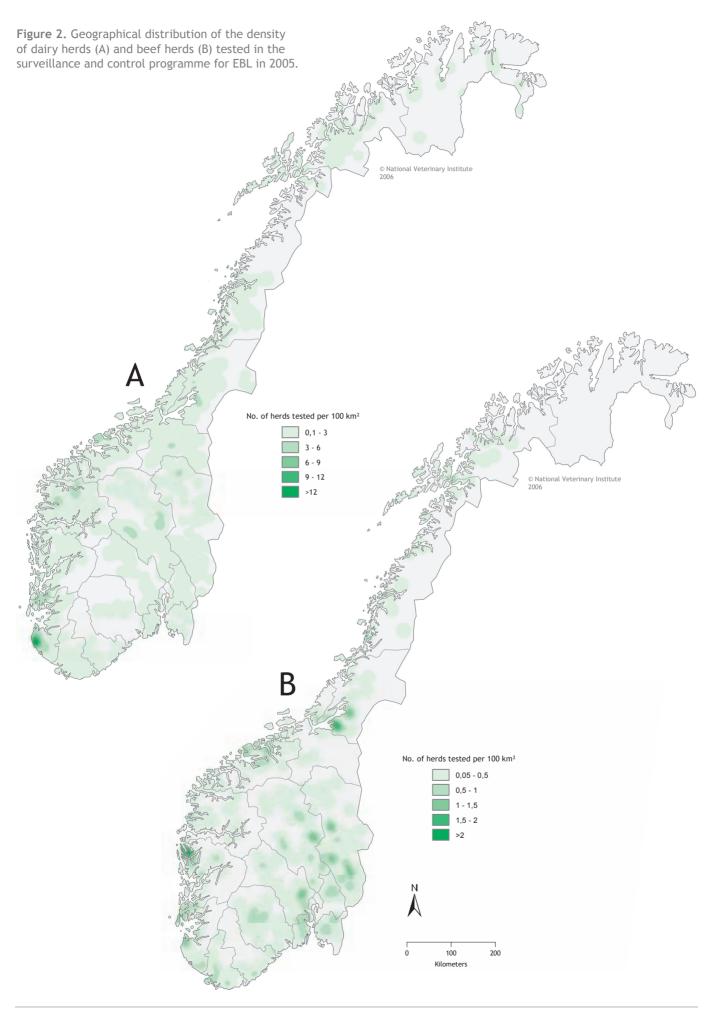
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# The surveillance and control programme for *Brucella abortus* in cattle in Norway

Annual report 2005

Annette Hegermann Kampen Berit Djønne Bjørn Bratberg Jorun Tharaldsen Trude Lyngstad Ola Nyberg

Eradication of bovine brucellosis in Norway was achieved in 1950. An extensive eradication campaign had been launched in Norway in 1935, and about 350,000 blood samples were tested during the subsequent 15 years (1, 2).

Since 1994, the EFTA Surveillance Authority (ESA) has recognised Norway as a state officially free from brucellosis as described in ESA Decision 66/94/COL, later replaced by ESA Decision 227/96/COL. In 2000, the Norwegian Animal Health Authority (from 2004; the Norwegian Food Safety Authority) launched a surveillance and control programme for Brucella abortus in which milk, blood and foetuses from dairy and beef herds were examined for evidence of Brucella abortus infection (Table 1). All investigations on Brucella abortus were negative in 2000, 2001, 2003 and 2004 (2, 3, 4, 5). In 2002, two bulk milk samples were antibody positive. Blood samples from animals older than two years were collected from these herds. Two cows in one farm and one cow in the other farm were positive in three different tests in two consecutive samplings six weeks apart. All three cows were culled. Autopsy did not indicate brucellosis, and bacterial examination was negative for Brucella abortus. Serological examinations of the animals in both herds 30 and 90 days after culling were negative. It was concluded that the positive serological results most likely were false positive reactions, most likely due to serological cross reactions (6).

During the years 2000-2004, the programme consisted of an active surveillance part, where 20 % of the Norwegian cattle population were sampled each year, and a passive surveillance part, where aborted foetuses and blood samples from their dams were investigated. Since 20 % of the Norwegian cattle population had been tested annually for five consecutive years and thereby fulfilled the requirements from the EU, the programme in 2005 was reduced to passive surveillance only. The Norwegian Food Safety Authority is responsible for carrying out the programme. The National Veterinary Institute is in charge of planning the programme, performing the analyses and reporting the results. The samples are collected by inspectors of the Norwegian Food Safety Authority.

#### Aim

The aim of the programme is to document freedom from *Brucella abortus* in cattle according to demands in Directive 64/432/EEC with amendments and to contribute to the maintenance of the present favourable situation.

# Material and methods

#### Passive clinical surveillance

- Herd criteria for submission of clinical material are:
- abortions occurring between the fifth month of pregnancy and 14 days before expected birth
- at least two abortions within this pregnancy period the last twelve months

Material for submission:

- foetus and the foetal membranes
- blood sample from the cow at the time of abortion and a second blood sample collected 14-21 days later

#### Post-mortem investigations

Foetuses are subjected to a full autopsy. Specimens from lungs, myocardium, liver, kidneys, (whole) brain, and foetal membranes are fixed in 10 % neutral phosphate-buffered

		Dairy cattle		Beef cattle		Total	
Year	Material	Samples	Herds	Samples	Herds	Samples	Herds
2000	Bulk milk/blood	4,228	4,228	5,695	677	9,923	4,905
	Foetuses					17	14
2001	Bulk milk/blood	5,128	5,128	7,027	868	12,155	5,996
	Foetuses	21	18	0	0	21	18
2002	Bulk milk/blood	4,664	4,664	7,296	915	11,960	5,579
	Foetuses	18	17	10	6	28	23
2003	Bulk milk/blood	3,684	3,684	7,905	887	11,589	4,571
	Foetuses	30	25	4	3	34	28
2004	Bulk milk/blood	3,138	3,138	7,986	813	11,124	3,951
	Foetuses	25	21	2	2	27	23
	Blood samples related to abortions	28	19	2	2	30	21

 Table 1. Number of bulk milk samples, blood samples and foetuses examined for brucellosis in the Norwegian cattle population during the years 2000-2004

formalin. The specimens are processed according to a standard routine protocol, sectioned at 5  $\mu m$  and stained with haematoxylin and eosin.

#### Bacteriological investigations

Foetal membranes and organs from the aborted foetus (liver, spleen and stomach contents) are sampled. Direct smears from these materials are examined following Gram and Modified Ziehl-Neelsen staining. Samples are cultured on bovine blood agar containing 5 % bovine blood, Skirrows medium and Tryptone Soy Agar at 37 °C in a 10 % CO2 atmosphere. The media are examined regularly and incubated for up to 14 days. Suspicious bacterial colonies are tested for motility, nitrate reduction, and for the production of catalase, indol, cytochrome oxidase, and urease. Non-motile, nitrate-reducing, indol-negative, and catalase-, cytochrome oxidase- and urease-producing isolates are sent to a reference laboratory for further identification.

#### Serology

Individual, paired blood samples are tested for antibodies against Brucella abortus in an indirect ELISA (Svanova®). The initial screening is performed using a single well per sample, and doubtful or positive reactions are retested in duplicates. If the result is negative when retested, the sample is concluded to be negative for antibodies against Brucella abortus. If the result still is doubtful or positive, the sample is tested with a competitive ELISA (C-ELISA, Svanova®). Positive samples in this test are subjected to a complement fixation test (CF). If the CF test also is positive, the result is reported with recommendation of a new blood sample from the suspected animal four to six weeks after the initial sampling. If this is positive, or if there should be a need for immediate follow-up, the animal will be tested with an intracutane test using Brucellergene OCB from Brucella melitensis (Synbiotics®).

# **Results and discussion**

A total of 24 foetuses from 21 different herds and 96 blood samples from 56 cows (paired samples from 39 cows and 18 single samples) were analysed in 2005 (Table 2).

Post-mortem investigations on foetuses in 2005 did not reveal pathological changes indicative of brucellosis. All bacteriological investigations for *Brucella abortus* and all blood samples tested for antibodies against *Brucella abortus* in 2005 were negative.

In conclusion, there was no detection of *Brucella abortus* in cattle in Norway in 2005. With the exception of a single relapse in 1953, bovine brucellosis has not been detected in Norway since 1950 (1, 2, 3, 4, 5, 6).

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Table 2. Number of samples tested for Brucella abortus in dairy herds and beef herds in 2005

	Dairy cattle		Beef cattle		Total	
Material	Animals	Herds	Animals	Herds	Animals	Herds
Foetuses (autopsy and bacterial culture)	16	14	8	7	24	21
Cows (serology)	48	26	8	4	56	30



# The surveillance and control programme for bovine virus diarrhoea in Norway

Annual report 2005

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Bovine virus diarrhoea (BVD) is a notifiable disease in Norway. Preliminary investigations, conducted between 1984 and 1986, indicated that nearly 30 % of Norwegian dairy herds contained animals with antibodies to bovine virus diarrhoea virus (BVDV) (1). Due to its high prevalence and the economic losses associated with BVD a surveillance and control programme was started in December 1992. The Animal Health Authority (from 2004: the Norwegian Food Safety Authority) was in charge of the programme and were responsible for blood sampling and for imposing control measures in BVD-positive herds. The National Veterinary Institute performed the laboratory analyses (2, 3). The government and the industry financed the programme.

During the programme period, the number of farms with restrictions because of infection with BVDV, has decreased from 2,950 in 1994 to one by the end of 2005 (Figure 1). The progress was considered excellent in the first years, but less so during the later period as demonstrated by the long "tail" of herds with restrictions (Figure 1). The main reason for this tail was that the number of new infected herds was relatively high (Figure 2). These herds were mostly located in the same areas as the remaining herds with restrictions.

The programme was initially divided into a three-step operation for dairy farms:

- 1. Bulk milk from all dairy herds was tested for antibodies, and the herds were classified from 0 to 3 according to the BVDV antibody level (Table 4).
- 2. In herds with an antibody titre above a certain minimum level, pooled milk from primiparous cows was examined for BVDV antibodies.
- 3a. If the pooled milk in step 2 was antibody positive, blood samples from three to five approximately one year old animals were collected, and a pooled sample was examined for BVDV antibodies.
- 3b. Beef cattle herds joined the programme in this step with testing of pooled blood samples of three to five animals (7 - 12 months of age).

The testing for antibodies in bulk milk and pooled samples from primiparous cows was usually performed once a year as a minimum, but pooled serum samples were tested more often in many herds. Tables 1-3 show the results of the tested herds in the programme during the period 1993-2004.

Year	No. of herds	% of herds in class 0 (S/P ratio<0.05)	% of herds in class 1 (0.05≤S/P ratio<0.25)	% of herds in class 2 (0.25≤S/P ratio<0.55)	% of herds in class 3 (S/P ratio≥0.55)
1993	26,424	63.0	14.1	15.9	7.1
1994	26,148	63.4	12.2	14.5	9.9
1995	25,577	63.7	10.6	12.5	13.2
1996	25,167	70.5	15.4	10.7	3.5
1997	24,862	74.3	15.7	8.7	1.2
			% of herds in class 1 (0.05≤S/P ratio<0.15)	% of herds in class 2 (0.15≤S/P ratio<0.55)	
1998	24,038	81.3	9.1	9.2	0.4
1999	23,584	85.6	8.8	5.6	< 0.1
2000	21,796	88.3	6.3	5.3	0.1
2001	19,910	91.9	4.7	3.2	0.2
2002	18,771	94.4	3.1	2.2	0.3
2003	17,549	96.7	2.1	1.1	0.02
2004	7,365*	95.8	2.8	1.3	0.1

Table 1. Distribution of Norwegian dairy herds in relation to BVDV antibody level in bulk milk during the period 1993-2004

\* 44 % of the total number of dairy herds

**Table 2.** Herds positive for antibodies against BVDV in pooledmilk from primiparous cows during the period 1993-2001(This test has not been in use after 2001)

Year	No. of herds examined	% antibody positive herds
1993	5,031	70.7
1994	3,228	54.5
1995	3,191	44.3
1996	1,849	44.1
1997		
1998	1,415	21.5
1999	924	24.2
2000	100	13.0
2001	53	9.4

**Table 3.** Pooled serum samples from young stock positive forantibodies against BVDV during the period 1993-2004

Year	No. of samples examined	% antibody positive samples
1993	5,000	46.5
1994	4,107	38.2
1995	5,347	23.5
1996	3,163	21.9
1997	3,292	16.0
1998	3,407	10.8
1999	3,060	8.6
2000	1,610	8.6
2001	4,198	2.5
2002	2,854	1.8
2003	2,100	1.0
2004	1,351	1.4

\* The data from 1997 are not available.

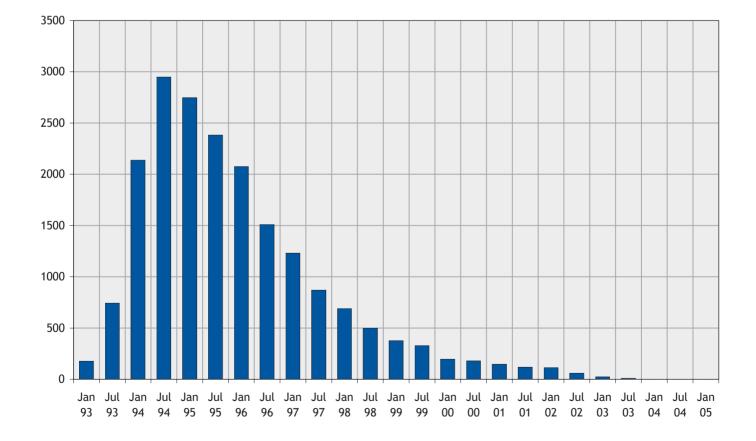


Figure 1. Number of herds with imposed restrictions because of BVDV infection during the period 1993-2005.

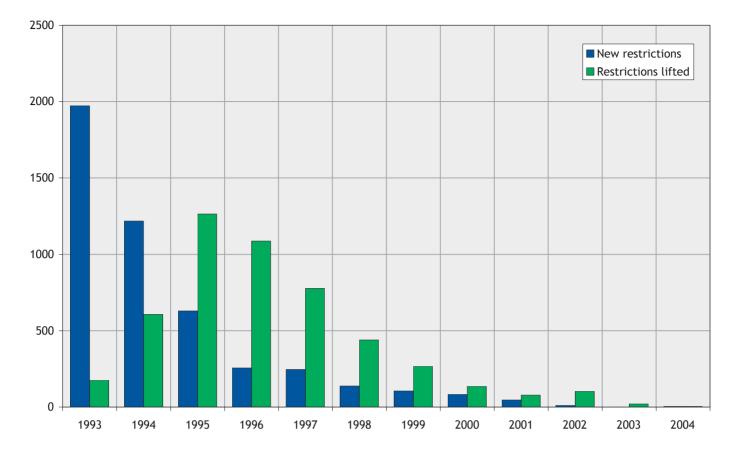


Figure 2. Number of new herds with restrictions imposed/restrictions lifted per year because of BVDV infection during the period 1993-2005.

#### Aim

The ultimate goal of the programme is to eradicate BVDV from the Norwegian cattle population.

#### Material and methods

An indirect ELISA test (SVANOVIR®, Svanova Biotech AB, Uppsala, Sweden) was used to measure antibodies against BVDV in milk and blood (4). Up until 2003, an antigencapture ELISA test (Moredun Animal Health, Edinburgh, Scotland) was used for the detection of BVD virus (5, 6). After this, the need for virus tests declined to less than 1,000 a year, and the "Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus" from IDEXX Laboratories has been used.

