

# Surveillance and control programmes for terrestrial and aquatic animals in Norway

Annual report 2004

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*Any use of the present data should include specific reference to this report.*

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## Contributors and participants

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### The following institution was responsible for the programmes:

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### The following people are responsible for the administration of the programmes at the Veterinary Institute:

Tormod Mørk (terrestrial animals)  
Hege Hellberg (aquatic animals)

### A large number of samples have been collected by:

Official inspectors in the Norwegian Food Safety Authority  
Farmers  
Dairy and slaughterhouse employees  
County Environmental Departments

### The major parts of the programmes were State funded.

#### Additional financial support was provided by:

Norwegian Meat Research Centre  
Centre for Poultry Science  
The poultry industry

### The following people have been most helpful in revising the manuscripts:

Edgar Brun  
Duncan Colquhoun  
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Sincere thanks are expressed to Berit Tafjord Heier for processing the data, Peder Andreas Jansen who was responsible for all the maps and Hanne Mari Jordsmyr who finalized the report and was responsible for creative initiative and contribution with reference to the design and layout of the report.

## Maps

New in the present annual report are the density projections of populations and herds in many of the maps. Map layers that show population densities are derived from kernel density interpolation of point data on the location of herds or populations. Accordingly, the density layers mirror the true geographic distribution of the respective populations, and are not bound to municipalities or any other administrative level.

Population densities were classified manually, but should be interpreted as dynamic variations in the population densities projected on the maps. The kernel density interpolations of population densities were estimated using ArcGIS Spatial Analyst with a search radius of 20 km and a cell size of 4 km<sup>2</sup>. All the maps were produced in ArcView 9.

## Contents:

Introduction.....	6
Main results from the surveillance and control programmes in 2004.....	8
The livestock and aquaculture populations in Norway.....	11
The surveillance and control programmes for <i>Salmonella</i> in live animals, eggs and meat in Norway.....	19
Examinations of residues of prohibited substances in live animals in Norway.....	27
Examinations of residues of veterinary drugs, prohibited substances and environmental contaminants in animal products in Norway.....	31
The surveillance and control programme for paratuberculosis in Norway.....	37
The surveillance and control programme for bovine spongiform encephalopathy (BSE) in Norway.....	43
The surveillance and control programme for infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) in Norway.....	53
The surveillance and control programme for enzootic bovine leukosis (EBL) in Norway.....	59
The surveillance and control programme for bovine brucellosis in Norway.....	65
The surveillance and control programme for bovine virus diarrhoea (BVD) in Norway.....	73
The surveillance and control programme for bovine tuberculosis in Norway.....	81
The surveillance and control programme for maedi in Norway.....	85
The surveillance and control programme for scrapie in Norway.....	93
The surveillance and control programme for <i>Brucella melitensis</i> infection in sheep flocks in Norway.....	103
The surveillance and control programme for specific virus infections in swine herds in Norway.....	109
The surveillance and control programme for infectious laryngotracheitis (ILT) and avian rhinotracheitis (ART) in poultry flocks in Norway.....	115
The surveillance and control programme for <i>Campylobacter</i> in broiler flocks in Norway.....	121
The surveillance and control programme for viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) in Norway.....	127
The surveillance and control programme for <i>Gyrodactylus salaris</i> in Atlantic salmon and rainbow trout in Norway.....	135
The surveillance and control programme for bonamiosis and marteiliosis in European flat oysters ( <i>Ostrea edulis</i> L.) in Norway.....	141

## 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 the movement of diseases into countries. A number of these diseases are zoonosis.

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 become expanded by new agreements. This political and economical progress represents a zoonosanitary challenge for authorities responsible for the health of humans and animals.

The agreement on the European Economic Area (EEA) established 1 January 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

The trade directives address several communicable diseases, which are controlled by restrictions on trade with infected herds and regions. 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.

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 free-status 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 *Bonamia 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 2004 (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) <i>Salmonella</i> (1995)	Paratuberculosis (1996) BVD (1992)
Swine	Residual substances (1999)	AD (1994) <i>Salmonella</i> (1995)	TGE (1995) PRRS (1995) Swine influenza (1997)
Small ruminants	Scrapie (1997) Brucellosis (2004)		Maedi (1997)
Poultry	Residual substances (1999) Newcastle disease <i>Mycoplasma</i> <i>Salmonella</i> (1995-breeding flocks)	<i>Salmonella</i> (1995-96)	ILT (1997) ART (1997) <i>Campylobacter</i> (2001)
Farmed deer	Tuberculosis (2000)		
Llama			Paratuberculosis (2000)
Fish	VHS/IHN (1994)		<i>Gyrodactylus salaris</i> (2000)
Shellfish	<i>Bonamia/Marteilia</i> (1995)		

BSE=bovine spongiform encephalopathy, EBL=enzoitic 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, 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 scrapie 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.

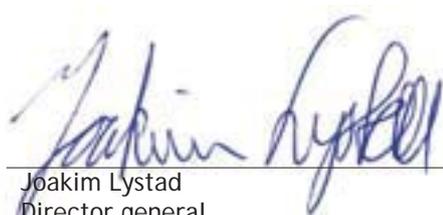
The economic funding for the programmes in 2004 was provided by the Ministry of Agriculture and Food, and the Ministry of Fisheries and Coastal Affairs with some contribution from the industries.

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

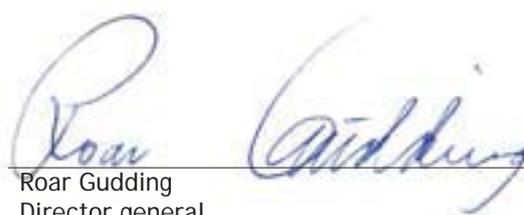
### Impact of the programmes

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.



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## Main results from the surveillance and control programmes in 2004

Due to the fact that consumption of poultry meat has been identified as a significant risk factor for human campylobacteriosis, an action plan including a surveillance programme encompassing all Norwegian broiler flocks was launched in 2001. Since then the prevalence of campylobacter-positive flocks has steadily decreased from 7.7% in 2001 to 3.3% in 2004.

Until 2003, avian rhinotrachitis (ART) had never been diagnosed in Norway, but antibodies against ART were for the first time detected in a commercial broiler breeder holding in 2003 and a commercial layer breeder holding in 2004. Clinical signs, however, were not observed.

A serologically based surveillance programme for maedi-visna has been performed in the counties of Rogaland and Hordaland since 1997. In November 2002, maedi-visna was diagnosed in two farms in Trøndelag. One of the infected flocks was a central breeding flock, and follow-up investigations revealed 45 flocks with seropositive animals. The spread of maedi-visna virus precipitated the authorities to launch a nationwide surveillance programme for maedi-visna. According to the programme, all sheep breeding stations and all breeding flocks were to be tested between 2003 and 2005. Seropositive animals were identified in one of 456 breeding flocks analysed during 2003 and in one of 1,230 breeding flocks analysed in 2004.

In 1994, based on the absence of a historical diagnosis, Norway achieved a *Brucella melitensis*-free status in small ruminants. To maintain this status a surveillance and control program for *B. melitensis* in sheep was established in 2004. A total of 50,501 blood samples from 1,655 sheep flocks were analysed for antibodies against *B. melitensis*; all were negative.

In 2004, a total of 37,884 animals were investigated in the surveillance and control programmes for BSE (n=23,115) and scrapie (n=14,769). Scrapie was diagnosed in 16 sheep. With the exception of two cases of classical scrapie in one flock, all cases were caused by the scrapie-strain Nor98, first detected in 1998.

At the turn of the year 2004/2005, only three cattle herds were subject to restrictions on account of BVD. Due to the good cooperation between the National Veterinary Institute, the authorities and the industry over a period of more than 10 years, it appears that extermination of this virus from the cattle population may be possible within a relatively short time.

The results from the surveillance programme for 2004 give additional documentation of freedom from specific virus infections (AD, TGE, PRRS and swine influenza) in the Norwegian swine population which is currently unique in an international context.

The *Salmonella* situation within the domestic animal population remains very good. In 2004, *Salmonella* was identified in two samples from cattle and one sample from swine and poultry, respectively. In addition, four swab samples from sheep carcasses were *Salmonella* positive.

In 2004, the paratuberculosis bacterium was found in four new goat flocks, but no infection was found in samples from cattle, sheep or llama.

The health situation is also satisfactory as far as important diseases of fish and shellfish are concerned. In 2004, Norway was granted status as free from bonamiosis and marteiliosis. *Gyrodactylus salaris* reappeared in two rivers in 2004. The rivers had been treated with rotenone to exterminate *G. salaris* in 1996 and 2003, respectively.

Otherwise, no A- or B-classified diseases have been detected in the surveillance and control programmes for terrestrial and aquatic animals (see overview).

Species	Infection	Start	Extent of prog
Cattle	IBR/IPV	1992	10% of dairy ca 10% of beef cat
	Brucellosis	2000	20% of dairy ca 20% of beef cat
		2000	In cases of abo
	BVD	1992	20% in most are All herds in cen
	EBL	1994	10% of dairy ca 10% of beef cat
	Bovine tuberculosis	2000	Inspection of ca suspected lesio
	BSE	1998	Investigation of
2000		Testing of impo	
2001		Testing of falle animals	
2001	2001	Testing of anim	
	2001	Testing of rand	
Swine	AD	1994	All breeding he fattening herds
	TGE	1994	
	PRRS	1995	
	Swine influenza	1997	
Poultry	Newcastle disease	1993	All chicken and
	ILT	1997	All chicken bre
	ART	1997	All chicken and
	<i>Campylobacter</i>	2001	All broiler flock
Small ruminants	Scrapie	1997	Testing of clini
		2002	Testing of falle
		1997	Random sampli
			Testing of prim
	Maedi	1997	All breeding flo 2003-2005
Brucellosis	2004	All breeding flo 2004-2005	
Several species	Salmonellosis	1995	Cattle: 3,000 ly Swine: 3,000 ly from all breed Poultry: faecal broilers or >25
	Paratuberculosis	1996	Testing of clini Testing of all ll goat and sheep
Fish	VHS/IHN	1994	Sampling of ap turbot farms (a course of a two
	<i>Gyrodactylus salaris</i>	2000	Sampling of ap salmon and rain Atlantic salmon approximately
Oyster	Bonamiosis	1995	Sampling of sel twice annually
	Marteiliosis	1995	Sampling of sel twice annually

Programmes in 2004	Number of samples examined in 2004	Positive samples in 2004	Previous positive results
Cattle herds	1,573 bulk milk samples	None	1992: 1 positive herd
Cattle herds	3,364 blood samples from 402 herds	None	
Cattle herds	3,138 bulk milk samples	None	
Cattle herds	7,986 blood samples from 813 herds		
Parturitions	Foetuses from 25 cows from 23 herds	None	
Meas	7,365 bulk milk samples	1998-2002: restrictions lifted in 1085 herds and imposed on 412 herds	
Certain areas	1,373 pooled blood samples	2003: restrictions lifted in 12 herds and imposed on 1 herd 2004: restrictions lifted in 4 herds and imposed on 4 herds	
Cattle herds	1,573 bulk milk samples	None	1995-1996: 7 positive herds
Cattle herds	3,364 blood samples from 402 herds		2002: 1 positive herd
Carcasses at slaughter, submission of organs for testing	Organs from 4 individuals	None	1984: 1 positive herd 1986: 1 positive herd
Of clinically suspect animals	3 samples	None	None
Imported animals and their progeny	24 samples	None	None
On stock and emergency slaughtered	11,297 samples	None	None
Animals selected at <i>ante mortem</i> control	1,353 samples	None	None
Randomly selected slaughtered animals	10,438 samples		
Herds and a selection of integrated and are tested	4,926 samples from 492 herds	None	None
«	4,908 samples from 492 herds	None	None
«	4,926 samples from 492 herds	None	None
«	4,921 samples from 492 herds	None	1998: 1 positive herd (H3N2)
Turkey breeder flocks	6,891 samples from 79 holdings	None	None
Breeder flocks	3,240 samples from 73 holdings	None	None
Turkey breeder flocks	3,360 samples from 76 holdings	2 positive flocks (1 holding)	2003: 2 positive flocks (1 holding)
Flocks	Samples from 3,626 flocks	118 (3.3%) positive flocks	2001: 7.7% positive flocks, 2002: 6.3% positive flocks, 2003: 4.9% positive flocks
Clinically suspect animals	19 samples	3 positive individuals	1997-2002: 20 positive individuals 2003: 1 positive individual
On stock	3,537 samples	4 positive individuals	2002: 3 positive individuals 2003: 8 positive individuals
Sampling of slaughtered animals	10,593 samples	8 positive individuals	2001-2002: 3 positive individuals 2003: 5 positive individuals
Primary and secondary flocks	620 samples	1 positive individual	2003: 1 positive flock
Flocks of sheep once during the period	36,911 samples from 1,230 flocks	1 positive flock	1998-2002: 2 positive flocks 2003: 1 positive flock
Flocks of sheep once during the period	50,501 samples from 1,655 flocks	None	
Lymph node samples	2,302 lymph node samples	3 positive samples (2 cattle, 1 swine)	1995-2002: Only a few positive samples each year 2003: 5 positive (2 cattle, 2 swine and 1 broiler)
Lymph node samples, faecal samples	2,662 lymph node samples and 2,635 faecal samples from 164 herds		
Sampling herds	7,033 faecal samples from 1,479 holdings		
Samples from all flocks of >50 layers/breeders			
Clinically suspect animals	Organ and faecal samples from 483 cattle, 1,312 goats, 176 sheep and 45 llamas	4 goat herds	1997: 4 cattle herds (imported animals) 1998-2002: 5 cattle herds, 10 goat herds and 2 sheep flocks, 2003: 3 goat herds
Approximately half of all salmonid and all farms should be tested in the 5-year period)	11,410 samples from 375 sites	None	None
Approximately half of all fresh water rainbow trout farms. Sampling of fingerlings/parr/smolts from 130 rivers	1,017 fish from 34 salmonid farms 4,509 fish from 120 rivers	No positive salmonid farms 2 positive rivers	1975-2003: 39 positive salmonid farms 1975-2003: 45 positive rivers
Selected farms and wild populations	420 oysters from 7 sampling points	None	None
Selected farms and wild populations	420 oysters from 7 sampling points	None	None



# The livestock and aquaculture populations in Norway



Annual report 2004

Berit Tafjord Heier



**Veterinærinstituttet**  
*National Veterinary Institute*



## 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 2004.

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 2003 and 2004.

Table 1. The livestock population in Norway and the number of slaughtered animals in 2004

Animal category	Number of		
	herds	animals <sup>1</sup>	slaughtered animals <sup>2</sup>
Cattle	22,500 <sup>1</sup>	936,600 <sup>1</sup>	334,100 <sup>2</sup>
Dairy cow**	15,677 <sup>1</sup>	253,200 <sup>1</sup>	-
Suckling cow**	3,793 <sup>1</sup>	44,700 <sup>1</sup>	-
Combined production (cow)**	934 <sup>1</sup>	25,200 <sup>1</sup>	-
Goat	1,090 <sup>1</sup>	71,000 <sup>1</sup>	18,400 <sup>2</sup>
Dairy goat**	568 <sup>1</sup>	44,650 <sup>1</sup>	-
Sheep	-	2,412,700 <sup>1</sup>	1,264,200 <sup>2</sup>
Breeding sheep > 1 year**	17,439 <sup>1</sup>	918,500 <sup>1</sup>	-
Swine	3,762 <sup>1</sup>	800,400 <sup>1</sup>	1,469,200 <sup>2</sup>
Breeding animal > 6 months**	2,199 <sup>1</sup>	61,800 <sup>1</sup>	-
Fattening pig for slaughter	3,344 <sup>1</sup>	424,100 <sup>1</sup>	-
Poultry			
Egg laying hen (> 20 weeks of age)	2,650 <sup>1</sup>	3,432,100 <sup>1</sup>	2,469,200 <sup>2</sup>
Flocks > 250 birds**	916 <sup>1</sup>	-	-
Broiler	489 <sup>2</sup>	-	42,851,700 <sup>2</sup>
Turkey, duck and goose for slaughter	191 <sup>1</sup>	365,800 <sup>1</sup>	1,035,200 <sup>2</sup>
Flocks > 25 birds**	67 <sup>1</sup>	-	-
Ostrich	18 <sup>1</sup>	190 <sup>1</sup>	-

<sup>1</sup> Register of Production Subsidies as of 31 July, 2004, <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 live animals and animal products to Norway in 2003 and 2004

Species	Imported product	2003 <sup>1</sup>		2004 <sup>2</sup>	
		No. of consignments	No. of animals or products	No. of consignments	No. of animals or products
Cattle	Live animals	1	17	-	-
	Semen (doses)	47	<180,000	-	40,000
	Embryos	20	<100	-	69
Swine	Live animals	2	6	-	-
	Semen (doses)	21	<200	-	200
Sheep	Live animals	-	-	2	11
	Semen (doses)	-	-	-	750
Goat	Live animals	7	92	2	26
Reindeer	Live animals for slaughter	-	-	2	350
Fur animal	Live animals	3	59	1	213
Poultry	Day-old chicks	19	8,500	16	157,357
Turkey	Day-old chicks	-	-	7	14,326
Duck and goose	Live birds	-	-	2	840
Halibut	Live fish	1	30,000	-	- <sup>1</sup>
Turbot	Live fish	2	750	2	600 <sup>1</sup>
Atlantic salmon	Live fish	-	-	2	429,480 <sup>1</sup>

<sup>1</sup> Data from the Norwegian Animal Health Authority, <sup>2</sup> Data from KOORIMP.

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. The interest to import live animals increased in general 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 16,600 dairy herds were registered in Norway in 2004 of which approximately 934 also kept suckling cows. The average number of dairy cows per herd was 16.3. The number of specialized beef herds

with at least one suckling cow was about 3,793 with a mean number of ten suckling cows per herd. There has been a decrease in number of Norwegian dairy herds over the last 14 years (Figure 1). However, the number of cattle in the beef and dairy production industry has been stable during the last three years.

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 2004, no live cattle were imported to Norway (Table 2).

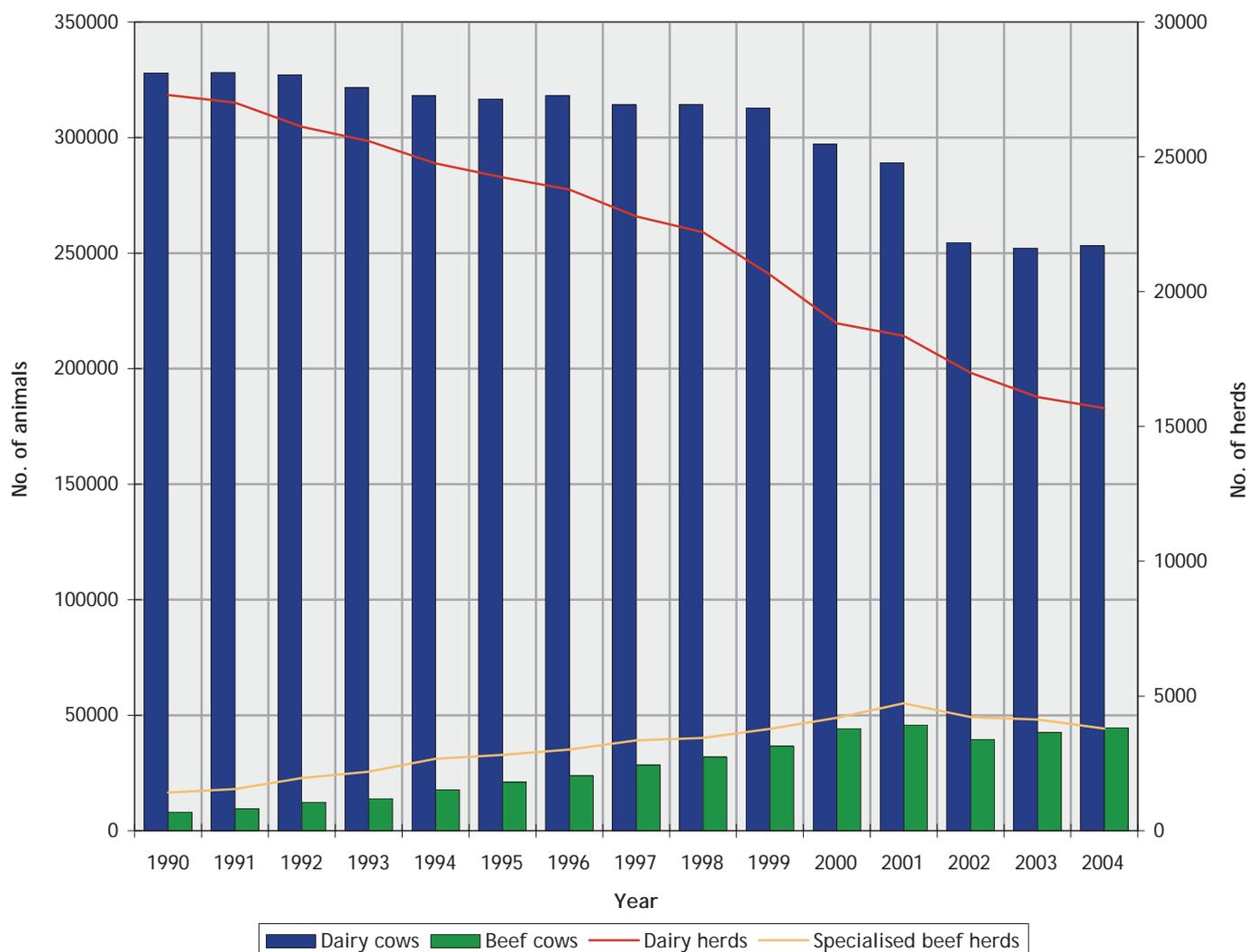


Figure 1. The number of dairy and beef cows in holdings with specialized dairy and beef production during the time period 1990-2004 (Statistics Norway and Register of production subsidies (RPS) for 2004).