Depending on the level of antibodies in bulk milk, the herds were grouped in four classes (Table 4). The results are expressed as S/P-ratio (Sample to positive ratio) (7).

The group of dairy herds sampled in 2005 did not include all dairy herds in Norway. From the special zones implemented in 2001 (see discussion), all dairy herds were sampled twice, as they were in 2004. From the rest of the country, 25 % of the herds were sampled. In total 45 % of the dairy herds were sampled.

Pooled milk samples from primiparous cows were not collected in 2005 (Table 2).

**Table 4.** Classification of bulk milk samples after testing for antibodies against BVDV according to the "sample to positive ratio" of antibodies (AB) in the sample

Class		S/P ratio
0	Not detected AB	< 0.050
1	Detected a small amount of AB	0.050 - 0.149
2	Detected a moderate amount of AB	0.150* - 0.549
3	Detected a great amount of AB	≥ 0.550

 $^{*}$  Before January 1 1998 the cut off value between class 1 and 2 was set at S/P ratio=0.250. The cut-off was reduced to =,150 to be able to discover newly infected herds at an early stage.

Table 5. Norwegian dairy herds classified according to BVDV antibody level in bulk milk in 2005

Year	No. of herds	% of herds in class 0 (S/P ratio<0.05)	% of herds in class 1 (0.05≤S/P ratio<0.15)	% of herds in class 2 (0.15≤S/P ratio<0.55)	% of herds in class 3 (S/P ratio≥0.55)
2005	7,481	98.0	1.2	0.8	0.03

Table 6. Antibodies against BVDV in pooled serum samples from young stock in 2005

Year	No. of herds examined	No. pooled serum samples examined	No. of pooled serum samples with positive result	% AB positive samples
2005	1,230	1,230	3	0.3

Table 7. Examination of individual blood samples for BVDV antigen during the period 1998-2005

	No. of individual	No. of herds	Virus positi	ve samples	Virus posi	tive herds
Year	samples examined	examined	No.	%	No.	%
1998	7,091	780	198	2.8	98	12.6
1999	7,619	648	224	2.9	92	14.2
2000	6,947	423	129	1.9	72	17.0
2001	6,287	386	174	2.8	56	14.5
2002	3,962	284	43	1.1	28	9.9
2003	1,135	149	22	1.9	9	6.0
2004	1,017	84	6	0.6	2	2.4
2005	356	48	4	1.1	1	2.1

Pooled serum samples from 1,230 different dairy (6 %) and beef cattle herds (94 %) were examined in 2005, and the results are shown in Table 6.

Positive results for antibodies in a pooled serum sample from young animals (seven to twelve months) indicate that BVDV was present in that herd less than one year ago. There is a great risk that one or more animals in such herds are persistently infected and, therefore, restrictions are imposed on the farm. Identification of such animals must be done by i) testing blood samples from every individual in the herd for antibodies, and ii) testing for the presence of virus in antibody negative individuals. In 2005, a total of 356 animals from 48 herds were investigated.

In 2001, nearly all beef herds with at least two suckler cows were tested with pooled blood samples from young animals. Very few samples were antibody positive. This indicated a very low prevalence of BVDV in beef herds and lead to a reduced testing in such herds. From 2002, counties that had been free of herds with restrictions for more than one year were subject to reduced testing. In these counties, only 20 % of the beef herds were tested.

The number of counties with this reduced testing scheme was in 2005 increased to 16 of a total of 18 counties.

# Results

A total of 7,481 dairy herds were tested for antibodies against BVDV in 2005, and nearly 98 % of these were negative regarding antibodies against BVDV (Table 5).

Of a total of 1,231 pooled serum samples from 1,230 different dairy and beef cattle herds, 0.3~% was antibody positive (Table 6).

BVDV was found in 1.1 % of the individual blood samples tested (Table 7).

# Discussion

Special zones were established in 2001 in areas with a particularly high number of BVDV infected herds. In these zones, specific testing schemes were imposed before animals could be sold or allowed access to common pastures. In addition, information to veterinarians, other advisors and farmers about the disease and how to act to avoid re-infection was provided (8). Figures 1 and 2 indicate that these new measures were effective in helping to shorten "the tail" of infected herds. The ultimate goal of eradicating BVD in Norway is now considered achieved (9). Only one herd had restrictions at the end of 2005. In 2005,

restrictions were imposed in only two new herds because of suspected BVDV infection (Figure 2). Active infection was found only in one of these herds. This herd had also had restrictions because of BVDV-infection earlier, but the restrictions were lifted late in 2002. In spite of positive test results for antibodies in bulk milk over the following two years, blood test of young animals were not performed in this farm until 2005. When all the animals in the herd were tested four persistently infected animals were identified, and all the other animals were antibody positive. Luckily, this herd had no close neighbours and did not sell live animals.

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# The surveillance and control programme for bovine tuberculosis in Norway

Annual report 2005

Annette Hegermann Kampen Ola Nyberg

A national campaign to control bovine tuberculosis was introduced in Norway as early as 1895. From 1895 to 1920, 43,565 herds and 359,587 heads of cattle were examined, and many herds became free from bovine tuberculosis (1, 2). In 1932, a new eradication campaign was started, and the number of infected herds declined from more than 150 in 1934 to less than 20 in 1942. The campaign was terminated in 1936 (2, 3). After completion of the eradication programme in 1963, bovine tuberculosis has been monitored through continuous, compulsory veterinary meat inspection, with laboratory investigation of suspicious materials. Apart from two single-herd outbreaks in Sogn og Fjordane county in 1984 and 1986 Norway has been considered free from bovine tuberculosis since 1963 (1, 2, 4).

Since 1994, the EFTA Surveillance Authority (ESA) has recognised Norway as a state officially free from bovine tuberculosis, as described in ESA Decision 225/96/COL replacing ESA Decision 67/94/COL. In 2000, the Animal Health Authority (from 2004: the Norwegian Food Safety Authority) launched a surveillance and control programme for bovine tuberculosis. The programme includes compulsory veterinary inspection of all bovine carcasses at slaughter, with submission of suspicious materials to the National Veterinary Institute for further examination.

#### Aims

The aims of the programme are to document absence of bovine tuberculosis, according to the criteria of Directive 64/432/EEC with amendments, and to contribute to the maintenance of this favourable situation.

#### Material and methods

**Submission of material from slaughterhouses** Lung tissue, lymph nodes and other organs with pathological lesions where bovine tuberculosis can not be excluded, are submitted for examination.

The Food Safety Authority collects the samples during routine meat inspection.

#### Histopathological examination

Tissues are fixed in 10 % neutral phosphate-buffered formalin for more than 24 hours, processed according to a standard routine protocol, embedded in paraffin and sectioned at 5  $\mu$ m. All samples are stained with haematoxylin and eosin and Ziehl-Neelsen (5).

#### Bacteriological examination

Samples are examined as described in the OIE manual (5). Samples are homogenised, decontaminated with 5 % oxalic acid and centrifuged. The top layer of the sediment is used for culturing and microscopic examination. The sediment is inoculated onto slopes of Petragnani medium, Stonebrink's medium and Middelbrook 7H10 medium. The slopes are incubated aerobically at 37 °C for two months and checked every week for growth of acid-fast bacilli, determined by the Ziehl-Neelsen method.

#### **Results and discussion**

Table 1 shows the number of samples collected by the Food Safety Authority for the monitoring of bovine tuberculosis and the results since the programme started in 2000. In 2005, one sample was submitted and was found to be negative for Mycobacterium sp.

The low number of submitted samples from the slaughterhouses indicates a low prevalence of suspicious pathological lesions. Continuous surveillance by veterinary meat inspection, early and effective eradication campaigns, combined with restricted import of live cattle, have contributed significantly to this favourable situation. With the exception of two single cases in 1984 and 1986, bovine tuberculosis has not been diagnosed in Norway since 1963 (1, 2, 4).

Table 1. Number of samples tested for bovine tuberculosisduring the period 2000-2005

	No. of	No. of	No. of positive			No. of positive
Year	samples	herds	Samples	Herds		
2000	0	0	0	0		
2001	3	3	0	0		
2002	0	0	0	0		
2003	1	1	0	0		
2004	4	4	0	0		
2005	1	1	0	0		

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# The surveillance and control programme for maedi in Norway

Annual report 2005



Ola Nyberg Jorun Tharaldsen Jorunn Mork Peder Andreas Jansen Annette Hegermann Kampen

Maedi is a progressive viral pneumonia in sheep first described in Iceland in 1939 (1). The disease occurs in several European countries as well as on other continents. The disease visna is caused by the same virus as maedi, but is a neuropathogenic manifestation of the infection (1, 2). Maedi-visna is classified as a list B disease in Norway and is notifiable to the Office International des Epizooties (former list B).

In Norway, maedi was officially reported for the first time in 1972 (3). The infection was introduced to the sheep population with imported Texel sheep in the 1960s. The increased incidence observed in the years from 1972 to 1975 led to a nationwide disease control programme launched by the Norwegian Animal Health Authority in 1975 (from 2004: the Norwegian Food Safety Authority).

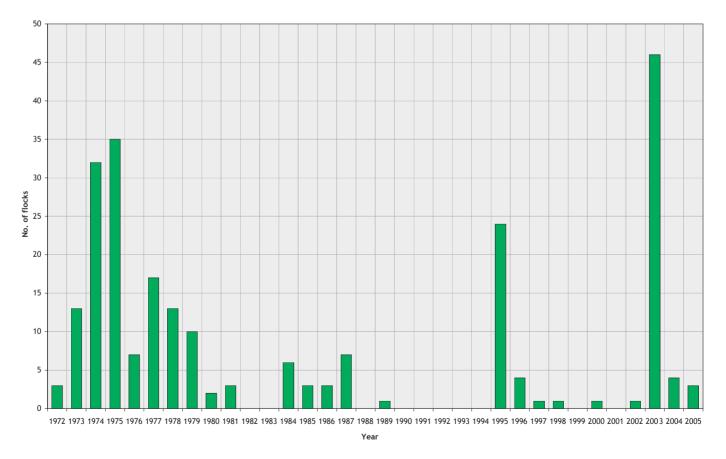
As no new infected flocks were detected during the early 1990s, the restrictions were lifted in all flocks by the end of 1994. In 1995, maedi was again diagnosed at slaughter in a ram from a flock in Hordaland county. During the period 1995 to 1997, 29 infected flocks were detected in the counties of Rogaland and Hordaland in western Norway.

A control programme for maedi was initiated in July 1997, including serological testing for maedi-visna in all flocks in high-risk regions (Rogaland and Hordaland counties) during a seven-year period (4).

In November 2002 and January 2003, post mortem examinations of lungs from two diseased sheep from two different farms in Nord-Trøndelag county showed histopathological changes consistent with maedi. The diagnosis were confirmed by serological tests of blood samples. The prevalence of positive animals was high in both flocks (55 % and 64 % respectively). One flock played a major role as a supplier of breeding animals, and in all there had been contact with about 250 flocks. During the following investigations more than 15,000 sheep in 300 flocks were serologically examined for maedi-visna infection. Restrictions were imposed on 250 flocks and amongst these, 50 flocks were found to be seropositive.

The outbreak demonstrated that maedi-visna infection was more widespread in Norway than previously anticipated, and necessitated a new nationwide control programme.

Further details on the programs during the period 1975 to 2003 have been reported previously (6), and an overview of the number of new affected flocks registered each year up to 2005 is given in Figure 1.



**Figure 1.** The number of new flocks infected with maedi registered during the period 1972 to 2005. The bars for 2003 - 2005 show both seropositive flocks detected through the investigations after the outbreak in Nord-Trøndelag county and seropositive flocks discovered in the programme.

## The present surveillance and control programme for maedi

In April 2003, the National Veterinary Institute was asked by the Norwegian Animal Health Authority to make a draft for a new nationwide surveillance and control programme for maedi. It was a prerequisite that it should be able to detect infected flocks more efficiently than the old programme. The expenses, however, should not exceed the costs of the existing programme to any great extent. These conditions limited the annual number of flocks and animals to be included in the programme. Thus, the flocks participating in ram circles seemed to be a suitable population for the purpose. The ram circles represent the top of the breeding system, and many rams used for breeding in other Norwegian sheep flocks are recruited from the ram circles. Approximately 2,400 flocks were part of this breeding system in 2005, of a total of 16,500 sheep flocks. It was decided to start with the population participating in the ram circles, and then gradually include more of the other flocks as the examined flocks were declared free from maedi-visna infection.

All breeding flocks would be tested during the period 2003 to 2005, with all flocks belonging to the same ram circle tested the same year. The programme was made in collaboration with the Norwegian Food Safety Authority (previously the Norwegian Animal Health Authority), the Norwegian Sheep and Goat Breeders Association (NSG) and the Norwegian Sheep Health Service. This programme started in November 2003.

#### per flock were tested in flocks with more than 200 animals. All rams and the oldest sheep among those more than oneand-a-half years old were sampled in each flock.

The programme in 2005 was based on serological examination of blood samples from the selected sheep for antibodies against maedi-visna virus with the ELISA from Pourquir (ELISA CAEV/MAEDI-VISNA serum verification kit, Institut Pourquier, Montpellier, France). Sero-positive ELISA-results were verified by another ELISA (ELITEST - MVV # CK104A, Hyphen BioMed, Andrésy, France) and an agar gel immunodiffusion test (AGIDT, Meditect, Veterinary Laboratories Agency, Weybridge, UK). In the case of inconclusive results (including single reactors), new blood samples from the animals were taken one to two months after the first sampling. These samples were doubly tested in all three tests.

Due to the known cross reactions in the serological tests between maedi-visna virus and caprine arthritis encephalitis virus (CAEV) infection, blood samples from sero-positive flocks with both sheep and goats were tested with a PCRmethod developed at the National Veterinary Institute. The PCR-method was designed to amplify sequences from both CAEV and maedi-visna virus, followed by sequencing to differentiate between the two virus types.

The meat inspectors at the abattoirs still play an important role in the programme by monitoring sheep and especially sheep lungs for detection of suspicious cases consistent with maedi-visna virus infection.

#### Aim

The aims of the surveillance and control programme for maedi are to document the status for maedi-visna virus infection in sheep in Norway, and to identify infected flocks to support disease control.

#### Materials and methods

The NSG's register of ram circles and their member flocks constituted the basis population for the programme, and 848 of these were selected for testing. In addition, sheep from 200 randomly selected flocks not belonging to any ram circle were included. Thirty animals per flock were sampled in flocks with less than 100 sheep, 35 animals were sampled in flocks with 100 to 200 sheep, and 40 animals

#### **Results**

Samples from a total of 940 flocks, constituting approximately 28 % of the breeding flocks and 6 % of the total Norwegian sheep flocks, were analysed in 2005 (Table 1). The geographical distribution of the Norwegian sheep population and the tested flocks at the municipality level is shown in Figure 2.

In 2005, six samples from two different flocks in different parts of the country were positive for antibodies against maedi-visna virus. Sheep from 5 flocks with close contact to goats tested positive in the serological tests. Eight sheep from two flocks were confirmed to be infected by CAEV and two sheep were confirmed to be positive for mædi-visna virus by PCR.

Table 1. The number of flocks and sheep tested in the Norwegian surveillance and control programme for maedi

Year	Total no. of sheep flocks*	No. of flocks included in the programme	No. of flocks sampled	No. of animals tested	No. of positive flocks
2003	18,400	2,227	456**	13,951	1
2004	17,439	2,600	1,230	36,911	1
2005	16,500	2,519	940	29,248	2

\* Based on data from the register of production subsidies as of 31 July 2004, \*\* Sampling period: November 20 to December 31.