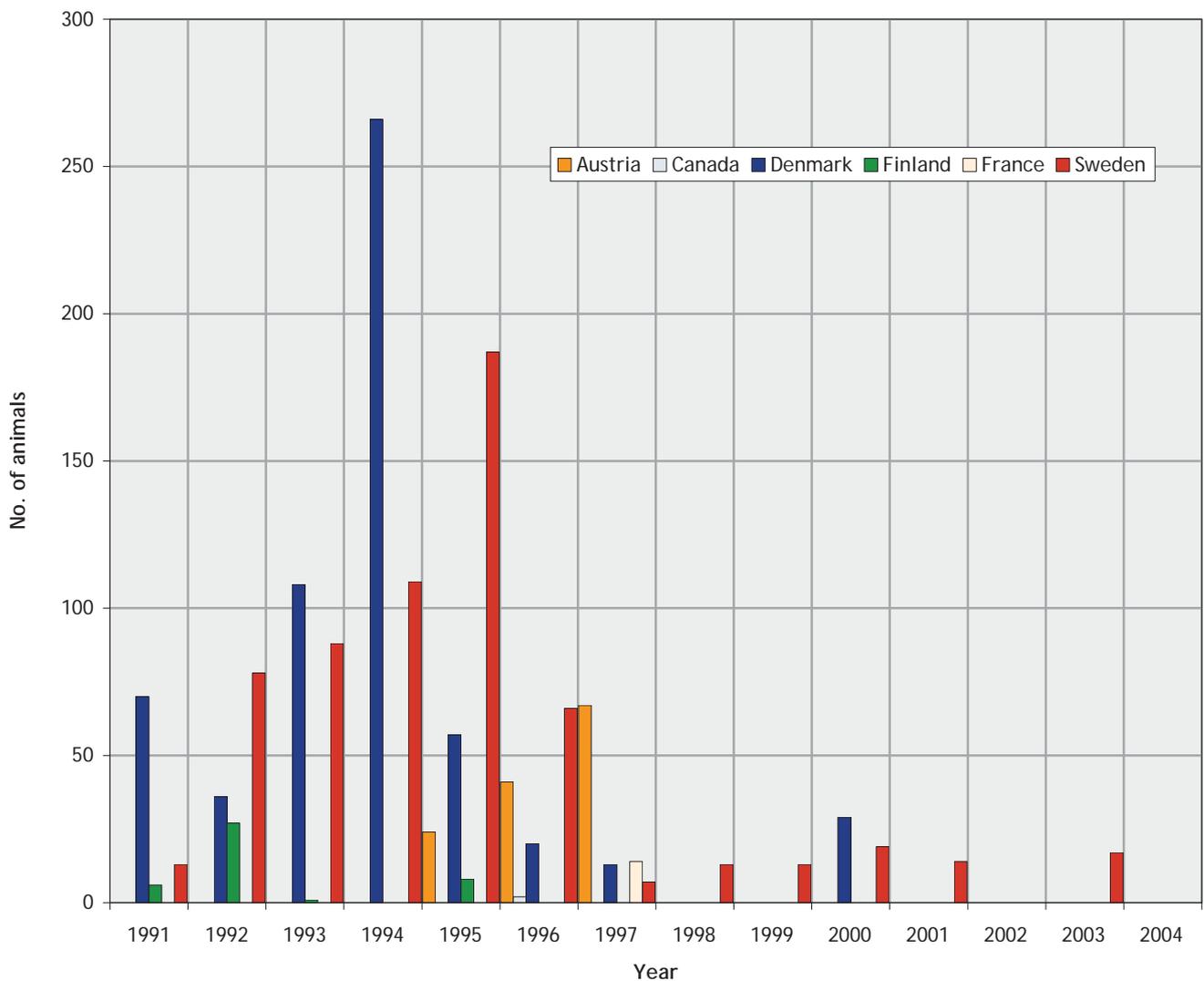


Figure 2. Imports of live cattle to Norway during the time period 1991-2004.

### The swine population

The Norwegian swine population is relatively small with a production destined for the domestic market. In 2003, about 1.5 million swine were slaughtered.

The population consists of approximately 62,000 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 2004 are given in Figure 3.

In 2004, no live swine were imported to Norway.

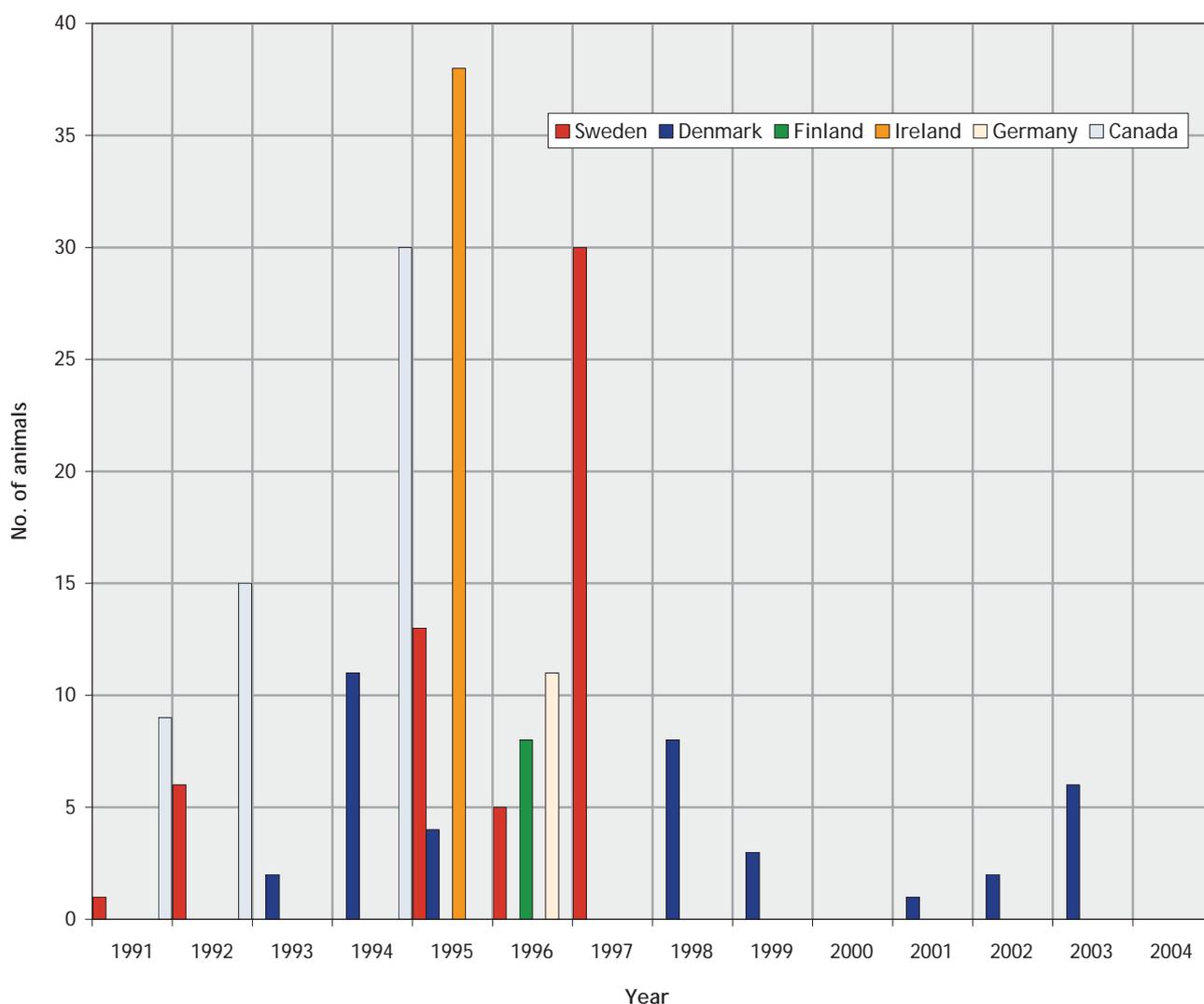


Figure 3. Import of live swine to Norway during the time period 1991-2004.

### The sheep population

The Norwegian sheep population consists of approximately 920,000 sheep older than one year. 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 breeds predominating. Each year about 1.3 million sheep are slaughtered and approved for human consumption. Only a few live animals have been imported since the 1970s. Eleven live animals were imported in 2004.

### The goat population

The Norwegian goat population is comprised of approximately 45,000 dairy goats and 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 18,000 goats are slaughtered and approved for human consumption each year. Only a small number of live animals have been imported since the 1970s.

### 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 2A shows the location and structure of the Norwegian layer population comprising two hatcheries, about 15 pullet rearing farms and about 900 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 is located in the northern part of Norway as shown in Figure 2B. 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

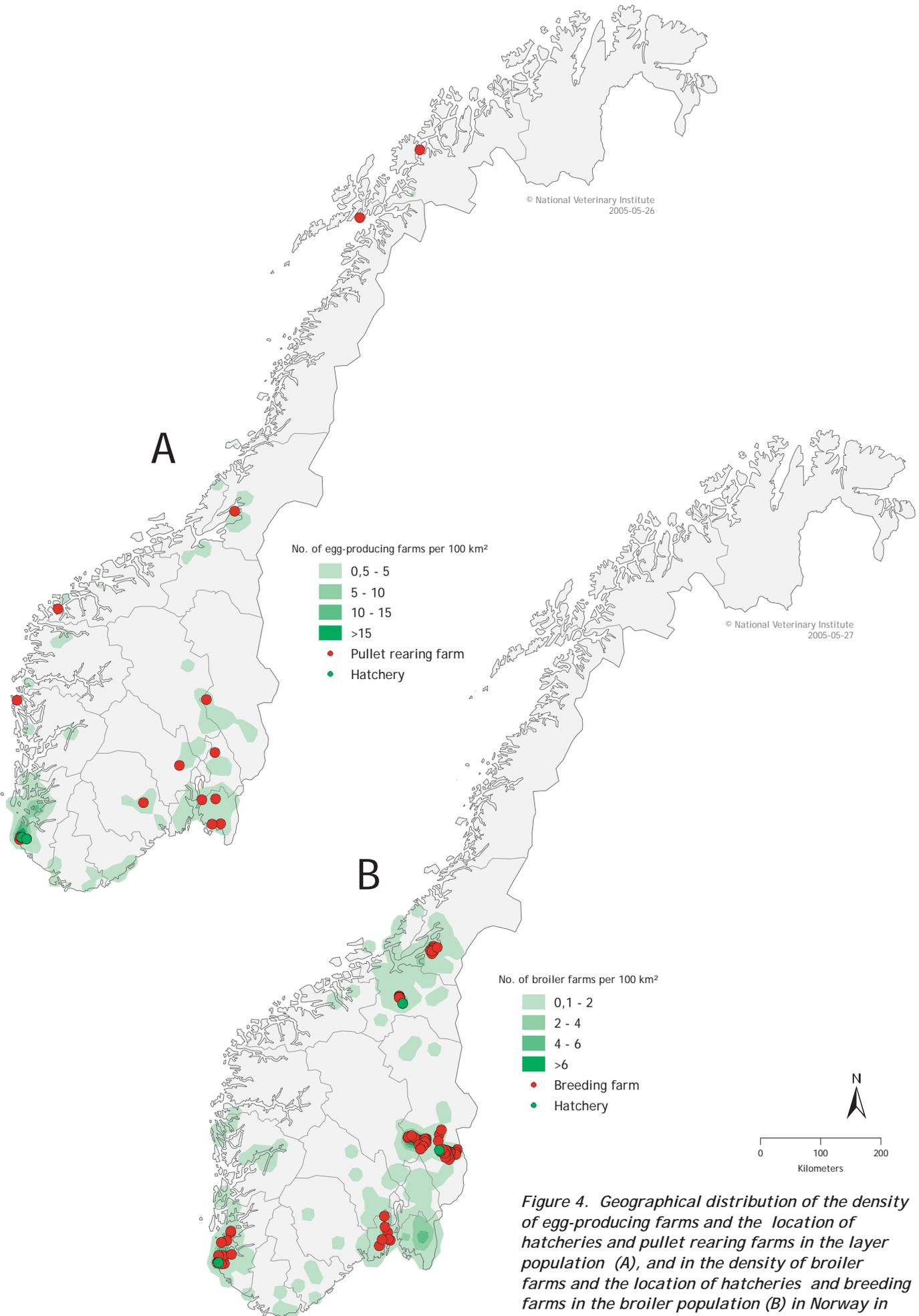
represents about 2% of the total value of all exports. Atlantic salmon is the most important species in the fish and shellfish farming industry. The counties of Hordaland and Nordland are the major counties for seawater farms producing Atlantic salmon. The production volume of Atlantic salmon increased with 3% from 2003 to 2004. A small reduction was observed in rainbow trout production volume in 2003 and 2004 (Table 3).

The import of live fish in 2004 consisted only of a few consignments of Atlantic salmon and turbot for the aquaculture industry (Table 2).