#### Discussion

The aim of the programme, which started in 2003, was to increase the sensitivity in discovering infected flocks compared to the previous programme without increasing the costs per flock to any extent. Two measures were established to achieve this. The number of sampled animals per flock was increased, and a more sensitive, but less labour-intensive test was introduced.

The sample size per flock was adjusted so that if none of the tested animals were seropostive, the prevalence of maedi-visna infected animals in a flock would be less than 6 %, given a confidence level of 95 % and 100 % test sensitivity.

The ELISA employed in this programme is considered more sensitive than the traditionally used agar gel immunodiffusion test. The ELISA is also more objective and less dependent of the operator's skill than the AGIDT. The ELISA is claimed to be as specific as the AGIDT. In spite of this, to gain experience with the different tests and to ascertain the sensitivity and the specificity for the ELISA from Pourquier, another ELISA and the AGIDT were used when the first test was positive. The disadvantage with this test regimen is that in some cases the results are difficult to interpret, which leads to more inconclusive results and requires testing of new blood samples. Experience from the test regimen implemented during the recent outbreak, however, showed that the proportion of inconclusive/false positive results was less than one percent.

Results from the new programme, including data from November 2003 through 2005, showed a preliminary prevalence of 0.2 % positive flocks (5, 6). However, considering the relatively small proportion of flocks tested and the low number of positive reagents, this prevalence has to be interpreted carefully. Knowledge about the distribution of the disease so far indicates that it is regionally clustered, and that a more extensive spread of maedi-visna virus has probably been prevented by the restrictions on transfer of sheep across county borders.

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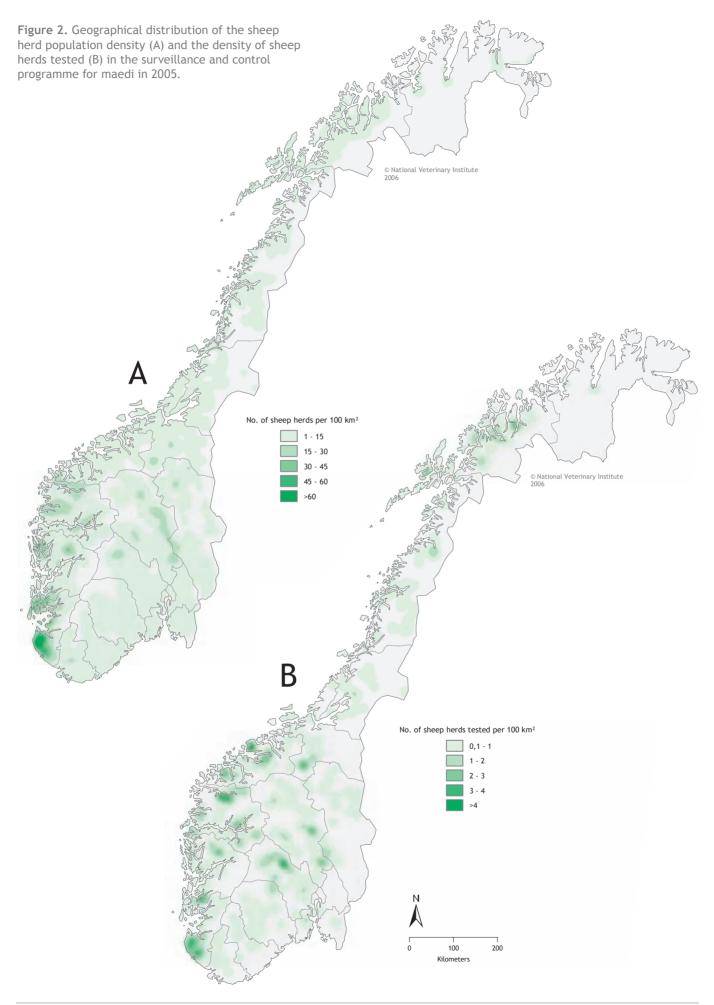
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Surveillance and control programmes in Norway · Maedi · Annual report 2005

# The surveillance and control programme for *Brucella melitensis* in sheep in Norway

Annual report 2005



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Responsible institutions National Veterinary Institute Norwegian Food Safety Authority

ansen

Brucellosis in sheep and goats is mainly caused by *Brucella melitensis*, although infection with *Brucella abortus* and *Brucella ovis* can also occur. The infection usually results in abortion in pregnant ewes and can cause orchitis and epididymitis in affected rams (1). *Brucella melitensis* infection is a zoonosis, and the bacterium causes a serious infection in humans characterised by undulant fever, chills, sweat and debilitation, also known as Malta fever (2).

*Brucella melitensis* is prevalent in sheep and goats in several Mediterranean countries (1), but has never been diagnosed in animals in Norway or any of the other Nordic countries (3, 4). Brucellosis is classified as a list A disease in Norway and is notifiable to the Office International des Epizooties (former list B).

After the agreement on the European Economic Area in 1994, Norway achieved status as free from *Brucella melitensis* in small ruminants on a historical basis. However, documentation is required to maintain the status, and as a result of this, a surveillance and control programme for *Brucella melitensis* in sheep was established in 2004.

The Norwegian Food Safety Authority is responsible for carrying out the programme. The National Veterinary Institute is in charge of planning the programme, performing the analyses and reporting the results. The samples are collected by inspectors from the Norwegian Food Safety Authority.

#### Aims

The aims of the programme are to document freedom from *Brucella melitensis* in sheep according to the demands in EU Directive 91/68/EEC with amendments and to contribute to the maintenance of this favourable situation.

#### Material and methods

In the surveillance and control programme for *Brucella melitensis* in sheep, ram circles registered by the Norwegian Sheep and Goat Breeders Association and their associated flocks constituted the main test population. Approximately 2,400 flocks were part of this breeding system in 2005, out of a total of more than 16,500 sheep flocks. Samples were collected from 735 flocks in the breeding system. In addition, sheep from 200 randomly selected flocks not belonging to any ram circle were included in the programme.

All individuals were sampled in flocks of less than 30 animals. In flocks of 30 to 100, 100 to 200, and more than 200 sheep, samples from 30, 35, and 40 animals were analysed, respectively. The number of herds in the surveillance and control programme for *Brucella melitensis* in sheep in 2005 is given in Table 1.

The programme was based on serological examination of blood samples from the selected sheep for antibodies against *Brucella melitensis*, using the rose bengal plate agglutination test (RBT) for the initial screening. A competitive ELISA (C-ELISA, Svanova Biotech AB, Uppsala, Sweden) was used to follow up unclear or positive reactions due to cross reactions.

#### **Results**

A total of 28,406 samples from 935 sheep flocks were analysed in 2005. The results from the surveillance and control programme for *Brucella melitensis* in sheep in 2004-05 are shown in Table 1. All samples tested for antibodies against *Brucella melitensis* in 2005 were negative, except for one sample being positive in both tests. A follow-up sample from the same sheep was negative in both tests, and there were no clinical signs of the disease in the flock. It was concluded that the seropositive result most likely was false positive due to a non-specific reaction.

The geographic distribution of the total number and the number of tested sheep flocks in 2005 are shown in Figure 1. The flocks tested in 2005 constituted 28 % of the breeding flocks and 6 % of the total number of Norwegian sheep flocks.

**Table 1.** Results and total number of sheep flocks within the frame of the Norwegian surveillance and control programme for *Brucella melitensis* in sheep in 2004 and 2005

Year	Total no. of sheep flocks*	Total no. of sheep >1 year of age	No. of flocks tested	No. of animals tested	No. of positive samples
2004	17,439	918,500	1,655	50,501	0
2005	16,500	927,400	935	28,406	1**

\* Based on data from the register of production subsidies as of July 31 2004. \*\* Probably unspecific reaction.

#### Discussion

Norway has, on a historical basis, been regarded as free from *Brucella melitensis* in small ruminants since 1994. However, the maintenance of the status depends on a continuous surveillance of the Norwegian sheep and goat population based on serological examination. A considerable proportion of the Norwegian sheep flocks were tested in 2004 (4). In total, approximately 90 % of the Norwegian breeding flocks have been screened for antibodies against *Brucella melitensis* during the period 2004-05.

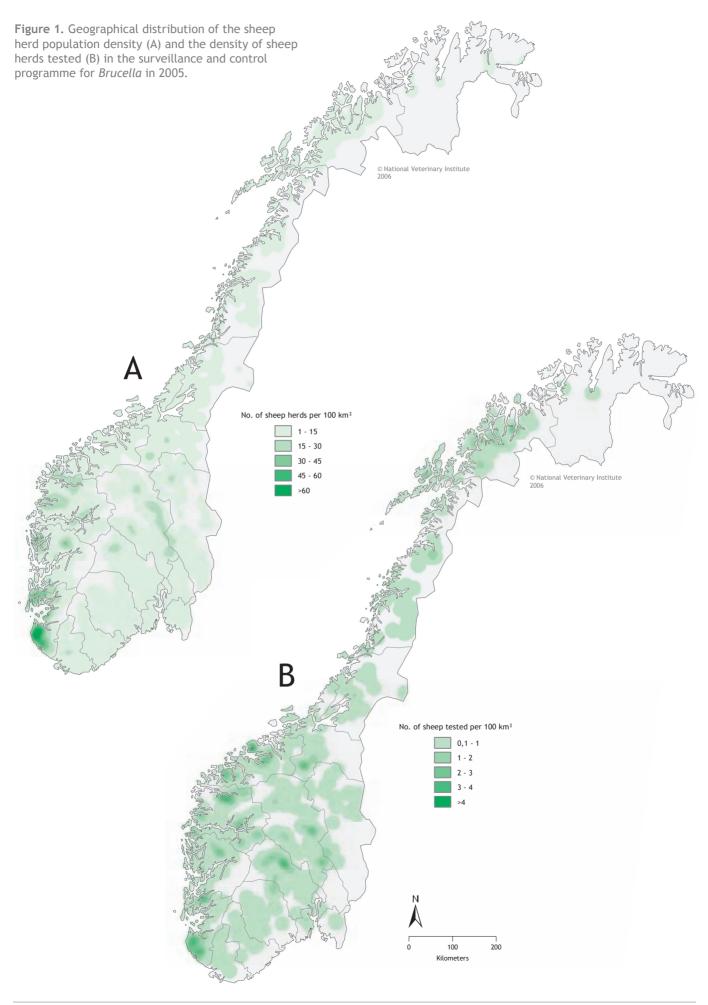
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# The surveillance and control programme for scrapie in Norway

Annual report 2005



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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 2004, scrapie had been diagnosed in a total of 106 sheep flocks. Scrapie has never been diagnosed in goats in Norway (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. A national scrapie surveillance and control programme was launched by the National Animal Health Authority in 1997 (from 2004: the Norwegian Food Safety Authority) (2).

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

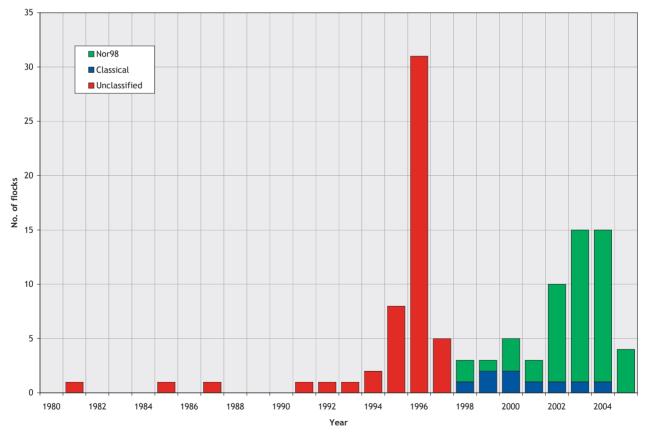
#### Aims

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

#### Materials and methods

In 2005, 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
- 1,000 goats older than 18 months which had died or been killed on the farm, but not slaughtered for human consumption (fallen stock)
- 5,000 randomly sampled healthy goats older than 18 months slaughtered for human consumption



**Figure 1.** Annual number of sheep flocks diagnosed with classical scrapie and scrapie Nor98 during the time period 1980-2005. Before 1998 the cases were not classified according to type of scrapie, but the majority of the scrapie cases are supposed to be the classical type.

The sheep and goat farmers were responsible for reporting to the local Norwegian Food Safety Authority; all sheep and goats with clinical signs consistent with scrapie, and small ruminants older than 18 months that died or were killed on the farm due to disease. The local Norwegian Food Safety Authority evaluated the reported cases and if indicated, either a post mortem examination at a laboratory, or a collection of a brain sample at the farm for laboratory examination was performed. The Norwegian Food Safety Authority carried out inspections of goat herds and sheep flocks, all of which should be inspected every second or third year. The Norwegian Food Safety Authority also sampled slaughtered sheep and goats at the abattoirs, while the National Veterinary Institute was responsible for the laboratory examinations and the reporting of the results.

#### Animals with clinical signs consistent with scrapie

A total of eight sheep and one goat with clinical signs consistent with scrapie were subject to clinical evaluation. The animals were either subject to 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

Samples from approximately 3,600 sheep and 300 goats found dead, or which were killed on the farm, but not slaughtered for human consumption, were submitted for examination. The majority of the samples consisted of retropharyngeal lymph nodes, and unfixed *medulla oblongata* obtained through the *foramen magnum* using a metal spoon specially designed at the National Veterinary Institute. Alternatively the samples consisted of formalinfixed and unfixed brain halves and unfixed retropharyngeal lymph nodes. The samples were examined at the National Veterinary Institute in Oslo.

#### Abattoir surveillance

Approximately 10,900 randomly collected brain samples from apparently healthy sheep and 2,500 randomly collected brain samples from apparently healthy goats older than 18 months were collected. The sheep samples were collected at 29 abattoirs, which process all the commercially slaughtered sheep in Norway.

The samples were obtained throughout the year, with approximately 42 % of the samples collected in September and October, which is the main slaughtering season for sheep in Norway. To ensure an appropriate distribution of the samples, the Veterinary Officers 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 the origin, species, age, breed, production type or 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 in Sandnes, Trondheim and Harstad.

#### Laboratory examination procedures

Clinically suspect animals were subject to histopathological examination of brain tissue and immunohistochemical examination of brain and lymphoid tissue for PrP<sup>sc</sup>. In addition a rapid test (TeSeE® Bio-Rad) was performed on brain and lymphoid tissues. From fallen stock a pooled brain tissue sample (obex and cerebellum) was initially examined by the rapid test. The abattoir samples (obex) were also initially examined by the rapid test. The TeSeE® Bio-Rad test was performed according to the protocol given by the manufacturer. Immunohistochemistry and Western blot were used as confirmative tests on the samples from fallen stock and the abattoirs. Immunohistochemistry was performed using a monoclonal anti-PrP-antibody (F89/160.1.5) (5). A commercially available kit (Envision+® System HRP [AEC] DakoCytomation) was used to enhance the sensitivity of the method. The confirmative tests, immunohistochemistry and Western blot analyses for PrPsc (TeSeE™ sheep/goat Western Blot Bio-Rad) were carried out at the National Veterinary Institute in Oslo, which is the national reference laboratory for TSEs.

#### 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 16<sup>th</sup> sheep slaughtered and examined for PrP<sup>Sc</sup> 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 scrapie Nor98 prevalences in the fallen stock and abattoir populations were estimated assuming a beta-distribution when using an uninformed prior. were apparently healthy animals slaughtered for human consumption (Table 1). Scrapie was not diagnosed in goats (Table 1).