Table 3. Production volume of the most important species in Norwegian aquaculture during the time period 1992-2004<sup>1</sup>

Year	Atlantic salmon (ton)	Rainbow trout (ton)	Cod (ton)	Arctic char (ton)	Halibut (ton)	Blue mussels (ton)	Scallops <sup>2</sup> (1,000 pieces)	Oysters (1,000 pieces)
1992	141,000	-	-	-	-	-	-	-
1993	170,000	-	-	-	-	-	-	-
1994	207,000	-	569	262	63	542	14	1,085
1995	249,000	-	284	273	134	388	206	325
1996	292,000	40,000	191	221	138	184	92	526
1997	316,000	34,000	304	350	113	502	159	147
1998	343,000	47,000	199	190	290	309	159	510
1999	412,000	50,000	149	426	450	542	1,600	365
2000	424,000	47,000	200	300	400	659	2,200	583
2001	418,000	60,000	300	300	500	851	3,150	833
2002	450,000	83,000	1,500	319	424	2,000	2,800	300
2003	520,000	71,000	2,500	272	426	2,600	-	-
2004	539,000	65,000	3,000	300	1,000	-	-	-

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



*Figure 4. Geographical distribution of the density of egg-producing farms and the location of hatcheries and pullet rearing farms in the layer population (A), and in the density of broiler farms and the location of hatcheries and breeding farms in the broiler population (B) in Norway in 2004.*

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

Annual report 2004



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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 salmonella infections.

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), and 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 19.06.95), 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 and fresh meat and poultry meat are based on bacteriological examination for *Salmonella*. Isolation of any *Salmonella* sp. is notified to the authorities responsible for the programmes. The Norwegian Food Safety Authority 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 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. 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 minced 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 total 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).

Table 1. Sampling of poultry breeders (simplified) in the *Salmonella* surveillance and control programme in 2004

Category of poultry		Time of sampling	Sample material
Grandparents	Day old	Day 1	Organs or meconium
	Rearing	1-2 weeks, 4 weeks, 9-11 weeks and 13-14 weeks	Faecal samples
	Egg production* - from the house	Monthly	Faecal samples
	- in the hatchery	Every 2 <sup>nd</sup> week of production	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 2 <sup>nd</sup> week of production	Organs or meconium

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

### 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 slaughterhouse 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 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 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 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 serotype, 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 slaughtering. 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 of 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

Pieces of neck skin from broilers, turkeys, ducks and geese are tested for *Salmonella*. At each slaughterhouse, a minimum of five neck skin samples is collected per day and at least one sample must be taken from each flock slaughtered on a single day. This corresponds to approximately 9,000 annual samples.

#### Food products

The surveillance and control programme for cutting plants and cold stores are based upon samples of minced meat taken from the equipment or from trimmings. Each sample consists of 25 grams of meat. 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

The lymph node sample from each animal is homogenized and one half of the sample is pooled together with four other samples 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 the lymph node and neck skin samples are stored separately at 4°C until the results of the bacteriological examination are ready. If the pooled sample is confirmed positive for *Salmonella*, the individual 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 2,627 faecal samples from 164 elite and multiplier breeding herds (including AI centres and testing stations) were examined in 2004 (Table 2). *Salmonella* was not detected in any of the samples. A total of 2,662 lymph node samples from slaughtered pigs were examined. Approximately 34% of the samples were taken from sows and 66% from slaughter pigs. One sample was positive for *Salmonella* (Table 3) giving an estimated *Salmonella* prevalence of 0.04% (95% confidence interval: 0.001% - 0.2%) at the individual carcass level.

#### Cattle

In 2004, a total of 2,302 lymph node samples from cattle were examined (Table 3). Two samples were positive for *Salmonella* (Table 3) giving an estimated *Salmonella* prevalence of 0.09% (95% confidence interval: 0.01% - 0.31%) at the individual carcass level.

#### Poultry

A total of 7,033 faecal samples from 1,479 different holdings were examined (Table 4). *Salmonella* was not detected in any of the samples.

### Fresh meat and fresh poultry meat

#### Swab samples from cattle, sheep and swine carcasses

A total of 6,856 swab samples from 40 slaughterhouses were examined in 2004 (Table 5). *Salmonella diarizonae* was detected in four pooled samples taken from sheep at two different slaughterhouses.

#### Neck skin samples from poultry

A total of 7,239 neck skin samples from poultry were examined in 2004. The samples represented all the eight poultry slaughterhouses in Norway. Nearly 75% of the samples came from broilers, 11% from layers and 14% 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 1,791 samples of minced meat from 92 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 2004

Herd category	No. of herds sampled (total*)	No. of samples examined	No. of positive samples	<i>Salmonella</i> serotype
Elite breeding herds	66 (66)	1,052	0	
Multiplier herds	96 (101)	1,523	0	
A.I. centres and testing stations	2 (4)	60	0	

\* Total number of herds is estimated as elite and multiplier breeding herds per 1 January 2004 excluding herds which ended breeding activity during 2004 before being tested.

Table 3. Number of individual lymph node samples from cattle and swine examined in the *Salmonella* surveillance and control programme in 2004

Species	No. of slaughterhouses sampled (total*)	No. of samples examined	No. of positive samples	<i>Salmonella</i> serotype
Cattle	31 (42)	2,302	2	<i>S. Typhimurium</i> (4,5,12:i:1,2)
Slaughter pigs	22 (32)	1,769	0	
Sows	18 (35)	893	1	<i>S. Typhimurium</i> (4,12:i:1,2)

\* Slaughterhouses where the number of slaughtered animals of a species is less than 100 according to the Slaughter Statistics for 2004 are not included in the sampling scheme.

Table 4. Sampling of poultry in the *Salmonella* surveillance and control programme in 2004

Poultry breeding flocks	No. of samples tested	No. of holdings tested	No. of positive holdings	<i>Salmonella</i> serotype
Grandparents				
Layers	22	3	0	
Broiler production	3	3	0	
Parents				
Layers	261	7	0	
Meat production - Broilers	445	78	0	
- Turkeys	143	5	0	
- Ducks	5	1	0	
- Geese	0	0	0	
<b>Total - Breeders</b>	<b>901</b>	<b>91</b>	<b>0</b>	
Other commercial poultry				
Pullets	254	26	0	
Layers	1,627	836	0	
Meat production - Broilers	3,772	528	0	
- Turkeys	347	74	0	
- Ducks	48	6	0	
- Geese	3	3	0	
<b>Total - Non breeder holdings</b>	<b>6,132</b>	<b>1,413</b>	<b>0</b>	
<b>Total</b>	<b>7,033</b>	<b>1,479</b>	<b>0</b>	

\* The poultry category for some of the samples might have been misclassified due to insufficient information on the form following the samples.

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 2004

Species	No. of slaughterhouses sampled (total*)	No. of samples examined	No. of positive samples	<i>Salmonella</i> serotype
Cattle	31 (42)	2,136	0	
Swine	24 (32)	2,456	0	
Sheep	27 (35)	2,264	4	<i>S. diarizonae</i> (61:k:1,5,(7))
Poultry	8 (8)	7,239	1	<i>S. Senftenberg</i>

\* Slaughterhouses where the number of slaughtered animals of a species is less than 100 according to the Slaughter Statistics for 2004, are not included.

## Discussion

The results from the *Salmonella* surveillance programme in 2004 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. diarizonae* is found most frequently from sheep (swabs). *S. Enteritidis* has never been found by 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* in the wild fauna (wild birds and hedgehogs) represented a risk for human infection, especially for children under four years of age. Such wild animal reservoirs may also be considered a risk of infection in farm animals. As no increase in prevalence of *Salmonella* has been demonstrated in the programme, it may be assumed that farm animal populations 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 not reached for any of the populations and a closer follow up of the personnel taking and reporting the sample is needed. Never the less, the programme was able to document a very low *Salmonella* prevalence in the examined populations.

There has been a slight decrease in the number of examined neck skin and minced meat samples. This reduction is probably explained by the structural changes in the poultry industry and the manufacturing plants.

## References

1. Hofshagen, M., Nygård, K., Kruse, H. (Eds.), 2004. Trends and sources of zoonotic agents in animals, feedingstuffs, food and man in Norway 2003. Norwegian Zoonosis Centre.
2. Anonymous, 1998. The Norwegian *Salmonella* surveillance and control programmes for live animals, eggs and meat. Veterinary and Food Department, Norwegian Ministry of Agriculture.
3. Anonymous, 1995. The Swedish *Salmonella* control programmes for live animals, eggs and meat. National Veterinary Institute, Swedish Board of Agriculture, National Food Administration.
4. Anonymous, 1994. The Finnish *Salmonella* control programmes for live animals, eggs and meat. Veterinary and Food Department, Finnish Ministry of Agriculture and Forestry.
5. Forskrift om overvåking av og kontroll med forekomsten av *Salmonella* hos levende dyr av 31.01. 1995 nr. 107. (Provision concerning surveillance and control of incidence of *Salmonella* in live animals.) <http://www.lovddata.no/for/sf/ld/xd-19950131-0107.html>
6. Instruks til det kommunale næringsmiddeltilsynet om overvåking av og tiltak mot *Salmonella* i ferskt kjøtt og ferskt fjørfekjøtt av 20.02.1996 nr 1474. <http://www.lovddata.no/cgi-wift/ldles?doc=/sf/sf/sf-19960220-1474.html>
7. Fredriksen, B., Bergsjø, B., Bruheim, T., Nyberg K., Flesjå, K.I., Skjervheim, M., 2002. The surveillance and control programmes for *Salmonella* in live animals, eggs and meat in Norway. In: Fredriksen, B., Mørk, T. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001. National Veterinary Institute, Oslo, Norway, pp. 19-32.
8. Jarp, J., Bergsjø, B., Bruheim, T., Nyberg K., Flesjå, K.I., Skjervheim, M., 2003. The surveillance and control programmes for *Salmonella* in live animals, eggs and meat in Norway. In: Heier, B.T. (Ed.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002. National Veterinary Institute, Oslo, Norway, pp. 20-28.
9. Hopp, P., Bergsjø, B., Bruheim, T., Flesjå, K., Nilsen, H., Nyberg K., 2004. The surveillance and control programmes for *Salmonella* in live animals, eggs and meat in Norway. In: Mørk, T., Hellberg, H. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003. National Veterinary Institute, Oslo, Norway, pp. 19-25.



# Examinations of residues of prohibited substances in live animals in Norway

Annual report 2004



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## Introduction

The programme for monitoring residues of prohibited substances in live animals has been administered by the Norwegian Animal Health Authority since 1999 (1, 2). Prior to this, surveying residues in both animal products and live animals was carried out by the Norwegian Food Control Authority. The programmes for residues in live animals, fish, and animal products were taken over by the Norwegian Food Safety Authority from 1 January 2004. 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 government food control authorities.

Sampling is carried out in accordance with EU Directive 96/23 (3), which determines the sampling frequencies in bovines, pigs and poultry based upon slaughter statistics from 2002.

The following prohibited substances are included in Group A and are covered by the programme:

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

## Aim

The aim of the present programme is to ensure food safety by monitoring live animals for the presence of prohibited substances.

## Materials and methods

The sampling plan for the various animal species is determined by the Norwegian Food Safety Authority, on the basis of earlier production (Table 1).

The programme includes sampling of muscle tissues, urine, and blood.

A national database consisting of all swine and cattle producers who apply for production subsidies (RPS) constitute the sampling frame for swine and cattle herds included in the programme for live animals. The RPS database includes information about the herd owners, herd localisation and the number of animals in different age categories. The register is owned by the Ministry of Agriculture and updated twice a year. The selection of herds was performed by simple random sampling by an automatic routine (SAS-PC System® Version 8e for Windows, SAS Institute Inc., Cary, NC, USA, 1999-2000). The sampling was also randomly distributed in time.

Information on each sample is registered in a protocol at the time of sampling and sent to a central registration unit. All samples are analysed within three months. Any prohibited substances detected are reported immediately.

## Results

All analyses are carried out by national reference laboratories. The Norwegian laboratories are accredited by the Norwegian Accreditation and meet the requirements of the standard ISO/IEC 17025. Substances A1, A3, A4 and A5 are analysed at the Hormone Laboratory, Aker University Hospital. Substances A2 are analysed at the Ghent University, Belgium, and sub-stances A6 are analysed at the Laboratory for Veterinary Drug Residue Analysis in Food, Norwegian School of Veterinary Science.

Table 2 presents an overview of the number of samples tested in 2004 with respect to the sampling plan, and grouped according to substances.

No traces of prohibited substances were detected in any of the animals sampled.

Table 1. The sampling plan for 2004 based on the number of animals slaughtered or tons produced in 2002

Categories	Animals slaughtered 2001	Total no. of animals to be tested	No. of animals to be tested for Group A substances - live and slaughtered
Bovine	343,584 *	1,374 ** (0.4%)	855 (430 live, 425 slaughtered)
Porcine	1,319,976 *	660 ** (0.05%)	298 (78 live, 220 slaughtered)
Poultry	45,656 tons	228 ** (1 per 200 tons)	168 (48 live, 120 slaughtered)

\* Total number of approved carcasses.

\*\* Includes both Group A and Group B substances, while only Group A substances are tested in this programme.

Table 2. The number of samples tested vs. planned in 2004

Substances	Bovines		Pigs		Poultry	
	Sampled	Planned	Sampled	Planned	Sampled	Planned
A1 Stilbenes	84	85	12	13	3	8
A2 Thyrostatics	45	45	20	13	8	8
A3 Steroids	78	85	17	13	2	8
A4 Resorcylic acid lactones	82	85	11	13	6	8
A5 Beta-agonists	85	85	9	13	6	8
A6 Annex IV substances*	43	45	13	13	-	8
<b>Total A</b>	<b>417</b>	<b>430</b>	<b>82</b>	<b>78</b>	<b>25</b>	<b>48</b>

\* A6: Annex IV: chloramphenicol; nitrofuranes; dimetridazole, metronidazole.

## Comments

Deviations from the sampling plan are due to inadequate implementation of the plan. Attempts to obtain suitable tissue samples from poultry have increased the implementation for 2004. Sampling routines will be further revised in 2005.

## References

1. Børsum, J., 2002. Examinations of residues of prohibited substances in live animals in Norway. In: Fredriksen, B., Mørk, T. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001. National Veterinary Institute, Oslo, Norway, pp. 33-36.
2. Børsum, J., 2003. Examinations of residues of prohibited substances in live animals in Norway. In: Heier, B.T. (Ed.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002. National Veterinary Institute, Oslo, Norway, pp. 29-32.
3. Council Directive 96/23/EF, 1996. Control measures to be implemented with respect to specific substances and their residues in live animals and animal products, and repeal of Council Directives 85/358/EØF and 86/469/EØF and resolutions 89/187/EØF and 91/664/EØF.

# Examinations of residues of veterinary drugs, prohibited substances and environmental contaminants in animal products in Norway

Annual report 2004



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## Introduction

Surveying residues in animal products has been carried out in Norway since 1985, starting with 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 milk, eggs, honey and fish, and by substantially increasing the number of samples and substances tested for in the programme. The programmes for residues in live animals and fish were taken over by the Norwegian Animal Health Authority and the Directorate of Fisheries, respectively.

The programmes for residues in live animals, fish, and animal products were taken over by the Norwegian Food Safety Authority from 1 January 2004. 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 government food control authorities.

To prevent consumption of animal products that contain potentially harmful residues, the Residue Control Regulation (RCR) was introduced (1). 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 (2).

The RCR determines MRLs for veterinary drugs. The use of veterinary drugs without MRLs in production animals is prohibited.

## 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.

## Materials and methods

### Group of substances

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

Table 1. The number of animals slaughtered and production figures for animal products in Norway in 2002

Categories	Production
Bovine	343,584 *
Porcine	1,319,976 *
Sheep	1,235,384 *
Equine	2,406 *
Reindeer	1,787 tons
Poultry	45,656 tons
Milk	1,504 mill litre
Eggs	47,400 tons
Honey	1,300 tons

\* 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 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 the Ghent University, Belgium. Substances A6, B1, B2b, e, and f 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

Table 2 presents an overview of the number of animals/foods sampled in 2004.

### General

In general, there were few deviations from the sampling plan.

### Heavy metals

Residues of environmental contaminants (cadmium and lead) exceeding MRLs were detected in samples from one bovine, 15 sheep, 32 reindeer, and 57 wild game animals, and in one cow's milk sample. Samples of wild game were taken from the species elk (39), roe deer (7), and red deer (13). The bovine sample was taken by the Norwegian Food Safety Authority's District office (DK) - Haugaland. The milk sample was sampled by DK Sør-Østerdal. Three of the sheep samples was sampled by DK Haugaland, five by DK Midt-Rogaland, three by DK Oslo, three by DK Sør-Innherrad, and one by DK Gauldal. Eight of the reindeer samples were sampled by DK Midt-Finnmark, ten by DK Øst-Finnmark, ten by DK Sør-Innherrad and four by DK Gauldal.

Analyses of heavy metals are initially carried out on liver and kidney samples from bovines, pigs, sheep, and reindeer. When residues exceed MRLs, samples of muscle from the same animals are analysed. All but nine of samples exceeding MRLs were in liver and kidney samples. In horses, only samples of muscle are analysed.

Analyses on heavy metals in wild game were carried out on samples of liver, kidney, and muscle. One of the roe deer samples was sampled by DK Hallingdal, four by DK Gjøvik, Toten og Land, and two by DK Nedre Telemark. Two of the red deer samples was sampled by DK Hallingdal, one by DK Orkdal, four by DK Sunnhordland, three by DK Haugaland, two by DK Nedre Telemark, and one by DK Midt og Vest Telemark. Three of the elk samples was sampled by DK Nord-Helgeland, one by DK Midt-Finnmark, one by Sør Helgeland, five by DK Vest-Agder, two by DK Hallingdal, four by DK Namdal, six by DK Hardanger, six by DK Romerike, one by DK Tromsø, three by DK Vesterålen, five by DK Gjøvik, Toten og Land, and two by DK Midt og Vest Telemark.

Cadmium and lead were found in combination in all but three of the animals. In three animals, lead was found only in muscle. This may imply contamination of the samples by the sampler or the laboratory.

Heavy metals are found in variable concentrations, both naturally and as a result of contamination. Heavy metals may accumulate in organs throughout life as a result of environmental contamination.

Table 2. The total number of animals/foods in the surveillance and control programme in 2004

Substances	Bovines		Pigs		Sheep		Horses		Poultry		Reindeer		Milk		Eggs		Honey		Wild game		
	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	
A1 Stilbenes	72		49		25				17		2										
A2 Thyrostatics	44		11		10				12												
A3 Steroids	123		41		25				10		2										
A4 Resorcylic acid lactones	75		46		20				12		2										
A5 Beta-agonists	70		30		25				10		4										
A6 Annex IV substances	55		48		20				42		6										
<b>Total A</b>	<b>439</b>		<b>225</b>		<b>125</b>				<b>103</b>		<b>16</b>										
B1 Tiamulin (pigs); penicillin (milk)			30										46								
B1 Quinolones	20		25		20																
B1 Sulfonamides	30		30		30				65		30		38		40						
B1 Tetracyclines									61		30		38		40						
<b>Total B1</b>	<b>50</b>		<b>85</b>		<b>50</b>				<b>126</b>		<b>60</b>		<b>122</b>		<b>80</b>						
B2a Anthelmintics	65		40		50				10		30		49								
B2b Anticoccidials	10		10		20				110						50						
B2c Carbamates and pyrethroides	20		10		35				10		10										
B2d Sedatives	36		30		35																
B2e NSAIDs	20		10		10				10												
B2f Glucocorticoids	20		20						10												
<b>Total B2</b>	<b>171</b>		<b>120</b>		<b>150</b>				<b>140</b>		<b>40</b>		<b>70</b>		<b>70</b>						
B3a Organochlorine compounds	20		20		13				13		10		19		15						
B3b Organophosphorous compounds	20		20		13				5				18								
B3c Chemical elements	20	1	15		79	14	25		3		33	32	24	1	20						76
B3d Mycotoxins	10		10		5				3				24								
<b>Total B3</b>	<b>70</b>	<b>1</b>	<b>65</b>		<b>110</b>	<b>14</b>	<b>25</b>		<b>24</b>		<b>43</b>	<b>32</b>	<b>85</b>	<b>1</b>	<b>35</b>						<b>76</b>
<b>Total B</b>	<b>291</b>	<b>1</b>	<b>270</b>		<b>310</b>	<b>14</b>	<b>55</b>		<b>290</b>		<b>143</b>	<b>32</b>	<b>277</b>	<b>1</b>	<b>185</b>						<b>76</b>
<b>Total A+B</b>	<b>730</b>	<b>1</b>	<b>495</b>		<b>435</b>	<b>14</b>	<b>55</b>		<b>393</b>		<b>159</b>	<b>32</b>	<b>287</b>	<b>1</b>	<b>195</b>						<b>76</b>

\*: 10 samples of honey are analysed for groups B2f in multiserries.

No.: Total number animals/foods in the covered period.

Pos.: Positive results (detection for banned substances or above MRLs or national limits for veterinary drugs and contaminants).

A6: Annex IV: chloramphenicol; nitrofuranes; dimetridazole, metronidazole.

Wild game: elk, roe deer, and red deer.

## References

1. Directive no 65, 2000. Control measures for residues of specific substances in foodstuffs, production animals and fish to ensure food safety (RCR). Norwegian Food Control Authority, Oslo, Norway.
2. Council Directive 96/23/EF, 1996. Control measures to be implemented with respect to specific substances and their residues in live animals and animal products, and repeal of Council Directives 85/358/EØF and 86/469/EØF and resolutions 89/187/EØF and 91/664/ EØF.
3. Veterinære lægemiddelrester i fødevarer 2003 - resultater fra den danske kontrol af veterinære lægemiddelrester, 2002. Ministeriet for Fødevarer, Landbrug og Fiskeri, Fødevedirektoratet, Fødevarerapport 2003: 13; ISBN: 87-91399-16-5.
4. Klaasen, C.D., 2001. Casarett & Doull's Toxicology. The basic science of poisons. 6<sup>th</sup> ed. McGraw-Hill, USA.
5. Adams, H.R., 1995. Veterinary Pharmacology and Therapeutics. 7<sup>th</sup> ed. Iowa State University Press, Ames, USA.
6. Food Residue Database, 2001. In: Research report no 46. The National Food Centre, Ireland.
7. Børsum, J., 2002. Examinations of residues of veterinary drugs, prohibited substances and environmental contaminants in animal products in Norway. In: Fredriksen, B., Mørk, T. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001. National Veterinary Institute, Oslo, Norway, pp. 37-43.
8. Børsum, J., 2003. Examinations of residues of veterinary drugs, prohibited substances and environmental contaminants in animal products in Norway. In: Heier, B.T. (Ed.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001. National Veterinary Institute, Oslo, Norway, pp. 33-39.

## Appendix

### Group A - Substances having anabolic effect and unauthorized substances

1. Stilbenes, stilbene derivatives, salts and esters
2. Thyrostatics
3. Steroids
4. Resorcylic 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
  - Anthelmintics
  - Anticoccidials
  - Carbamates and pyrethroids
  - Sedatives
  - NSAIDs
  - Other pharmacologically active substances
3. Environmental contaminants and other substances
  - Organochlorine compounds, incl PCBs
  - Organophosphorus compounds
  - Chemical elements
  - Mycotoxins

# The surveillance and control programme for paratuberculosis in Norway



Annual report 2004



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## Introduction

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 and most often culling of the herd when the infection is confirmed. 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).

Occurrence of the disease in Norway, control measures taken up to 1995, and results from the surveillance and control programmes from 1996 to 2002, are described in the annual report for 2001 (6) and 2002 (7).

## Aim

The aim of the surveillance programme for paratuberculosis in 2004 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 in 2004 included in the surveillance and control programme for paratuberculosis; 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 the dairies in the sampling period and all beef cattle herds receiving state support according to recordings of July 2003. One hundred herds were randomly selected and five faecal samples were collected from the five oldest cows in each herd.

In 1999, blood samples from animals older than 24 months in 287 randomly chosen herds (242 dairy herds and 45 beef herds) were examined for antibodies against *M. a. paratuberculosis* in an ELISA (6). Ten per cent of the animals were seropositive. These animals are monitored by faecal culture each year, and when slaughtered, organ samples are collected. The last animals from this lot were slaughtered this year.