The individual age and breed were registered and the prion protein genotype examined for all four scrapie cases (Table 2). All four scrapie cases were diagnosed as scrapie Nor98, based on the unique Western blot profile (Table 2).

#### **Results**

#### Sheep

Scrapie was diagnosed in four sheep. One case was reported because the sheep had shown clinical signs consistent with scrapie at the ante mortem control at the abattoir. One scrapie case was identified in fallen stock, and two cases The identity of the flock was reported for 14,030 (94.8 %) of the total of 14,794 samples from sheep. In the event of a positive sample from slaughtered animals, the flock identity of the remaining samples (5.2 %) could be traced via the carcass number. The 14,030 samples were collected from 5,813 different sheep flocks. The mean number of

Table 1. Brain samples from sheep and goats submitted for examination for scrapie in 2005

Reason for submission to the laboratory	No. of samples	No. of rejected samples	Negative	Positive
Sheep				
Animals with clinical signs consistent with scrapie	8	0	7	1
Fallen stock	3,644	22	3,621	1
Healthy slaughtered animals	10,894*	5	10,887*	2
Animals killed under scrapie eradication	248	0	248	0
Total sheep	14,794	27	14,763	4
Goats				
Animals with clinical signs consistent with scrapie	1	0	1	0
Fallen stock	309	7	302	0
Healthy slaughtered animals	2,507	0	2,507	0
Animals killed under scrapie eradication	0	0	0	0
Total goats	2,817	7	2,810	0

\* 133 samples 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 2005

Case no.	Year of birth	Reason for submission to laboratory examination <sup>1)</sup>	Breed <sup>2)</sup>	Prion Protein Genotype	Scrapie type
1	1999	Fallen stock	Spæl Sheep	AHQ/AHQ	Nor98
2	1999	Healthy slaughtered animals	Norwegian White Sheep	ARQ/ARR	Nor98
3	1998	Healthy slaughtered animals	Norwegian White Sheep	AHQ/AHQ	Nor98
4	1998	Suspect	Norwegian White Sheep	ARQ/ARR	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.

animals tested per flock was 2.4 (range 1-24, flocks eradicated due to scrapie are excluded). From 1,812 flocks more than two samples were tested.

#### Goat

The identity of the herd was reported for 2,694 (95.6 %) of the total of 2,817 samples from goats. In the event of a positive sample from slaughtered animals, the herd identity of the remaining samples (4.4 %) could be traced via the carcass number. The 2,694 samples were collected from 449 different goat herds. The mean number of animals tested per herd was 6.0 (range 1-81). From 260 flocks more than two samples were tested.

The geographical distribution on a municipality level of the sheep and goat populations is shown in Figures 2A and 2B. The origin of the sheep and goat samples and the origin of the scrapie cases are shown in Figures 3A and 3B.

The prevalence of scrapie in the fallen stock of sheep was estimated to 0.05 % (0.007-0.15 %), (95 % confidence interval [CI]), and the prevalence of scrapie in sheep slaughtered for human consumption was estimated to 0.03 % (0.006-0.07 %), (95 % CI).

PrP genotyping was performed on 600 sheep randomly sampled from the healthy slaughtered population. The PrP genotypes are grouped in accordance with the British National Scrapie Plan (NSP) (Table 3).

Table 3. PrP genotypes in the healthy slaughtered population in 2005 grouped in accordance with the British National Scrapie Plan (NSP)

Genotype category	Number	%
NSP1, genetically most resistant, ARR/ARR	75	12.5
NSP2, genetically resistant, ARR/ARQ, ARR/ARH, ARR/AHQ, VRR/ARQ	196	32.7
NSP3, genetically low level resistant, ARQ/ARQ	118	19.7
NSP3, genetically low level resistant, AHQ/AHQ, ARH/ARH, ARH/ARQ, AHQ/ARH, AHQ/ARQ	101	16.8
NSP4, genetically susceptible, ARR/VRQ	40	6.7
NSP5, genetically highly susceptible, ARQ/VRQ, ARH/VRQ, AHQ/VRQ, VRQ/VRQ	70	11.7
Total	600	100.0

#### Discussion

Scrapie Nor98 was diagnosed in four 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 scrapie Nor98 (6, 7, 8). There were two scrapie Nor98 cases which had genotypes considered relatively resistant (NSP2) towards classical scrapie, and two cases had genotypes less resistant (NSP3) towards classical scrapie. Examination of 38 scrapie Nor98 cases has shown that the PrP genotype distribution differs markedly from that of the previous cases with classical scrapie and that polymorphisms at codon 141 and 154 in the ovine prion protein gene are associated with scrapie Nor98 (4).

Following the EU Regulation (EC) No. 999/2001 Annex VII, as amended by Regulation (EC) No 1915/2003 all sheep in the four scrapie Nor98 flocks were genotyped. Animals with a VRQ allele and animals without at least one ARR allele were killed, and about 250 animals older than 18 months were examined for PrP<sup>Sc</sup>, but no additional animals with scrapie Nor98 were detected in these flocks. This result as well as the absence of additional scrapie Nor98 cases in the eradicated flocks previous years, suggests that scrapie Nor98 is, if contagious at all, less contagious than classical scrapie.

Scrapie Nor98 was diagnosed in several different breeds. The sheep were between six and seven years old, which is in agreement with the result from previous years with the mean age being six years (Table 2). In contrast, the mean age of cases with classical scrapie has been 3.5 years.

The scrapie Nor98 cases detected in 2005 were located in counties where the disease has previously been diagnosed. Scrapie Nor98 is diagnosed 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.

The prevalence of scrapie Nor98 in fallen stock was estimated to 0.05% (0.007-0.15%), (95% CI) and the prevalence has been decreasing since the rapid test programme was initiated in 2002. The prevalence of scrapie Nor98 in sheep slaughtered for human consumption was estimated to 0.03% (0.006-0.07%), (95% CI), which was lower than in 2004, but at the same level as in 2002 and 2003 (6, 7, 8). The results from the surveillance of slaughtered animals indicate that the prevalence in sheep population does not change. This is in contrast to the decrease in the prevalence in fallen stock, which might indicate that farmers are more reluctant to notify found-dead animals which might have shown signs typical for scrapie.

Classical scrapie was not diagnosed in 2005 and was last detected in one flock in 2004. When the classical form of scrapie is detected, the whole flock is killed. The fact that classical scrapie has only been detected by examination of clinical cases or follow up of contact flocks and not by the active surveillance programmes (comprising more than 80,000 samples from 2002) strengthens the opinion of a very low prevalence of this type of scrapie.

The difference between the number of examined sheep from fallen stock (3,644) 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. An additional explanation may be a lack of information to the sheep and goat farmers concerning their duty to report to The Norwegian Food Safety Authority all small ruminants that die, or are killed due to disease, on their farms. However, the numbers of animals examined in the sheep fallen stock and slaughtered populations are sufficient to estimate the prevalences of scrapie Nor98 in these populations.

For monitoring of sheep, between one and 24 animals have been tested for PrPSc in the same flock. This indicates that in some flocks more animals have been examined than expected after random sampling of the slaughtered population. The mean Norwegian flock size counts 56 breeding sheep older than 12 months. Sheep from 5,813 of the total of approximately 16,500 flocks have been examined.

#### Acknowledgment

The authors thank the Norwegian School of Veterinary Science for the PrP-genotyping and all who have contributed to sampling, preparation and examination of the samples.

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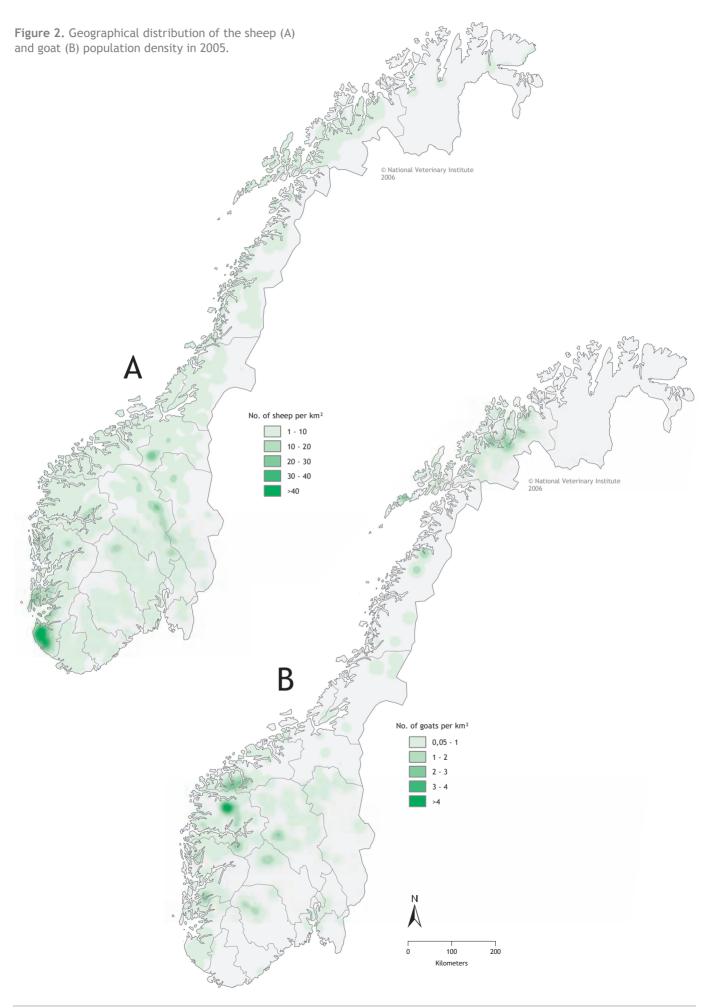
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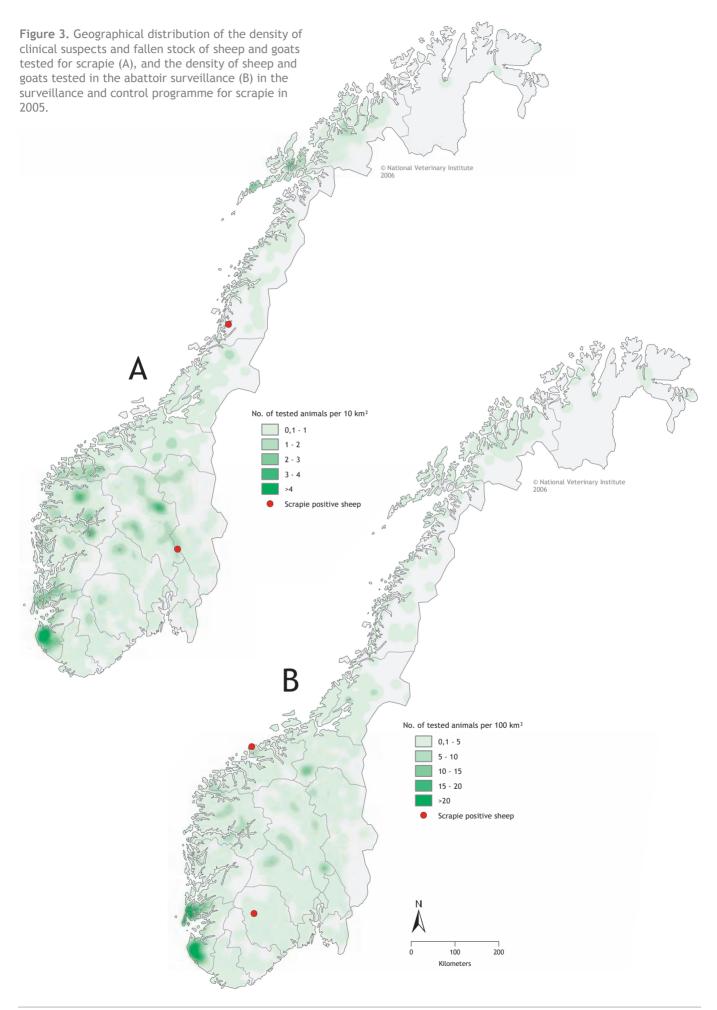
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The surveillance and control programme for specific virus infections in swine herds in Norway



Annual report 2005

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The national surveillance and control programme for specific virus infections in swine was launched in 1994 in order to document the status of Aujeszky's disease (AD), transmissible gastroenteritis (TGE), and porcine respiratory corona virus (PRCV) in the Norwegian swine population. Porcine respiratory and reproductive syndrome (PRRS) and swine influenza (SI) were included in the programme in 1995 and 1997, respectively. From 1997 to 2001 porcine epidemic diarrhoea was also included (1, 2, 3, 4).

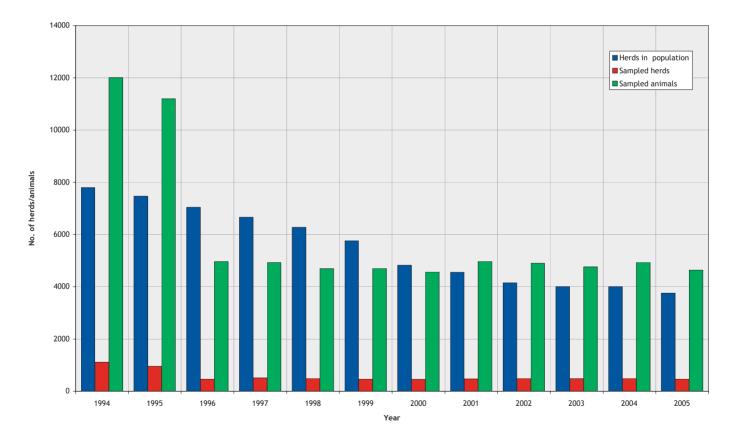
The EFTA Surveillance Authority (ESA) has recognised the swine population in Norway as free from AD since July 1 1994, and has defined additional guarantees to protect the swine health status in Norway. The additional guarantees relating to AD for pigs destined for Norway are described in ESA Decision 75/94/COL, amending ESA Decision 31/94/ COL, later replaced by ESA Decision 226/96/COL.

An overview of the material from previous years is presented in Figure 1. The Norwegian Food Safety Authority is responsible for running the programme, while the National Veterinary Institute is responsible for planning, laboratory analyses and reporting.

Until 2005, AD, PRRS, TGE and PRCV had never been detected in Norwegian pigs. Antibodies against Swine influenza (SI, H3N2) were detected once in 1998 in a pig multiplier herd tested in the national surveillance programme. No clinical signs of the disease were observed, and the titres were low. Infection from humans could not be excluded as the cause of the serological reactions in this case.

#### Aims

The aims of the programme are, through serological surveillance, to document absence of specific infectious diseases in the Norwegian swine population and to contribute to the maintenance of this favourable situation.



**Figure 1.** The size of the sampling frame and the number of sampled herds and animals in the Norwegian surveillance and control programme for specific virus infections in swine during the time period 1994-2005.

#### **Material**

The surveillance of swine herds is focused on the breeding population. All nucleus and multiplying herds were tested. In addition, the nucleus units of all the sow pools and a random selection of the remaining swine population were included in the programme. Because the counties Østfold, Akershus, Vestfold and Rogaland were considered to be "high risk areas", a relatively larger proportion of farms from these counties was tested.

The random selection was conducted from all swine herds receiving governmental production subsidies according to records of 31 July 2004. The register included a total of 3,762 commercial swine herds. Based on this, the sampling plan specified 170 nucleus herds and multiplying herds, 8 sow pools, 280 integrated and piglet-producing herds, and 60 fattening herds. Samples from nucleus herds, multiplying herds, nucleus units of the sow pools, and integrated and piglet-producing herds were collected at the farm, while samples from fattening herds were collected at six different abattoirs. From all herds, samples from ten pigs were to be collected.

#### **Methods**

All the serological analyses were performed at the National Veterinary Institute in Oslo.

#### Aujeszky's disease

All serum samples were tested for antibodies against AD virus in a commercial blocking ELISA (SVANOVIR<sup>TM</sup>). The test detects antibodies against glycoprotein B (previously glycoprotein II) on the surface of the virus.

### Transmissible gastroenteritis virus and porcine respiratory coronavirus

A combined blocking ELISA (SVANOVIR<sup>TM</sup>) was used for detection of antibodies against TGEv/PRCV. Depending on the reaction pattern of two different monoclonal antibodies against TGEv/PRCV and TGEv respectively, the test is able to distinguish between antibodies against TGEv and PRCV.

#### Porcine reproductive and respiratory syndrome

All serum samples were tested for antibodies against PRRS virus using the HerdChek PRRS 2XR Antibody Test Kit (IDEXX) which detects the most predominant European or American type of PRRS viruses. In the case of dubious or positive results, the samples were retested with blocking ELISAs and immune-peroxidase tests (IPT) at the Danish Institute for Food and Veterinary Research.

#### Swine influenza

To test for swine influenza, the samples were analysed for antibodies against the serotypes H1N1 and H3N2 in the hemagglutination inhibition test (HI). The reagents were produced at the National Veterinary Institute in Oslo.

All individual samples that gave an inconclusive or positive result in any of the ordinary routine tests, were followed up by specified reference tests.

#### Results

Blood samples from 4,644 individual animals were submitted to the National Veterinary Institute. The number of negative and rejected samples for AD, SI, PRRS, TGE and PRCV respectively, is presented in Table 1.

All serum samples were negative in all analyses, except for one serum sample from one pig in a fattening herd being positive for antibodies against the PRCV virus. The nine other pigs from the same farm were negative, as were follow-up samples from ten other pigs from the same farm. It was concluded that the seropositive result most likely was a false positive due to a non-specific reaction.

Table 1. Number of samples submitted to the laboratory and the test results for AD, swine influenza, and PRRS, PRCV and TGE in 2005

Disease	Received	Rejected	Negative	Positive
AD	4,644	0	4,644	0
SI	4,644	9	4,635	0
PRRS	4,644	7	4,637	0 *
TGE	4,644	9	4,635	0
PRCV	4,644	20	4,624	1 **

\* The result from one sample was inconclusive, \*\* Probably unspecific reaction

The distribution of tested herds in relation to type of production is given in Table 2. The mean number of animals tested per farm was 9.9 (range 2 - 22).

The geographical distribution of sampled herds relative to the geospatial distribution of the swine population is presented in Figure 2.

Table 2. Distribution of swine herds in the surveillance and control programme related to the type of production in 2005

Category	No. of herds tested	% of herds tested	Total no. of individual samples collected	% of individual samples collected
Nucleus herds and multiplying herds	156	33.3	1,563	33.7
Sow pools	9	1.9	90	1.9
Integrated and piglet-producing herds	253	54.1	2,496	53.7
Fattening herds	50	10.7	495	10.7
Total	468		4,644	

#### Discussion

The results from the surveillance and control programme provide additional documentation of freedom from specific virus infections in the Norwegian swine population. Antibodies against any of the specified viruses have been detected only twice since the start in 1994. A low level of antibodies against swine influenza (H3N2) was detected in samples from pigs in one herd in 1998, and one out of ten pigs from a fattening pig herd had antibodies against PRCV in 2005. To date, there have been no clinical recordings indicating the presence of any of the viral infections included in this surveillance and control programme (1, 2, 3, 4).

The Norwegian swine industry has been structurally changed during the last ten years. The number of herds has declined and the average herd size increased, while the produced tonnage of pork meat has been relatively stable. The number of sampled herds and animals was reduced in 1996 due to a modification of the EFTA Surveillance Authority (ESA) requirements to maintain the additional guarantees for AD. The EU has not approved the programmes for the other specific virus infections for granting of additional guarantees, so they are continuously based on national decisions. The fraction of sampled farms has not declined substantially since the start of the programme, the figures being 14.3 % and 12.4 % in 1994 and 2005, respectively. The geographical distribution of investigated farms is in accordance with the spatial distribution of the total swine herd population (Figure 2). Farmed wild pigs and pigs kept as pets are not included in the programme. No wild boar population is registered in Norway.

Due to restricted import of live swine and swine products, the Norwegian swine population is relatively isolated. In 2005, 49 live animals were imported from Finland and approximately 400 doses of swine semen were imported from Finland and Sweden. In some of the neighbouring countries which are potential trading partners for swine breeding material, some of the infectious diseases included in the programme occur. PRCV is present in Sweden, PRRS occurs in Denmark, and Swine influenza occurs in both countries. Several countries purchase swine breeding material from Norway. The surveillance and control programme for specific virus infections provides solid documentation of the favourable health situation in the Norwegian swine population in general and the breeding herds in particular.

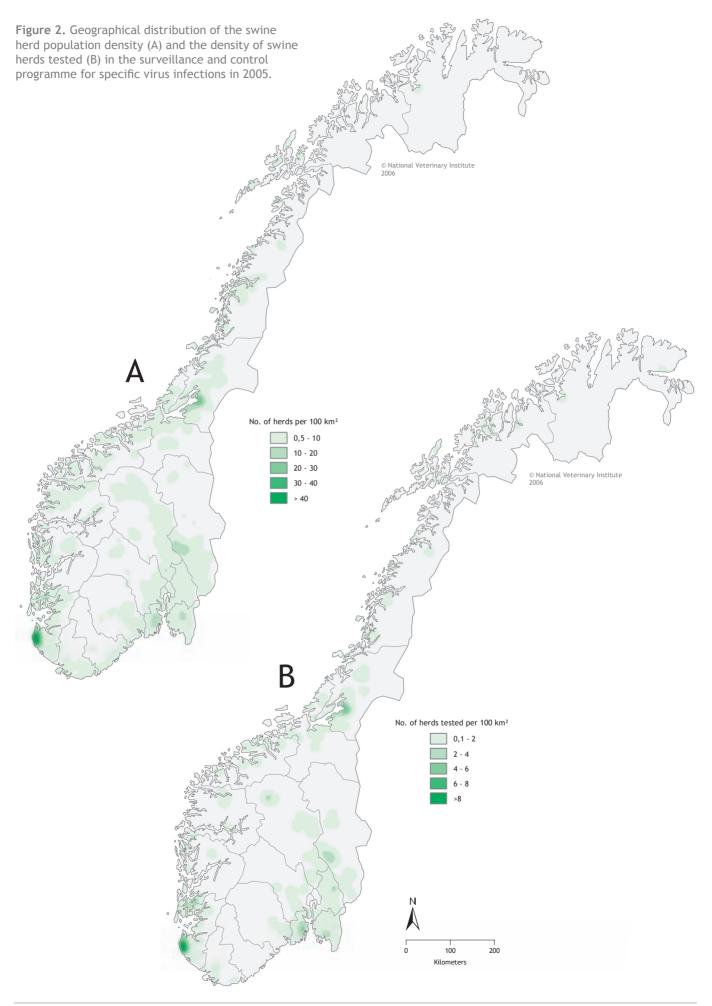
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The surveillance and control programme for chronic wasting disease (CWD) in wild and captive cervids in Norway

Annual report 2005



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Chronic Wasting Disease (CWD) is a unique transmissible spongiform encephalopathy (TSE) that occurs in wild and captive cervids. Naturally occurring disease has been demonstrated in Rocky Mountain mule deer (*Odocoileus hemionus*), black-tailed deer (*O. hemionus columbianus*), white-tailed deer (*O. virginianus*) and Rocky mountain elk (*Cervus elaphus nelsoni*) in the USA and Canada.

CWD is, like scrapie in small ruminants and bovine spongiform encephalopathy (BSE) in cattle, characterised by the accumulation of an abnormal form of the prion protein (PrP<sup>Res</sup> or PrP<sup>CWD</sup>) in the central nervous system. In most of the CWD-affected animals, PrP<sup>CWD</sup> is also detectable in the lymphoid tissues (1). The clinical features of CWD animals are dominated by weight loss and emaciation, behavioural change, depression, excessive salivation and oesophageal reflux, and polyuria/polydipsia (2). The age of the affected animals is generally greater than eighteen months (3). The histopathological changes are, like the other TSEs, characterised by vacuolation of the brain tissues. The diagnosis CWD relies on the detection of the PrP<sup>CWD</sup> by immunological methods such as immunohistochemistry, ELISA or Western Blot.

In Norway, TSEs are restricted to some cases of both the classical and the atypical type (Nor98) scrapie in sheep (4). Scrapie has never been diagnosed in goats. In 1994 a case of Feline Spongiform Encephalopathy (FSE) was detected.

Chronic wasting disease is yet to be diagnosed in cervids in Europe, but the number of animals tested is low, despite efforts from Germany, Belgium, Finland and Norway.

The population density of wild cervids varies in the various geographical areas in Norway. The density of red deer (*Cervus elaphus*) is high in the western counties, while moose (*Alces alces*) and roe deer (*Capreolus capreolus*) are most frequently located in the south eastern parts. The number officially hunted in 2005 was: 36,000 moose, 27,600 red deer, and 28,900 roe deer. Norway has both a wild and a semi-domestic population of reindeer (*Rangifer tarandus*), with 4,800 wild animals officially registered hunted in 2005 and the semi-domestic presently counting about 200,000 animals. The wild reindeer are found in relatively confined mountain areas in the southern parts of country while the semi domestic reindeer are mainly kept in the north.

The amount of captive deer herds in Norway is increasing and counts between 50-100 farms. Most of the farms keep red deer, and only a few keep fallow deer (*Dama dama*). Based on the fact that Norway has a large population of cervids, a number of them grazing in regions where scrapie is detected, a voluntary survey to investigate the possible occurrence of CWD in Norwegian wild and captive cervids has been set up. The populations to be surveyed comprise moose, red deer, roe deer and reindeer. A passive surveillance programme on CWD was initiated in 2003. During 2004 and 2005 a number of samples from slaughtered reindeer from several regions in the country also have been examined.

A small population (approximately 150) of wild-living musk oxen (*Ovibus moschatus*), lives in the mountainous area of Dovre. TSE has not been diagnosed in the musk ox, but the species has been included in the programme from 2004.

#### Aim

The aim of the programme is to investigate the possible occurrence of CWD in the Norwegian cervid population.

#### Material and methods

#### Material

Captive cervid older than one year that died or were euthanised were tested for CWD. Wild red deer and moose older than one year were also requested tested. Additionally, cervids older than one year received at the National Veterinary Institute for autopsy were subjected to CWD testing. Some musk oxen were also tested.

#### Laboratory examinations procedures

A rapid test (TeSeE® Bio-Rad) was used to screen brain samples for detection of the PrP<sup>Res</sup> (PrP<sup>CWD</sup>). All the samples were analysed at the National Veterinary Institute in Oslo, which is the National Reference Laboratory for TSEs in Norway.

The National Veterinary Institute is part of the group "Control for Cervids" within the NeuroPrion Network of Excellence aiming at optimising diagnostics tools in Europe for the detection of CWD.

#### Results

None of the samples analysed in 2005 tested positive in the rapid test for CWD.

**Table 1.** Animals tested for CWD with the rapid test andtheir geographic location. No positive samples were found

	No. of animals						
County	Moose	Deer	Musk	Rein- deer	Roe		
Akershus	2				12		
Aust-Agder	2				1		
Hedmark	2			1			
Hordaland		1					
Møre og Romsdal		1	1				
Nord- Trøndelag	2	3			1		
Oppland	1		3	92			
Oslo					1		
Rogaland	1						
Sogn og Fjordane		3		1			
Sør- Trøndelag	2	1	6				
Østfold	2						
Location not given		1			2		
Total	14	10	10	94	17		

A total of six of the tested cervids were captive: four red deer, one moose (zoo) and one roe deer (zoo). For two of the sampled red deer there was no information provided as to whether the animals were wild or captive. Ninety-three out of ninety-four tested reindeer were semi-domestic.

Seventeen of the tested animals were sent to the National Veterinary Institute exclusively for CWD testing, whereas the rest of the tested animals represent routine autopsy material.

The relatively high number of tested roe deer from Akershus is due to cooperation between the National Veterinary Institute and the local wildlife authority in the municipality of Vestby, whom have collected roe deer heads for CWD testing, mainly from animals killed in traffic.

#### Discussion

No animals were detected positive for CWD. The total number of samples collected and analysed is low, but constitutes a substantial effort as far as very few European cervids have been tested to date (5). The testing of cervids, in contrast to BSE and scrapie, is as yet not mandatory in the European Community countries.

Chronic wasting in cervids may be due to various reasons. The most variable factor is nutrition during winter. Clinical signs of ataxia and changes in behaviour may be due to infection of the worm *Parelaphostrongylus elaphus*, possibly especially in the moose, but this infection has not been diagnosed in Norway. Clinical signs in cervids reminiscent of chronic wasting disease have not been reported in Norway.

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# The surveillance and control programme for avian influenza (AI) in wild birds in Norway

Annual report 2005



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As a consequence of the epidemic avian influenza in southeast Asia, surveillance programs for this disease in poultry and wild birds have been implemented in the Member States of the European Union since 2002.

The Norwegian Food Safety Authority is responsible for the implementation of the active surveillance programme for avian influenza (AI) in wild birds. The programme, which was started in 2005, is based on virological investigations in healthy live or hunted birds. The National Veterinary Institute in Oslo is responsible for planning, laboratory investigations and reporting components of the programmes.

Al is a serious, highly contagious disease of poultry and other captive birds caused by many different subtypes of influenza type A viruses. The level of risks posed by the different subtypes for animal, and public health, is very variable and to a certain extent unpredictable. This is due to rapid virus mutation and possible re-assortment of the genetic material between different subtypes.

Wild waterfowls are the natural reservoir for all influenza A virus subtypes. The birds do not usually develop clinical disease, but shed large amounts of virus in their faeces upon infection (1). An avian influenza virus surveillance programme in wild waterfowl in Norway was started in 2005, and included the screening of 419 ducks and 200 geese. Here we present the results of these studies.

Al has never been diagnosed in poultry in Norway.

### Aims

The aim of the national surveillance and control programmes for AI in wild birds is to contribute to the knowledge on the threats posed by wild birds in relation to any influenza virus of avian origin.

# Materials and methods

The programme in 2005 consisted of a molecular screening of cloacal swabs from healthy birds shot during the 2005hunting season. Sampling equipment consisting of a sample tube containing a virus transport medium and swabs were sent out to voluntary hunters in the counties of Rogaland and Østfold. The hunters were also given written instructions on how to sample the animals and requested to fill in registration forms for individual birds. Cloacal swabs were taken from shot birds and the swab was placed in the transport medium and sent by overnight post to the National Veterinary Institute's laboratory in Oslo. The samples were frozen upon arrival. The sampling comprised the following species; greylag goose (*Anser anser*), mallard (*Anas platyrhynchos*), wigeon (*Anas penelope*), teal (*Anas crecca*), goosander (*Mergus merganser*), tufted duck (*Aythya fuligula*), common scoter (*Melanitta nigra*) goldeneye (*Bucephala clangula*) and redbreasted merganser (*Mergus serrator*) (2).