#### *Llamas*

The llama was recently introduced as a new species to Norway. 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 more than four years old should be collected each year. In addition, organ samples are collected from llamas at slaughter, and from animals that die at more than four years of age.

#### *Goats*

Hundred flocks, in which the kids were vaccinated, and 30 unvaccinated flocks were randomly selected for sampling by faecal samples from the ten oldest goats, or from sick goats.

#### *Sheep*

Twenty flocks 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 474 faecal samples and nine organ samples were collected from cattle, while 1308 faecal samples and four organ samples were collected from goats. A total of 176 faecal samples were collected from sheep, and 44 faecal samples and one organ sample were collected from llamas (Table 1).

Table 1. Number of samples collected for examination for *Mycobacterium avium* subsp. *paratuberculosis* in 2004

		Faecal samples no. of animals	Intestinal samples no. of animals	Total no. of animals	Total no. of herds
Cattle	Dairy and beef cattle	474	0	474	95
	Seropositive in 1999	0	2	2	1
	Suspected or imported cases	0	7	7	5
	Control of infected herds and contact herds	25	17	42	1
Goat	Vaccinated	974	0	974	97
	Unvaccinated	313	0	313	32
	Suspected cases	0	0	0	0
	Control of infected flocks and contact flocks	21	4	25	3
Sheep	Random sample	176	0	176	19
	Control of infected flocks and contact flocks	0	0	0	0
Llama		44	1	45	10

### 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 µg/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 26 cattle from seven different herds were examined with no positive results (Table 2).

A total of four goats from two different flocks were examined (Table 3). All four goats came from infected flocks, but neither granulomatous lesions nor acid fast bacteria were found in the intestines or the lymph nodes.

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

### Bacteriological examination

A total of 525 cattle in 108 herds were examined for paratuberculosis by bacteriological methods (Table 2). *M. a. paratuberculosis* was not found.

Table 2. Results of histopathological and bacteriological examination of cattle in 2004

Type of samples	Bacteriology			Histopathology		
	No. of samples	No. of herds	No. of pos. samples	No. of samples	No. of herds	No. of pos. samples
Faeces	499	101	0			0
Intestinal samples	26	7	0	26	7	0

Table 3. Results of histopathological and bacteriological examination of goats in 2004

Type of samples	Bacteriology			Histopathology		
	No. of samples	No. of flocks	No. of pos. samples	No. of samples	No. of flocks	No. of pos. samples
Faeces	1,308	131	17			
Intestinal samples	4	2	0	4	2	0

Table 4. Results of histopathological and bacteriological examination of sheep in 2004

Type of samples	Bacteriology			Histopathology		
	No. of samples	No. of flocks	No. of pos. samples	No. of samples	No. of flocks	No. of pos. samples
Faeces	176	19	0			
Intestinal samples	0	0	0	0	0	0

Table 5. Results of histopathological and bacteriological examination of llamas in 2004

Type of samples	Bacteriology			Histopathology		
	No. of samples	No. of herds	No. of pos. samples	No. of samples	No. of herds	No. of pos. samples
Faeces	44	10	0			
Intestinal samples	1	1	0	1	1	0

A total of 1,312 dairy goats from 131 flocks were examined for paratuberculosis by bacteriological methods (Table 3). *M. a. paratuberculosis* was isolated from 17 goats in ten flocks. Six of the flocks had been positive earlier, while four flocks had not been detected previously. The kids in these flocks are vaccinated against paratuberculosis since 1992-1993.

A total of 176 sheep from 19 flocks were examined for paratuberculosis by bacteriological methods (Table 4). *M. a. paratuberculosis* was not isolated from any of the samples.

A total of 45 llamas from ten 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 19 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 traced to one of two reasons; brought into the country with imported animals (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. Importation is therefore no longer any big risk for acquiring the infection, whereas the infected goat flocks are still a great risk for spreading the infection to other species and to new goat flocks.

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. Forty flocks (16%) 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 four new flocks this year. It is probable that even more flocks are infected because vaccination hides the symptoms. The surveillance programme for 2004 therefore gave priority to samples from vaccinated goat flocks while cattle and sheep were sampled less. By following this priority a few years more, our prevalence estimate could possibly come closer to the true prevalence in the endemic area and make a platform for an eradication programme.

## References

1. Horne, H., 1908. Kronisk pseudotuberkuløs tarmbetændelse hos kvæg konstateret i Norge. (Chronic pseudo-tuberculous intestinal inflammation demonstrated in Norway). *Nor. Vet. Tidsskr.* 20, 70-77.
2. Holmboe, F.V., Slagsvold, L., 1934. Paratuberkulose hos sau og geit. (Paratuberculosis in sheep and goats). *Skand. Vet. Tidsskr.* 24, 573-585.
3. Saxegaard, F., Fodstad, F.H., 1985. Control of paratuberculosis (Johne's disease) in goats by vaccination. *Vet. Rec.* 116, 439-441.
4. García Marín, J.F., Tellechea, J., Gutiérrez, M., Corpa, J.M., Pérez, V., 1999. Evaluation of two vaccines (killed and attenuated) against small ruminant paratuberculosis. In: *Proceedings of Sixth International Colloquium on Paratuberculosis*, pp. 234-241.
5. Djønne, B., Fredriksen, B., Nyberg, O., Sigurðardóttir, Ó.G., Tharaldsen, J., 2001. National bovine paratuberculosis program in Norway. *Bull. Int. Dairy Fed.* 364, 75-80.
6. Djønne, B., Nyberg, O., Fredriksen, B., Sigurðardóttir, Ó.G., Tharaldsen, J., 2002. The surveillance and control programme for paratuberculosis in Norway. In: *Surveillance and control programmes for terrestrial and aquatic animals in Norway*. Annual report 2001. National Veterinary Institute, Oslo, Norway, pp. 45-54.
7. Nyberg, O., Djønne, B., 2003. The surveillance and control programme for paratuberculosis in Norway. In: Heier, B.T. (Ed.) *Surveillance and control programmes for terrestrial and aquatic animals in Norway*. Annual report 2002. National Veterinary Institute, Oslo, Norway, pp. 41-47.
8. Berg Jørgensen, J., 1982. An improved medium for culture of *Mycobacterium paratuberculosis* from bovine faeces. *Acta Vet. Scand.* 23, 325-335.
9. Saxegaard, F., 1985. Isolation of *Mycobacterium paratuberculosis* from intestinal mucosa and mesenteric lymph nodes of goats by use of selective Dubos medium. *J. Clin. Microbiol.* 22, 312-313.
10. Sigurðardóttir, O.G., Press, C.M., Saxegaard, F., Evensen, O., 1999. Bacterial isolation, immunological respons and histopathological lesions during the early subclinical phase of experimental infection of goat kids with *Mycobacterium avium* subsp. *paratuberculosis*. *Vet. Pathol.* 36, 542-550.

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



Annual report 2004

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## Introduction

### 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), while active surveillance was 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 ELISA method for detection of resistant prion protein (PrP<sup>Sc</sup>) (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 OIE protocol (1, 2). The number of samples examined in each category 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 the Norwegian surveillance programme according to categories from 1998-2003

Reason for submission to the laboratory	1998-2000	2001	2002	2003
Clinically suspected	78	14	2	2
Fallen stock		1,352	1,482	1872
Emergency slaughtered		7,073	7,246	7,322
Ante-mortem animals		2,612	3,562	4,102
Imported slaughtered animals	19 *	88	39	39
Healthy slaughtered animals		2,400	9,907	10,726
<b>Total</b>	<b>97</b>	<b>13,539</b>	<b>22,238</b>	<b>24,063</b>

\* All the samples were examined in 2000.

## Surveillance programme

### Programme outline

For 2004 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 National Veterinary Institute, Oslo. Official inspectors at 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 µm 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<sup>Sc</sup> (TeSeE®, Bio-Rad) as described by the manufacturer.

Table 2. Examination for BSE in cattle sampled by the Norwegian surveillance programme according to categories in 2004

Reason for submission to the laboratory	No. of samples	No. of rejected samples	Negative	Positive
Clinically suspected animals	3	0	3	0
Fallen stock	2,145	60	2,085	0
Emergency slaughter	9,217	5	9,212	0
Ante-mortem animals*	1,355	2	1,353	0
Imported animals	24	0	24	0
Healthy slaughtered animals	10,443	5	10,438	0
<b>Total</b>	<b>23,187</b>	<b>72</b>	<b>23,115</b>	<b>0</b>

\* Abnormal findings at ante-mortem examination, rejected for human consumption, or which died at the abattoir or during transport.

### 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 µm, 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 23,187 cattle. Of these, 72 (0.3%) samples were unsuitable for examination. The categories and number of animals examined are presented in Table 2.

For 2.8% 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 22,595 samples originated from 12,156 herds (10,756 dairy cattle herds and 1,400 beef cattle herds). The mean number of examined animals per herd was 1.9.

### Clinically suspected animals (passive surveillance)

Only three animals have been investigated as clinical suspects. 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 2004 has increased, while the number of examined cattle from ante mortem category has decreased significantly compared to corresponding categories for 2003 (Table 1). The Norwegian cattle population approximates 415,000 cattle older than 24 months (Husdyrregistret per 31.12.04). Fallen stock older than 24 months is approximately 0.77% of the adult population (Husdyrregisteret per 31.12.03). The majority of samples from fallen stock was collected on the farms.

The difference between the examined number and the number of fallen stock may partly be 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 about their duty to report all cases of fallen stock older than 24 months to the NFSA.

The number of samples examined in each region is compared to the expected number of samples (estimated according to the total number of fallen stock older than 24 months and the cattle population in the regions). In most regions the number of animals sampled was low compared to the expected number to be sampled (Figure 1). In contrast, in the region Buskerud, Vestfold and Telemark, a region with a small cattle population, the number sampled and the expected number corresponded well. In the region Rogaland and Agder, a region with a large cattle population, there was a minor difference between the numbers sampled and the expected number. In this region a proportion of the samples are collected at a rendering plant, which makes the collecting of samples less cumbersome.