The samples were registered upon arrival and screened using a reverse transcriptase polymerase chain reaction (RT-PCR). The screening RT-PCR used was a pan-influenza A virus RT-PCR that reveals the presence of all subtypes of influenza type A virus. The method does not, however, give information as to which hemagglutinin (H) or neuraminidase (N) subtype is present in influenza positive samples. Therefore, the samples found to be positive in the initial pan-influenza A virus RT-PCR were further subtyped, using RT-PCRs specific for H5 and full-length RT-PCRs for the H and N genes. Samples positive for the pan-influenza A virus RT-PCR were also inoculated in embryonated eggs for virus isolation following the procedures described in the OIE Manual (3), with some minor modifications.

# Results

None of the greylag goose samples (0/200) were positive for influenza A virus, while 19.1 % of the ducks (80/419) were positive. The prevalence for the different duck species were as follows; mallard 20.4 % (58/284), widgeon 12.5 % (8/64), teal 30.9 % (13/42), goosander 0 % (0/5), tufted duck 0 % (0/4), common scoter 14.3 % (1/7), goldeneye 0 % (0/11), and red-breasted merganser 0 % (0/2). One mallard and one teal were found to carry H5N2 subtypes, and sequencing of the H gene identified both viruses as low-pathogens, closely related to subtypes evidenced in recent years in Sweden and the Netherlands. The other subtypes identified included H1N1, H3N2, H3N8, H6N2, H6N8, H8N4, and H9N2 in mallards, H3N2, H6N2 and H9N2 in teals, and H6N2 in wigeons and common scoter.

Virus isolation was successful in less than 20 % of the RT-PCR-positive samples, and did not give any additional information to what was found in the molecular biological sequencing.

# Discussion

In both mallards and teals, the prevalence of AI infection was rather high, 20.4 % and 30.9 % respectively. These two species accounted for almost 90 % of the positive results whilst comprising less than 80 % of the ducks sampled. Mallards were found to harbour the highest diversity of H and N subtypes. Subtype H5N1 was found in one mallard, and H5N2 was found in a mallard and a teal. Further sequencing of the H in these three viruses identified them as low pathogenic strains closely related to viruses recently isolated in Sweden and the Netherlands (4).

None of the greylag goose samples tested positive for influenza A virus. This finding is consistent with earlier surveys of this species in Norway in 2003 (5) and in 2004 (unpublished). Thus, the greylag goose does not seem to be a common reservoir of influenza A virus in Norway.

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The surveillance and control programme for infectious laryngotracheitis (ILT) and avian rhinotracheitis (ART) in poultry flocks in Norway

Annual report 2005





The Norwegian Food Safety Authority is responsible for the implementation of the surveillance and control programmes for infectious laryngotracheitis (ILT) and avian rhinotracheitis (ART) in poultry flocks. The programmes, which were started in 1998, are based on serological investigations. The National Veterinary Institute in Oslo (VI) is responsible for the planning, laboratory investigations and the reporting components of the programmes.

ILT is a severe respiratory disease in chickens, and was first described in the USA in the 1920s. Since then, the disease has been seen in most parts of the world, including most European countries (1). ILT has not been diagnosed in commercial chicken flocks in Norway since 1971, but clinical outbreaks of ILT have occurred sporadically in Norwegian hobby flocks since 1998 (2). ILT is an OIE listed disease, and in Norway it is a notifiable list A-disease.

ART is a highly contagious infection which affects the upper respiratory passages of poultry. The disease is called turkey rhinotracheitis (TRT) in turkeys and swollen head syndrome (SHS) or ART in chicken. The disease is caused by avian pneumovirus (APV), and was first described in South Africa in the 1970s. Since then, the disease has been diagnosed in most countries (1). ART has also been diagnosed sporadically in our neighbouring countries. ART had, until outbreaks in 2003 and 2004, never been diagnosed in Norway, where it is a notifiable list B-disease. The disease is not notifiable in the OIE-system.

In August and December of 2004, ART was diagnosed in samples from two separate flocks owned and operated by one large layer breeder company. No clinical symptoms were seen in any of these flocks. In spite of finding increased titres in a second sampling, several attempts at virus detection by the National Veterinary Laboratory in Norway and the Veterinary Laboratories Agency, Weybridge UK, using both RT-PCR and propagation in cell culture, were negative. Follow-up sampling conducted in January and February 2005 gave positive results from more flocks owned by the company. As a result of this, the Norwegian Food Safety Authority discontinued the practise of ART surveillance in chickens in the spring of 2005. Turkeys continue to be tested for TRT.

### Aims

The aims of the national surveillance and control programmes for ILT and ART are to document that the commercial poultry populations in Norway are free from these infections and to contribute to the maintenance of this status.

### Materials and methods

According to the national regulations for certification of poultry breeding farms (Forskrift om sertifisering av fjørfevirksomheter av 18.11.94), blood samples from 60 birds must be taken at least once a year from every breeding flock at the farms. These blood samples are to be tested for Newcastle disease, as Norway has status as a non-vaccinating country. Thirty of the 60 samples from chicken and turkey flocks are included in the national surveillance and control programmes for ILT and ART. Blood samples from chickens and pheasants are tested for antibodies against both viruses, the samples from turkeys are tested only against APV. Blood samples from other poultry flocks are not included in the programme. Figure 1 shows the number of farms tested during the time period 1998-2005 (from 2005: the Norwegian Food Safety Authority). Information from the Norwegian Animal Health Authority concerning farms which need to be certified in 2000, indicated that 89 broiler breeder farms, seven layer breeder farms and four turkey breeder farms should have submitted samples for investigations that year.

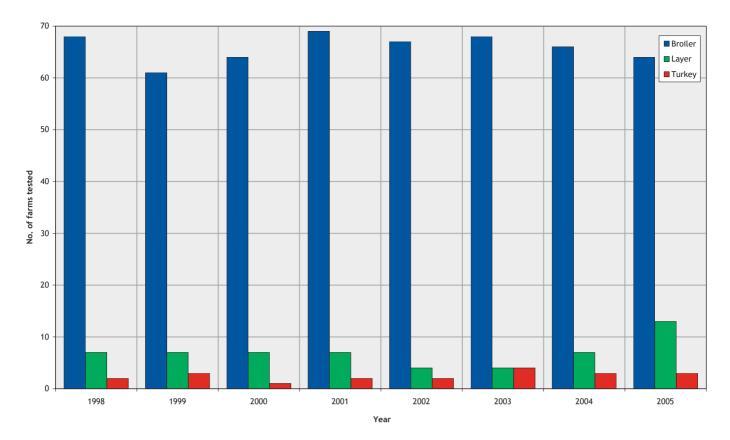
#### ILT

An indirect ELISA-test produced by Kierkegaard-Perry, Gaithersburg Maryland, USA, was used for the testing of antibodies against the ILT-virus.

#### ART

All serum samples were tested for specific antibodies against APV with a blocking-ELISA produced by SVANOVA, Uppsala, Sweden.

Flocks with single positive reactions are followed up by repeated sampling, and if false positive results can't be ruled out by this procedure, serum samples with a positive reaction in the ELISA-tests are submitted to the Veterinary Laboratories Agency (VLA), Weybridge, England for testing using virus neutralisation tests.



**Figure 1.** The number of farms tested in the surveillance and control programmes for infectious laryngotracheitis (ILT) and avian rhinotracheitis (ART) in poultry flocks in Norway during the time period 1998-2005.

Table 1. Number of farms, flocks and birds tested in the surveillance and control programmes for ILT in poultry in 2005

Production	No. of farms tested	No. of flocks tested	Total no. of birds tested	Flocks with seropositive samples
Broiler	64	101	2,940	1
Layer	13	26	750	0
Total	77	127	3,690	1

Table 2. Number of farms, flocks and birds tested in the surveillance and control programmes for ART in poultry in 2005

Production	No. of farms tested	No. of flocks tested	Total no. of birds tested	Flocks with seropositive samples
Broiler	26	33	1,047	0
Layer	3	5	210	1
Turkey	3	6	180	0
Total	31	44	1,437	1

### Results

Tables 1 and 2 show the number of farms, flocks and birds tested in the different poultry production types in the national surveillance and control programmes for ILT and ART, respectively, in 2005.

#### ART

Of the 44 flocks (1,437 samples in total) analysed for antibodies against APV in the surveillance programme, samples from one flock tested positive. This flock was from the large layer breeder company that tested positive on several occasions in 2004 and in six flocks under follow-up sampling in 2005. No clinical symptoms were seen in any of the flocks that tested positive. The company situated in Rogaland has its own and contract production in several houses spread over an area of approximately five kilometres in radius. All serum samples positive in the SVANOVA ELISA were sent to Veterinary Laboratories Agency, Weybridge UK for virus neutralisation (VN) testing using APV types A and B. In the VN-test, only one sample showed a borderline neutralising reaction. The others were negative. From the virus neutralisation results, it thus seems relatively unlikely that the infective agent was a typical poultry type A or B avian pneumovirus. The positive flock from August was stamped out, and the two houses where the flock had been hold were cleaned and disinfected. A follow-up screening of farms in the district revealed no spread of the infection to other farms. Pharyngeal swabs were taken from several chickens and examined both by the National Veterinary Institute, Oslo and the Veterinary Laboratories Agency, Weybridge UK for virus by RT-PCR and propagation in cell culture, but all attempts to identify the agent responsible for the positive serology were negative.

In May 2005, the Norwegian Food Safety Authority discontinued the practise of ART surveillance in chickens in the spring of 2005. Turkeys continue to be tested for TRT.

All the other samples analysed in the surveillance programme for ART were negative.

#### ILT

One of the 3,690 blood samples tested for antibodies against ILTV was positive. This sample originated from a broiler breeder flock situated in Hedmark. An additional sixty blood samples were taken from the flock, all of which were negative. On the basis of these results, the original positive test was deemed false.

All the other samples analysed in the surveillance programme for ILT were negative.

### Discussion

ART had never been diagnosed in Norwegian poultry before the demonstration of antibodies against APV in 2003 and 2004/2005. The two affected farms; one broiler breeder farm and one layer breeder farm are located in the same area, approximately four kilometres apart. However, a common infection source has not been identified. In spite of numerous failed attempts to isolate and identify the infectious agent causing the seroconversion, none have been found. The diagnosis has thus been based on serology only, as for ART in many other countries (1).

Clinical symptoms were not observed in any of the flocks that tested positive in 2004/2005. The use of stamping out measures was unable to check the spread of infection and as of May 2005, chickens are no longer tested for the presence of antibodies against APV. The national surveillance and control programme for ART continues in turkey flocks, as this is a disease which causes significant health and economical consequences predominantly in this species (1, 3). In Norway, ART is classified as a B-list disease and clinical outbreaks in chicken flocks will be treated as such. The national surveillance and control programme for ILT continues for chickens.

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The surveillance and control programme for *Campylobacter* in broiler flocks in Norway

Annual report 2005



Merete Hofshagen Torkjel Bruheim

Campylobacteriosis is currently the most commonly reported bacterial infectious disease in the Norwegian human population. The incidence increased by 145 % from 1997 to 2001 but has since then declined slightly. In almost half of the cases, the infection is acquired in Norway. Consumption of poultry meat purchased raw has been identified as a significant risk factor together with drinking un-disinfected water, eating at barbecues, occupational exposure to animals, and eating undercooked pork (1).

The action plan regarding *Campylobacter* in Norwegian broilers has been implemented since spring 2001 (2, 3). The objective is to reduce human exposure to thermophilic *Campylobacter* (mainly *C. jejuni*, but also *C. coli*, *C. lari* and others) through Norwegian broiler meat products. The action plan is a joint effort involving several stakeholder groups from "stable-to-table". The Norwegian Zoonosis Centre developed the action plan in co-operation with the authorities, the National Veterinary Institute, the Norwegian Institute of Public Health, the Norwegian School of Veterinary Science, the Centre for Poultry Science, and the poultry industry. The Norwegian Zoonosis Centre at the National Veterinary Institute coordinates the programme, and is responsible for the collection and analysis of data and the communication of results.

The action plan consists of three parts; a surveillance programme including all Norwegian broiler flocks slaughtered before 50 days of age, a follow-up advisory service on farms with *Campylobacter* positive flocks, and surveys of broiler meat products.

The surveillance programme is described below. The results from the surveys of broiler meat products and additional material from the Norwegian action plan regarding *Campylobacter* in Norwegian broilers can be found at the website www.zoonose.no.

### Materials and methods

The surveillance has been in effect since 27 April 2001. Pre-slaughter sampling of flocks is performed by the owner and consists of 10 swabs from fresh faecal droppings. The 10 swabs are pooled into one sample and submitted to the National Veterinary Institute's laboratory in Trondheim, where the samples are analysed. The samples are taken a maximum of four days before slaughter (before 1 March 2005; maximum eight days before slaughter). The carcasses from the positive flocks are either heat treated or frozen for a minimum of three weeks (before 1 May 2004; five weeks) before being marketed. All flocks are tested again upon arrival at the slaughter plant by sampling of 10 whole caecae (before 1 May 2004; 10 cloacal swabs) per flock at the slaughter line. Contents from the 10 caecae are pooled into one sample and analysed by local laboratories. Samples are analysed using the method described in NMKL no. 119, 1990, with minor modifications.

### **Results and discussion**

A total of 3,652 flocks from 506 broiler farms were tested. These flocks were slaughtered in 3,899 batches (a batch includes all chickens from one flock slaughtered on the same day). A total of 225 flocks were slaughtered in more than one batch. Most of these were slaughtered in two batches, a few were slaughtered in three or four batches.

Overall, 132 (3.6 %) flocks (134 (3.4 %) batches) were positive for *Campylobacter* sp. either at pre-slaughter, slaughter, or both sampling times.

Of the 132 positive flocks, 90 (68.2 %) tested positive at pre-slaughter sampling and were subject to sanitary measures at slaughter in order to prevent contaminated poultry from reaching the general market as fresh broiler meat. A total of 11 flocks tested positive at pre-slaughter only.

The positive flocks came from 95 (18.8 %) farms. Of these 95 positive farms, 76 (80.0 %) had only one positive incidence during 2005 (a positive incidence is defined as one positive flock or as several parallel positive flocks from different houses) and produced 87 (65.9 %) of the positive flocks. A total of 17 (17.9 %) farms had two positive incidences (producing 37 (28.0 %) of the positive flocks), one (1.1 %) had three and one (1.1 %) had five positive incidences. The 19 farms with two or more positive incidences in 2005 (representing 20.0 % of positive farms and 3.8 % of all farms) had 45 positive flocks, accounting for 34.1 % of all positive flocks.

The proportion of *Campylobacter* positive flocks has varied substantially since the action plan was launched, as has the proportion of flocks that only test positive at the slaughterhouse (Figure 1).

From 1 March 2005, all flocks had to be sampled maximum four days before slaughter. This contributed to the fact that in 2005, 31.8 % of the positive flocks were detected only at slaughter. This is in contrast to previous years, when the sample was taken approximately one week before slaughter, and where approximately 50 % of the positive flocks were detected only at slaughter. This has also contributed to the fact that in 2005, even though there were more positive flocks in total than in 2004, the reduction in the number of positive flocks potentially released untreated to the market has continued.

For those slaughterhouse samples where the reference laboratory confirmed *Campylobacter* sp., *C. jejuni* was isolated from 88 %, *C. coli* from 11 % and *C. lari* from 1 % of the samples. In 12 flocks, attempts to verify the positive diagnosis did not succeed. Five of these flocks were negative at the pre-slaughter sample.

Considerable regional differences in the proportions of positive flocks and farms have been revealed (Table 1, Figure 2).

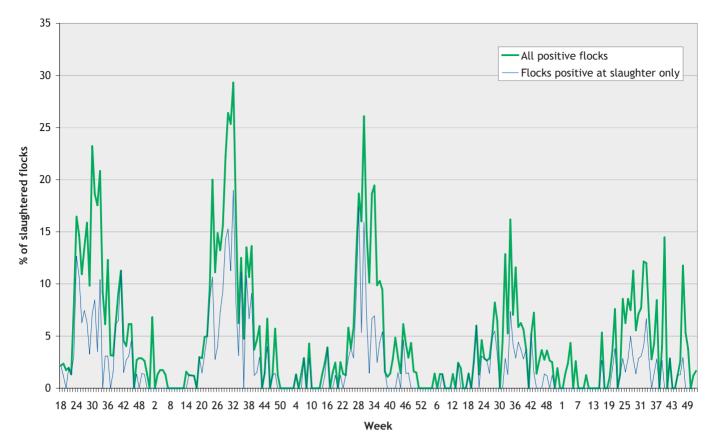


Figure 1. Weekly incidence of Campylobacter sp. in slaughtered Norwegian broiler flocks from week 18 in 2001 throughout 2005.

Most farmers follow the guidelines regarding time of preslaughter sampling. From 1 March 2005, a total of 350 (10.7 %) slaughter batches were sampled earlier than four days before slaughter, mostly in the beginning of the new regime, or in connection with holidays. In total, less than 0.4 % of the flocks were not sampled according to the action plan (i.e. sampled only once).

Table 1. Campylobacter positive farms and flocks by countyin Norway 2005

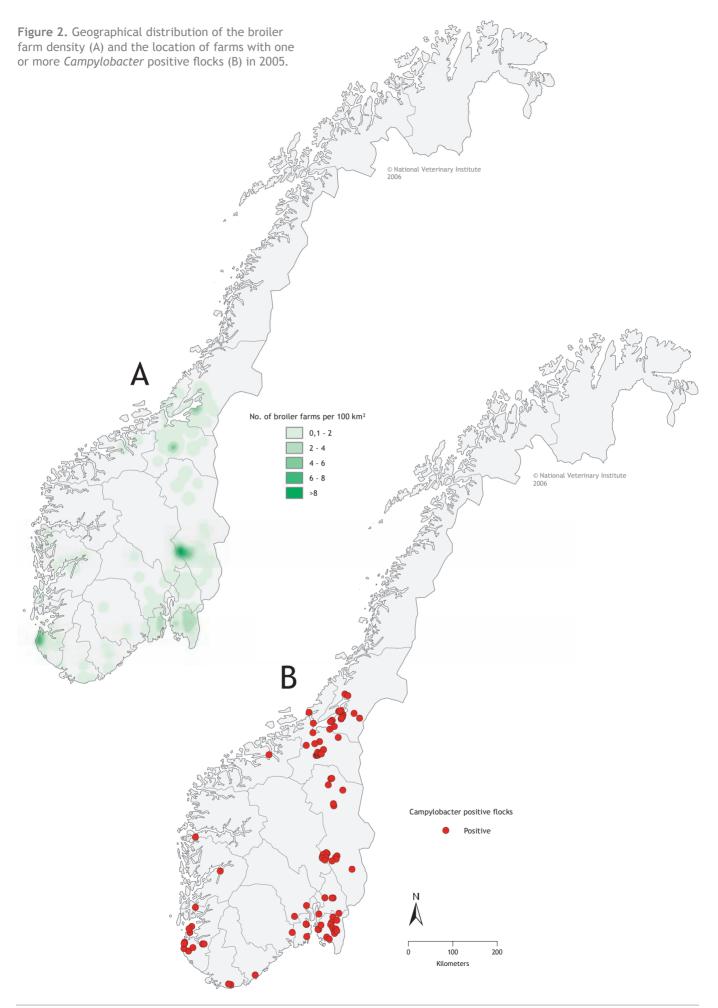
		Farms	;	Flocks		
County	N	No. positi	ve (%)	N	No. positiv	/e (%)
Østfold	80	14	(18)	624	20	(3)
Akershus	13	5	(38)	101	5	(5)
Hedmark	109	20	(18)	825	29	(4)
Oppland	9	0	(0)	52	0	(0)
Buskerud	10	2	(20)	62	2	(3)
Vestfold	37	3	(8)	237	4	(2)
Telemark	4	1	(25)	23	1	(4)
Aust-Agder	4	1	(25)	21	2	(10)
Vest-Agder	5	2	(40)	30	2	(7)
Rogaland	82	13	(16)	705	17	(2)
Hordaland	15	1	(7)	89	1	(1)
Sogn og Fjordane	1	1	(100)	5	2	(40)
Møre og Romsdal	3	1	(33)	26	1	(4)
Sør-Trøndelag	71	17	(24)	392	25	(6)
Nord-Trøndelag	63	15	(24)	460	121	(5)
Total	506	95	(18.8)	3,652	132	(3.6)

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The surveillance and control programme for viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) in Norway

Annual report 2005



Hege Hellberg Birgit Dannevig Petter Hopp

Viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) are two important rhabdovirus infections in salmonid fish. VHS occurs in continental Europe and is an important disease in rainbow trout farming due to its clinical and economic consequences. A specific strain of VHS virus (VHSV) has caused disease in Pacific cod (*Gadus macrocephalus* (Tilesius)) and Pacific herring (*Clupea harengus pallasi* (Valenciennes)) (1, 2, 3). This strain is not pathogenic to rainbow trout (*Oncorhynchus mykiss* (Walbaum)). VHS virus has been isolated from several marine fish species in North European coastal waters (the English Channel, the Baltic Sea, the North Sea, the Norwegian Sea, Skagerak) (1). VHS was reported for the first time in Norway in 1964 and until 1974, several clinical disease outbreaks were diagnosed.

Infectious haematopoietic necrosis has caused serious economic losses in farmed rainbow trout and salmon, and the disease has also had an impact on wild populations of Pacific salmon. The disease was first described in Europe in 1985, in France and Italy. The disease has been documented in several other countries in continental Europe, but has not yet been diagnosed in Norway. For more detailed information on VHS and IHN, reference is made to previous reports of the surveillance and control programmes (4, 5).

Norway achieved disease free status for VHS and IHN approved by ESA on historical grounds, based on health control information and virological examinations carried out in Norwegian fish farms since 1967. Norway has operated a surveillance programme in accordance with Directive 91/67 EEC since the autumn of 1994 (6). The Norwegian Food Safety Authority is responsible for the programme and for inspection and sampling. The National Veterinary Institute is responsible for laboratory procedures and analysis in accordance with Commission Decision 2001/183/EC (7) and prepares the report.

### Aim

The aim of the programme is to document the absence of VHSV and IHNV in Norwegian fish farms and maintain approved zone status for Norway.

### Materials and methods

#### Sampling

Sampling and inspection is carried out by the District Offices of the Norwegian Food Safety Authority in accordance with yearly sampling schedules covering approximately 50 % of farms producing susceptible species. According to Directive 91/67/EEC (6) and Decision 2001/183/EC (7), all fish farms producing species susceptible to VHS and IHN should be sampled over a two-year period. Inspection and sampling must be carried out when the water temperature is below 14 °C. A minimum of 30 fish must be sampled from each farm. Organ material for virological examination for VHSV and IHNV must contain spleen, anterior kidney, and either heart or encephalon (brain). For brood fish, ovarian fluid must be examined. For fry (<4 cm) the entire fish excluding the body behind the vent shall be examined. Ten fish may be pooled to form a single sample. If rainbow trout are kept on a farm, all samples shall be derived from this species. In farms without rainbow trout, the samples shall be taken on an even basis from all the different species present. Samples are collected in transport medium for virological analysis and sent to the National Veterinary Institute for analysis.

#### Analysis

Samples must arrive at the laboratory within 48 hours of sampling. According to the specifications of Decision 2001/183/EC (7), the samples must be kept cool during transport; the temperature shall not exceed 10 °C. At arrival, samples are homogenised and suspended in the original transport medium and centrifuged at 4 °C. Since infectious pancreatic necrosis (IPN) virus is ubiguitous in Norwegian fish farms, the sample material is neutralised with IPN antiserum prior to inoculation on cell cultures to avoid IPN virus masking VHS/IHN virus in the samples. Neutralized homogenate is then inoculated on BF-2 and EPC cell lines as specified (7). Inoculated cell lines are incubated at 15 °C for 7 to 10 days and observed for cytopathogenic effect (CPE). If no CPE is observed, subcultivation is performed to fresh cell cultures. If CPE is observed, virus is identified as specified by 2001/183/EC and the EU central reference laboratory in Århus.

### **Results**

In 2005, the National Veterinary Institute received 13,550 samples from 426 sites. 90 samples from 3 sites were deemed unsuitable for analysis and rejected. A total of 13,460 samples from 423 sites were examined (Table 1 and 2, Figure 1 and 2).

VHSV and IHNV were not detected.

Table 1.	Different	categories	of	fish	examined	for	VHS/IHN	in	2005	
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	Fry - smolt		On-growing		Brood fish		Total	
	No. of sites	No. of samples						
Atlantic salmon (Salmo salar L.)	98	3,320	238	7,230	10	330	345	10,880
Rainbow trout (O. mykiss)	19	700	40	1,200	3	90	61	1,990
Brown trout (Salmo trutta L.)	8	360					8	360
Arctic char (Salvelinus alpinus L.)	3	50	3	90	1	30	7	170
Turbot (Scophthalmus maximus L.)								
Sea trout (S. <i>trutta</i> L.)	1	30	1	30			2	60
Brook trout (Salvelinus fontinalis (Mitchill))								
Relict Atlantic salmon (S. <i>salar</i> L.)								
Total	125*	4,460	280*	8,550	14	450	417*	13,460

\* The total number of sites may be less than the sum of the different species as some sites produce more than one species.

 Table 2. Number of farms and species examined for VHS/IHN during the time period 1995-2005

Farm types	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
Per production type											
Hatcheries	71	169	162	30	27	45	30	32	54	51	125
On-growing farm	207	340	346	478	527	447	508	414	429	303	280
Brood stock farms				2	3	7	7	14	2	9	14
Per species											
Farms with Atlantic salmon	225	425	392	417	462	382	408	372	387	295	345
Farms with rainbow trout	31	63	69	66	62	83	93	61	74	48	61
Farms with brown trout	15	13	38	21	27	28	24	23	24	21	8
Farms with char	1	7	6	5	4	10	8	9	9	5	7
Farms with turbot	6	1	1		1	1	4		1	1	
Farms with sea trout				2	3	2	4	1	2	2	2
Farms with brook trout				2		1	1	2	1	2	
Farms with relict Atlantic salmon				1						1	
Total	278	509	506	510	554	494	534	468	498	375	417

# Discussion

In 2005, 13,460 samples from 423 fish farms were examined compared to 11,410 samples from 375 fish farms in 2004 (6). The Norwegian Food Safety Authority is responsible for selection of sites and sampling.

In 2004, when 450 samples from 15 sites were rejected; temperature had exceeded 10 °C during transport due to the use of unsuitable transport boxes or delays in postal service. To remedy the situation, sampling instructions were revised in late 2004 in cooperation with The Norwegian Food Safety Authority. In addition, standardised, insulated boxes for transport of samples were supplied to all inspectors by the National Veterinary Institute. In 2005, 90 samples from 3 sites were rejected. This represents a considerable improvement.

# Conclusion

No suspected or confirmed cases of VHS virus or IHN virus have been registered in Norwegian fish farms in 2005, based on the examinations carried out in the surveillance and control programme for VHS and IHN at the National Veterinary Institute.

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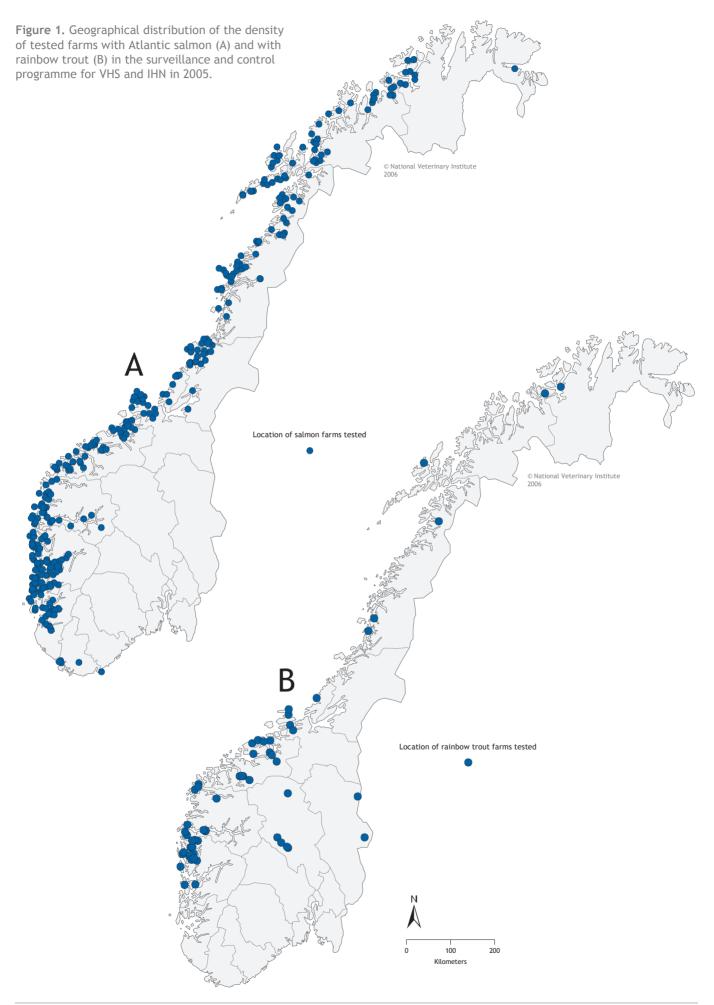
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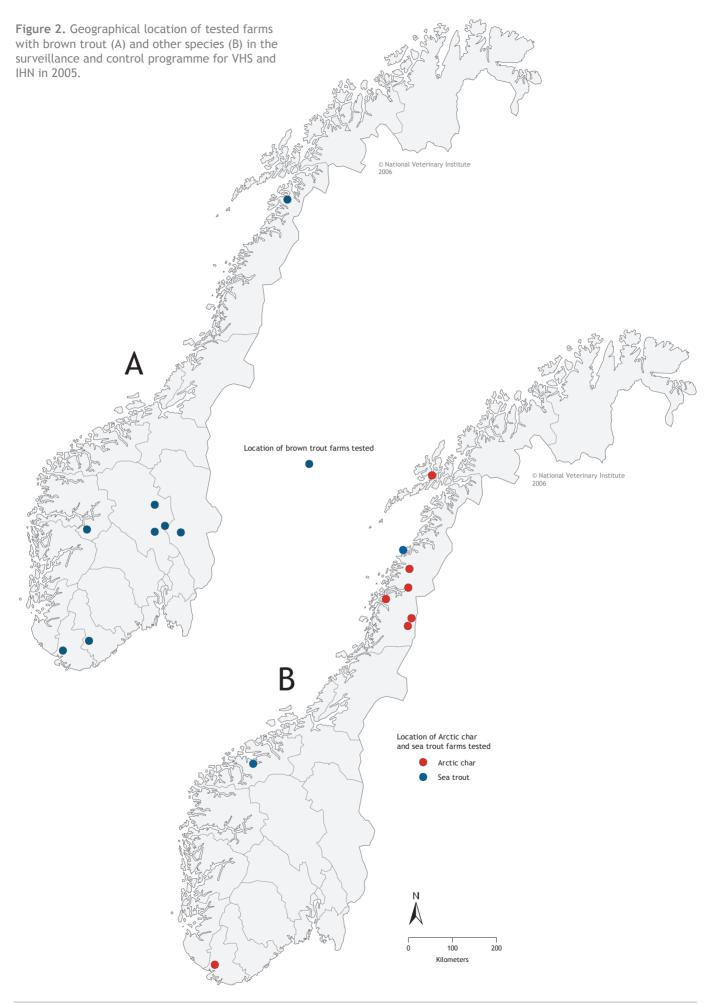
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The surveillance and control programme for *Gyrodactylus salaris* in Atlantic salmon and rainbow trout in Norway

Annual report 2005



Tor Atle Mo Kari Norheim

Gyrodactylus salaris was detected for the first time in Norway in Atlantic salmon (Salmo salar L.) parr from a hatchery in Sunndalsøra, Møre og Romsdal County in 1975. Later the same year, G. salaris was detected in the river Lakselva in Misvær, Nordland County. Altogether, the parasite has been detected in Atlantic salmon fingerlings/parr from 45 rivers, 13 hatcheries/farms with Atlantic salmon parr/smolts and 26 hatcheries/farms with rainbow trout (Oncorhynchus mykiss) during the period 1975 to 2005. The policy of the Environmental and Veterinary Authorities is to eradicate G. salaris from infected rivers and farms. The procedure is aimed at eliminating the hosts (salmon and rainbow trout) and thus also the parasite, which does not have specialized free-living stages or intermediate hosts. By 31 December 2005, G. salaris was confirmed eradicated from 15 rivers and from all hatcheries/fish farms. For ten additional rivers the result of the eradication procedure has not yet been confirmed. The parasite is known to be present in 20 rivers in Norway.