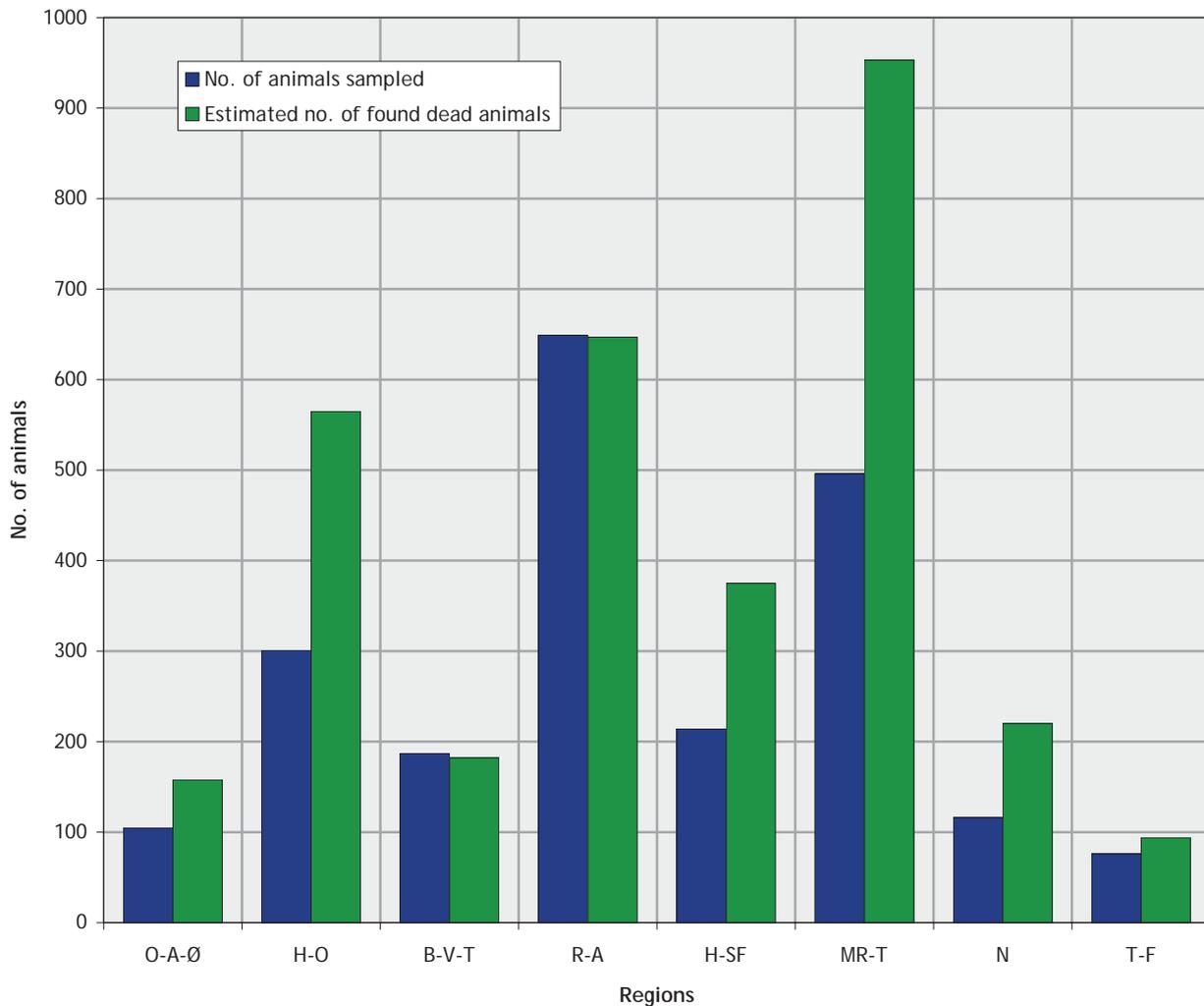


Figure 1. Number of fallen stock (with reported identity) sampled in each surveillance region in 2004, compared with what would be expected assuming 0.77% fallen stock of the respective regional cattle populations older than 24 months: Husdyrregistret per 31.12.04).

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 = Troms and Finnmark.

Norwegian cows are slaughtered at a low age, mean age is approximately 50 months for dairy cows and 68 months for suckling cows (suckling cows constitute only 13% of the cattle population older than 24 months) (National Production Recording Scheme 2000, Norwegian Beef Herd recording System 1999).

The low age at culling implies that 39.3% of the samples from dairy cattle and 34.3% 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 Figure 2.

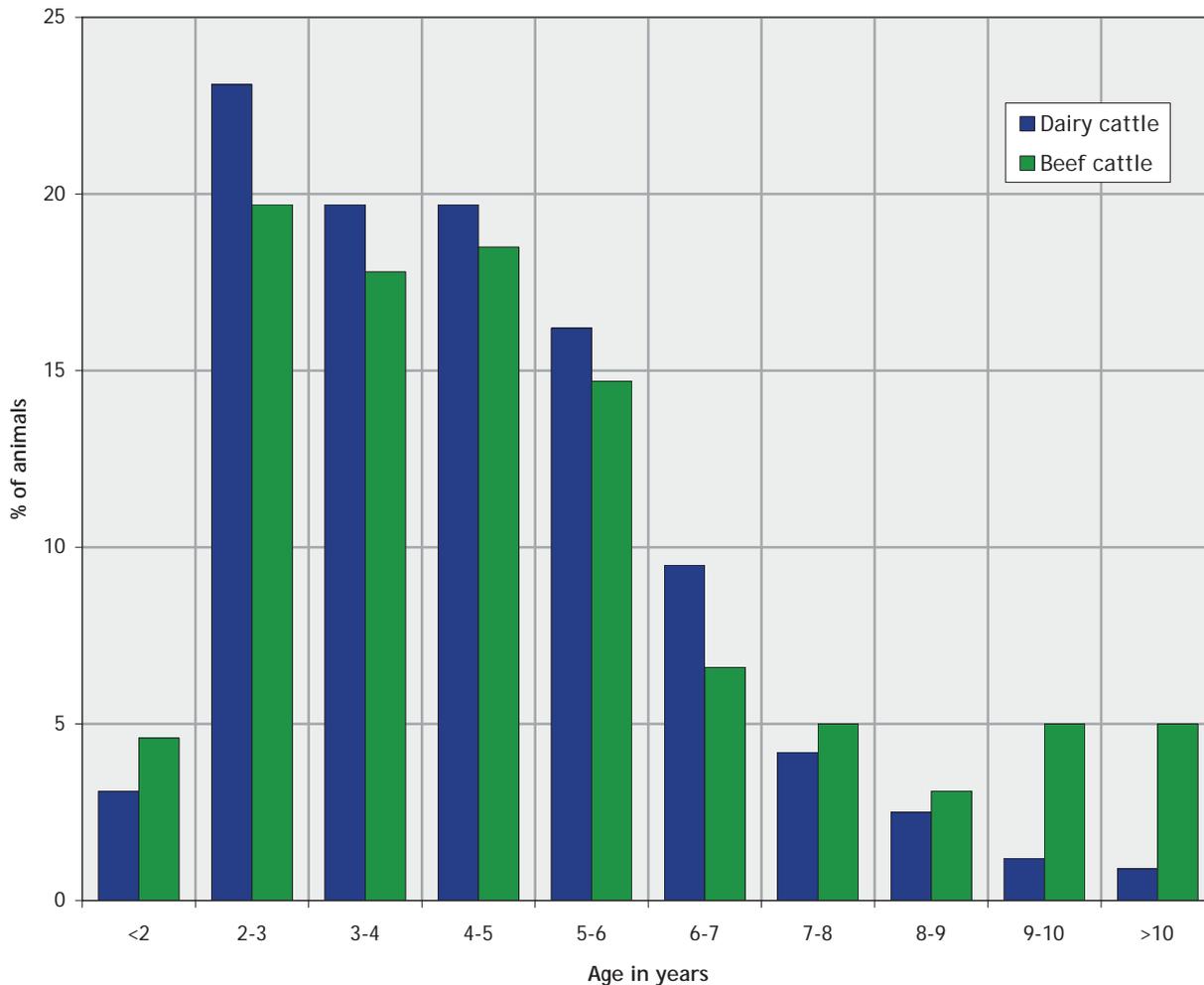


Figure 2. Age distribution of dairy cattle (n=1795) and beef cattle (n=259) sampled as fallen stock in 2004 (only animals with age information and assigned production type are included).

## Conclusion

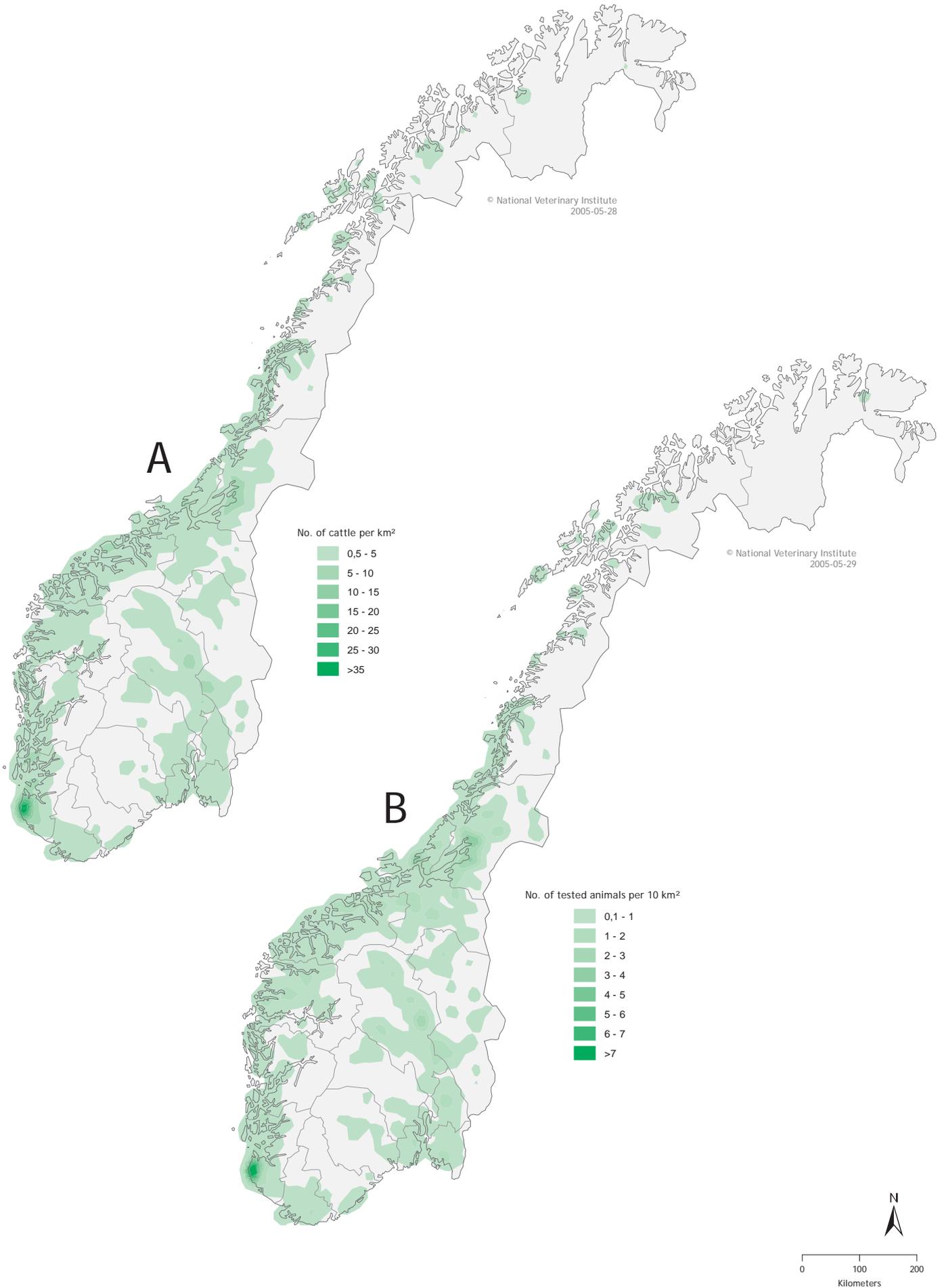
In the BSE-monitoring programme in EU 2003 only 7 (0.51%) of 1,364 verified cases of BSE were younger than 48 months, and 0.10 positive cases were detected per 10,000 tests in cattle 36-48 months, in contrast to 2.93 in cattle 73-84 months (3). These results indicate that BSE-monitoring of animals younger than 48 months is of low value.