G. salaris has been a notifiable (Group B) disease in Norway since 1983, while the disease has been listed as an "Other significant disease" in the Office International des Epizooties (OIE). The Directorate for Nature Management and the County Environmental Departments started surveillance of G. salaris in Norwegian salmon rivers during the late 1970s. By the mid 1980s, the National Veterinary Institute extended this surveillance to include fish farms, especially inland rainbow trout farms. During the 1990s the Veterinary Authorities gradually undertook the responsibility for all surveillance, and in 2000 a national surveillance programme was implemented by the Norwegian Animal Health Authority (from 2004: the Norwegian Food Safety Authority) (1, 2, 3, 4). In 2005 the programme was carried out accordingly for most selected rivers, but in relatively few hatcheries and farms.

The Norwegian Food Safety Authority is responsible for sampling rivers and fish farms. The Regional Food Safety Authorities have, however, commissioned the respective County Environmental Departments and other institutions/companies to perform river sampling. The National Veterinary Institute in Oslo is recognized as the OIE reference laboratory for the disease, and is responsible for examination of samples as well as taxonomical studies if *Gyrodactylus* is detected.

### Aim

The purpose of the surveillance programme is to trace any spread of *Gyrodactylus* salaris to new river systems or fish farms. Resources are not being used to carry out surveillance in rivers and fish farms already infected, unless measures for eradication of the parasite have been carried out or other circumstances justify surveillance.

### Materials and methods

The surveillance programme is based on sampling and examination procedures developed by the National Veterinary Institute. In rivers, at least 30 Atlantic salmon fingerlings/parr/smolts are caught by means of electrical fishing gear (it may be difficult in some rivers to sample this number of fish). The fish are killed and preserved in 96 % ethanol. The samples are sent to the National Veterinary Institute in Harstad where body surface and fins are examined by a magnifying microscope (10 - 15 times magnification). Fish from farms are caught by net and samples preserved and transported to the laboratory for examination as indicated above. However, only fins (with the exception of adipose fin) are sampled and preserved for examination from fish of 15 cm or longer.

### Results

Tables 1 and 2 show the results following examination of fish from different rivers and different fish farms, respectively. In some of the large rivers, sampling was done at different dates and at different sampling stations. Altogether, 3,833 fish specimens from 120 rivers were examined in 2005.

*G. salaris* reappeared in two rivers; Steinkjervassdraget and Figga, in Nord-Trøndelag county. Both rivers were rotenone treated in 2001/2002. Altogether, 2,503 fish specimens from 81 fish farms were examined in 2005 without any observation of *G. salaris*.

County	Rivers	Species	No. of fish examined	Detections
Finnmark	8	Atlantic salmon	245	0
Troms	11	Atlantic salmon	283	0
Nordland	16	Atlantic salmon	456	0
Nord-Trøndelag	17	Atlantic salmon	508	2 <sup>1</sup>
Sør-Trøndelag	5	Atlantic salmon	167	0
Møre og Romsdal	20	Atlantic salmon	678	0
Sogn og Fjordane	22	Atlantic salmon	690	0
Hordaland	5	Atlantic salmon	187	0
Rogaland	3	Atlantic salmon	92	0
Vest-Agder	3	Atlantic salmon	95	0
Aust-Agder	0			
Telemark	0			
Vestfold	2	Atlantic salmon	131	0
Buskerud	1	Atlantic salmon	41	0
Akershus	2	Atlantic salmon	60	0
Oslo	3	Atlantic salmon	94	0
Østfold	2	Atlantic salmon	106	0
Total	120		3,833	2

Table 1. Number of rivers in different counties examined for Gyrodactylus salaris in 2005

1 Reappearence after rotenone treatment.

County	Farms	Species	No. of fish examined	Detections
Finnmark	0			
Troms	3	Atlantic salmon	91	0
Nordland	10	Atlantic salmon	303	0
Nord-Trøndelag	11	Atlantic salmon	333	0
Sør-Trøndelag	10	Atlantic salmon, rainbow trout	296	0
Møre og Romsdal	16	Atlantic salmon	180	0
Sogn og Fjordane	9	Atlantic salmon, rainbow trout	279	0
Hordaland	15	Atlantic salmon, rainbow trout	459	0
Rogaland	2	Atlantic salmon	69	0
Vest-Agder	0			
Aust-Agder	0			
Telemark	0			
Vestfold	0			
Buskerud	0			
Akershus	0			
Oslo	0			
Østfold	0			
Oppland	4	Rainbow trout	120	0
Hedmark	1	Rainbow trout	30	0
Total	81		2,503	0

 Table 2. Number of fish farms in different counties examined for Gyrodactylus salaris in 2005

# Conclusion

In 2005, *Gyrodactylus salaris* was detected in Atlantic salmon parr in two rivers (Steinkjervassdraget and Figga, Nord-Trøndelag county), but not in fish farms. Both rivers had been rotenone treated in 2001/2002 to eradicate the parasite.

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The surveillance and control programme for bonamiosis and marteiliosis in European flat oysters (*Ostrea edulis* L.) in Norway

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Hege Hellberg



Notifiable diseases have not been reported from any European flat oyster (*Ostrea edulis* L.) population in Norwegian waters (1, 2). This is in contrast to the situation in most other oyster producing European countries, where infectious diseases cause great losses in previously highly productive flat oyster populations (3). The protozoan parasites *Bonamia ostreae* and *Marteilia refringens* are identified as the main disease-causing organisms (4, 5) and bonamiosis has caused a collapse in flat oyster production in affected regions. Bonamiosis and marteiliosis are classified as notifiable diseases by the OIE and as group A diseases in Norway.

In 2004 the entire coastline of Norway was recognized as an approved zone with regard to Bonamia ostreae and Marteilia refringens (6). The decision is based on the results of the surveillance and control programme for bonamiosis and marteiliosis which was initiated in the fall of 1995. The programme is based on directions given by the Commission Decision of November 6 2002 (7) referring to the OIE (International Office of Epizootics) "Manual of Diagnostic Tests for Aquatic Animals - 2003" (8), describing procedures for sampling and analysis of European flat oysters for bonamiosis and marteiliosis. The European flat oyster is found to latitude 65 °N in Norway, and wild populations are small and geographically limited due to climatic conditions. Since 1995, altogether 10 sites along the Norwegian coast have been included in the surveillance programme. However, not all sites have been included each year and selection of sampling sites has been based on the size of the wild populations and the structure of the oyster industry. To ensure sampling of wild oysters on the eastern coast after the decline in the population in the inner parts of Oslofjorden (site 1), sampling from site 2 recommenced in 2005. Also, one farm (site 4) stopped farming oysters in late 2004, leaving a total of seven sampling sites for 2005 (Figure 1).

The Norwegian Food Safety Authority is responsible for the programme, and responsible for inspection and sampling. The National Veterinary Institute in Bergen is responsible for laboratory procedures and analysis in accordance with the EU Decision, and also prepares the reports. In 2005, the National Veterinary Institute in Bergen was accredited by the Norwegian Board of Accreditation for the histological detection of *Bonamia* sp. and *Marteilia refringens* in flat oysters. A total of 4,810 oysters were examined during the initial two-year control period 1995-97. *Bonamia* sp. or *Marteilia refringens* were not observed. During the following years until 31 December 2004, a total of 3,750 oysters were examined and *Bonamia* sp. or *Marteilia refringens* were not observed (9).

### Aim

The goal of the programme is to document the absence of *Bonamia ostreae* and *Marteilia refringens* in Norwegian flat oysters and maintain approved zone status for Norway.

# Materials and methods

#### Sampling

The sample sites are inspected and oysters sampled in the spring and autumn of each year by the Food Safety Authority District Offices, or persons appointed by the District Offices. During the initial two-year period from 1995 to 1997, 150 oysters were sampled each spring and autumn at each site. From 1998 onwards, 30 oysters per site have been collected each spring and autumn. Live oysters are shipped to the National Veterinary Institute in Bergen.

#### Analysis

Oyster shipments arrive at the laboratory within 24 hours of sampling. The oysters are opened and sampled for histological examination according to section 3.1 of the OIE "Manual of Diagnostic Tests for Aquatic Animals - 2003". Tissue samples are fixed in Davidson's fixative for at least four days. The samples are dehydrated through an ascending ethanol series, embedded in paraffin and sectioned with a Reichert-Jung 2035 microtome. Sections (3-5 µm) are mounted on glass slides, stained with Haemotoxylin-Eosin in a SHANDON VARISTAIN 24, a coverslip applied and fastened with Eukitt. Two sections of each sample are prepared and examined in a Leitz Laborlux S or a Leica DM LB microscope at magnifications ranging from 100x to 1,000x. Samples may be stored for weeks in Davidson's fixative prior to processing and can be stored indefinitely when embedded in paraffin or on covered glass slides prior to analysis.

### **Results**

During 2005, the National Veterinary Institute in Bergen received a total of 378 oysters from seven sites (Table 1, Figure 1). All samples were examined. *Bonamia* sp. or *Marteilia refringens* were not observed.

Table 1. Number of sample sites tested for bonamiosis

Sample site	Spring 2005	Autumn 2005	Total 2005
1	*	*	0
2	30	30	60
3	30	30	60
4	*	*	0
5	30	30	60
6	30	30	60
7	18	#	18
8	30	30	60
9	30	30	60
Total	198	180	378

 $^{\ast}$  No longer included in surveillance programme. # Not sampled due to climatic conditions.

### Discussion

The results from the initial two-year period provide support for freedom from bonamiosis and marteiliosis in the Norwegian flat oyster population. Given a sample size of 150, the surveillance and control programme is designed to detect infected oysters at a prevalence of 2 % or higher at a 95 % confidence level. For subsequent samplings, a sample size of 30 gives a 95 % probability for detection of a 10 % prevalence of infected individuals.

Oyster production in Norway is limited and the present sampling programme covers the geographical area in which commercial production and harvesting is possible. Sampling is judged to be representative and the results from the continued surveillance support the findings that *Bonamia ostreae* and *Marteilia refringens* are not present in the Norwegian flat oyster population.

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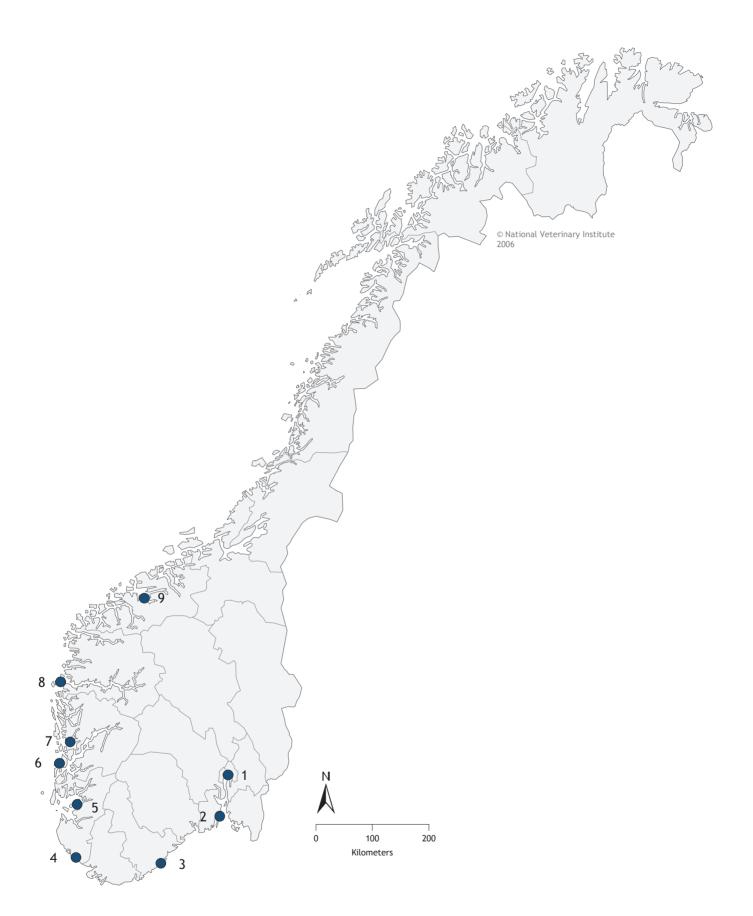
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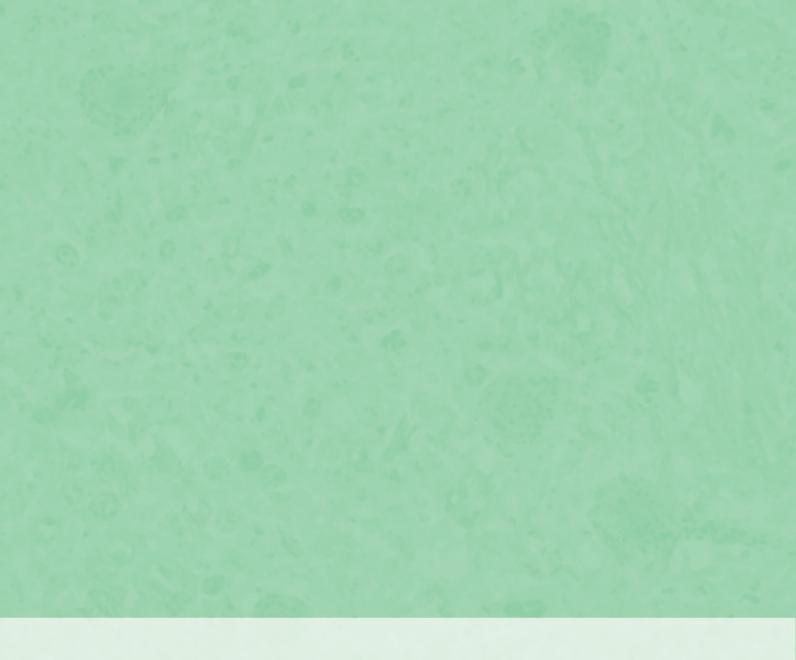
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**Figure 1.** Geographical location of the sample sites in the surveillance and control programme for bonamiosis and marteiliosis in European flat oysters (*Ostrea edulis* L.) in 2005.



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