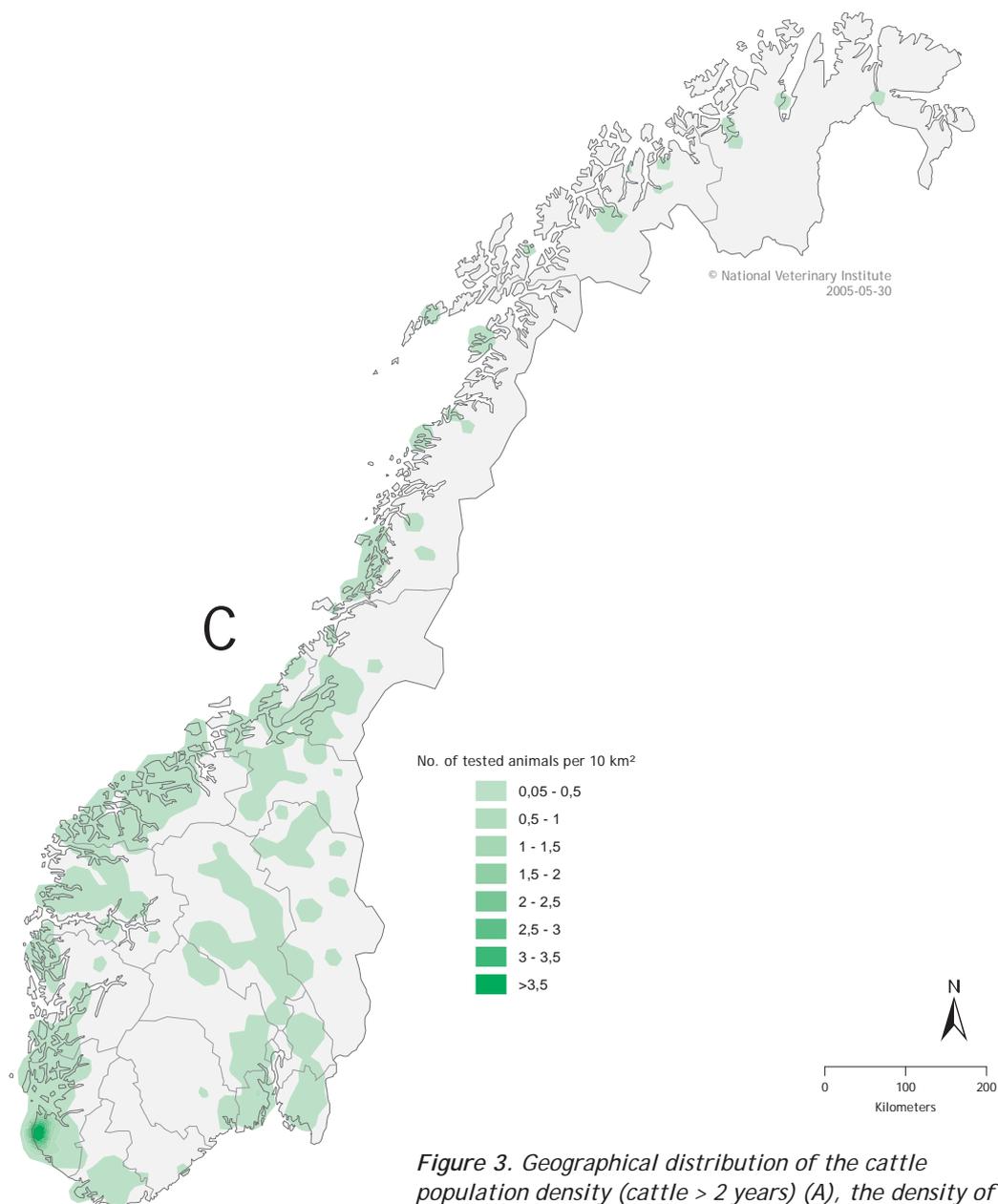
The geographical distribution of the cattle population and the animals tested are presented in Figure 3. The figure indicates that there is a variation in the following up of the BSE-surveillance programme.

The "Surveillance and control programme for bovine spongiform encephalopathy (BSE) in Norway, Report 2001" (4) suggested that the Norwegian cattle population has not been infected by BSE 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 2001, 2002 (5), 2003 and 2004 with more than 80,000 negative samples, strengthen the assumption that Norwegian cattle are not infected with the BSE-agent.

## References

1. Anonymous, 1996. Scrapie I. In: Manual of standards for diagnostic tests and vaccines. 3<sup>rd</sup> ed. Office International des Epizooties, Paris, pp. 673-677.
2. Anonymous, 2000. Scrapie I. In: Manual of standards for diagnostic tests and vaccines. List A and list B diseases for mammals, birds and bees. 4<sup>th</sup> ed. Office International des Epizooties, Paris, pp. 873-880.
3. Anonymous, 2004. Report on the monitoring and testing of ruminants for the presence of transmissible spongiform encephalopathy (TSE) in 2003, including the results of the survey of prion protein genotypes in sheep breeds. European Commission, Health & Consumer Protection Directorate-General, Brussel, Belgium.
4. Mørk, T., Bratberg, B., Hopp, P., Benestad, S.L., Høgåsen, H., Bruheim, T., 2002. The surveillance and control programme for bovine spongiform encephalopathy (BSE) in Norway. In: Fredriksen, B., Mørk, T. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001. National Veterinary Institute, Oslo, Norway, pp. 55-66.
5. Valheim, M., Bratberg, B., Høgåsen, H., Moldal, T., 2003. The surveillance and control programme for bovine spongiform encephalopathy (BSE) in Norway. In: Heier, B.T. (Ed.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002. National Veterinary Institute, Oslo, Norway, pp. 49-56.
6. Valheim, M., Bratberg, B., Bruheim, T., Høgåsen, H., Moldal, T., 2004. The surveillance and control programme for bovine spongiform encephalopathy (BSE) in Norway. In: Mørk, T., Hellberg, H. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003. National Veterinary Institute, Oslo, Norway, pp. 43-50.





*Figure 3. Geographical distribution of the cattle population density (cattle > 2 years) (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 2004.*



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



Annual report 2004

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## Introduction

In the early 1960s, two outbreaks of infectious pustular vulvovaginitis were diagnosed in cattle in Norway. Since then, no cases of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) were reported 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. Virus isolation attempts from organ samples gave negative results. Sixteen contact herds and all dairy herds in the same region were serologically negative (1, 4, 5, 6). Likewise 40 red deer, which were shot in the neighbourhood during the hunting season the same year, were also serologically negative (unpublished). IBR/IPV virus infection has not been demonstrated since then in Norway. All breeding bull candidates are tested serologically in quarantine before entering the breeding centres. All breeding bulls are subject to a compulsory test each year.

The Norwegian Food Safety Authority is responsible for carrying out the IBR/IPV surveillance and control programme. 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 official inspectors from the Food Safety Authority.

## Aim

EFTA Surveillance Authority (ESA) has recognised Norway as free from IBR since 1994. Decisions concerning the additional guarantees relating to IBR for bovines destined to Norway are described in ESA Decision 74/94/COL, amending ESA Decision 20/94/COL.

The ESA Decisions accepting the IBR-free status of Norway include requirements on annual reports of the surveillance of the disease.

## Material and methods

The surveillance of cattle 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 were made, consisted of all herds of cattle delivering milk to the dairies in the sampling period. In 2004, bulk milk samples from 1,573 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 2003. A total of 3,364 individual blood samples from 402 beef herds were analysed in pools with a maximum of 20 samples in each. The sampled herds represented approximately 9.7% of the Norwegian cattle herds.

The number of herds in the monitoring programme for IBR/IPV in 2004 is given in Table 1. The geographic distribution of the total number and the tested number of dairy and beef herds are shown in Figures 1 and 2.

All 1,573 bulk milk samples and 3,364 blood samples were tested for antibodies against bovine herpes virus 1 (BHV-1) using a blocking-ELISA (2) at the National Veterinary Institute, Oslo.

Table 1. Total number of dairy herds and beef herds within the frame of the Norwegian monitoring programme for IBR/IPV in 2004

Herd category	Total no. of cattle herds*	No. of herds tested	% tested of the total no. of herds
Dairy herds	16,611	1,573	9.5
Beef herds	3,793	402	10.6
<b>Total</b>	<b>20,404</b>	<b>1,975</b>	<b>9.7</b>

\* Based on data from the Register of production subsidies as of 31 July 2003.

## Results

All the samples tested for antibodies against BHV-1 in 2004 were negative. Table 2 shows the results of the testing during the period from 1993 to 2004.

Table 2. Antibodies against IBR/IPV virus in the Norwegian bovine population during the period 1993-2004

Year	Dairy herds	Beef herds		No. of positive samples
	No. of bulk milk samples tested	No. of beef herds sampled	No. of individuals tested	
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

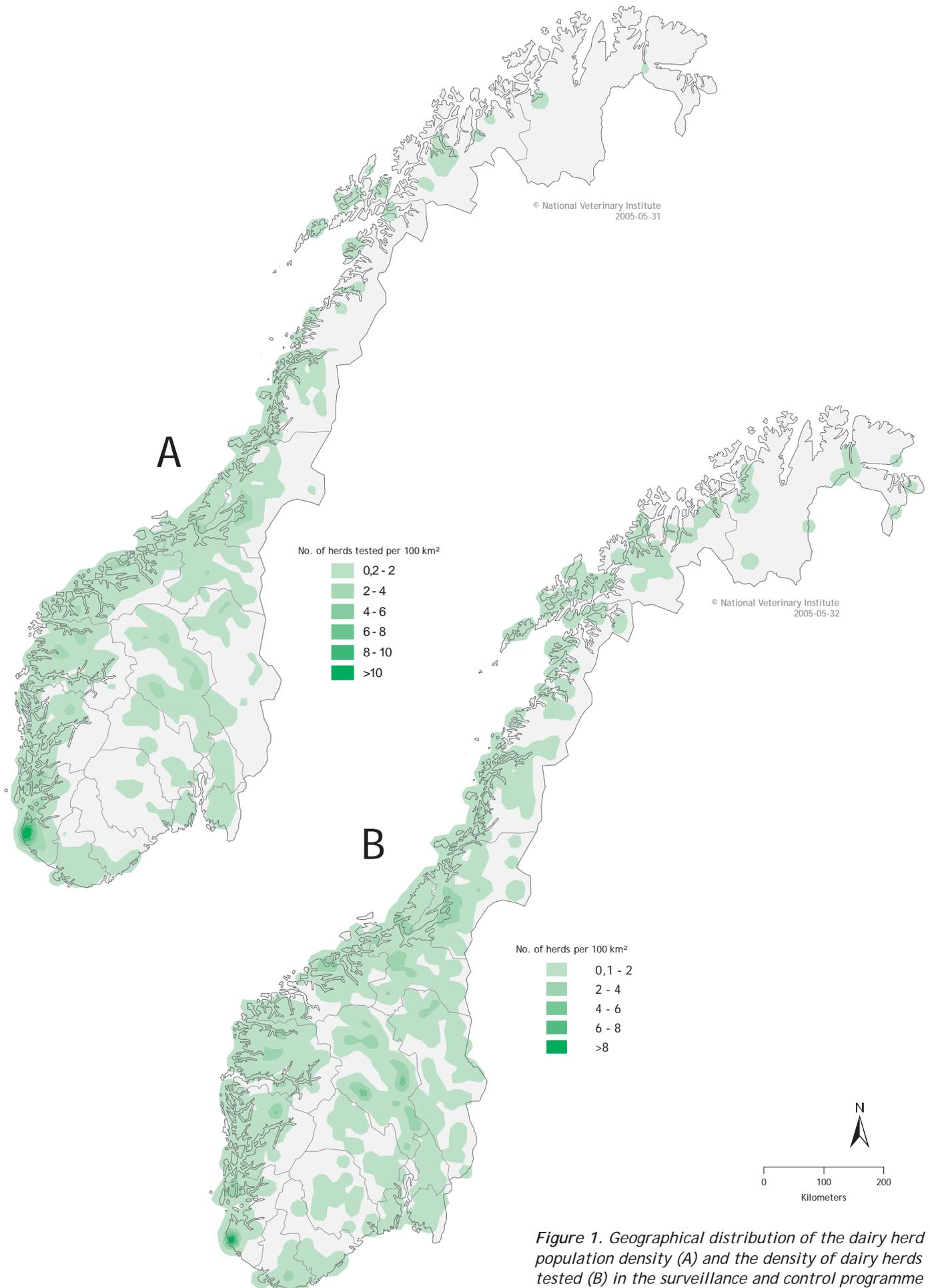
## Discussion

Norway has had 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 has been evaluated using Monte Carlo simulation models (3). The Danish ELISA-test is calculated to have a sensitivity of 82.9% when used for bulk milk testing in Denmark (2), but the sensitivity improves when the same test is used in Norway because of the smaller herds. The number of milking cows in an average Norwegian herd is 17, compared to 85 in Denmark. The sensitivity is even better when testing serum samples and Norwegian investigations have shown that the test has a specificity of 100% (3).

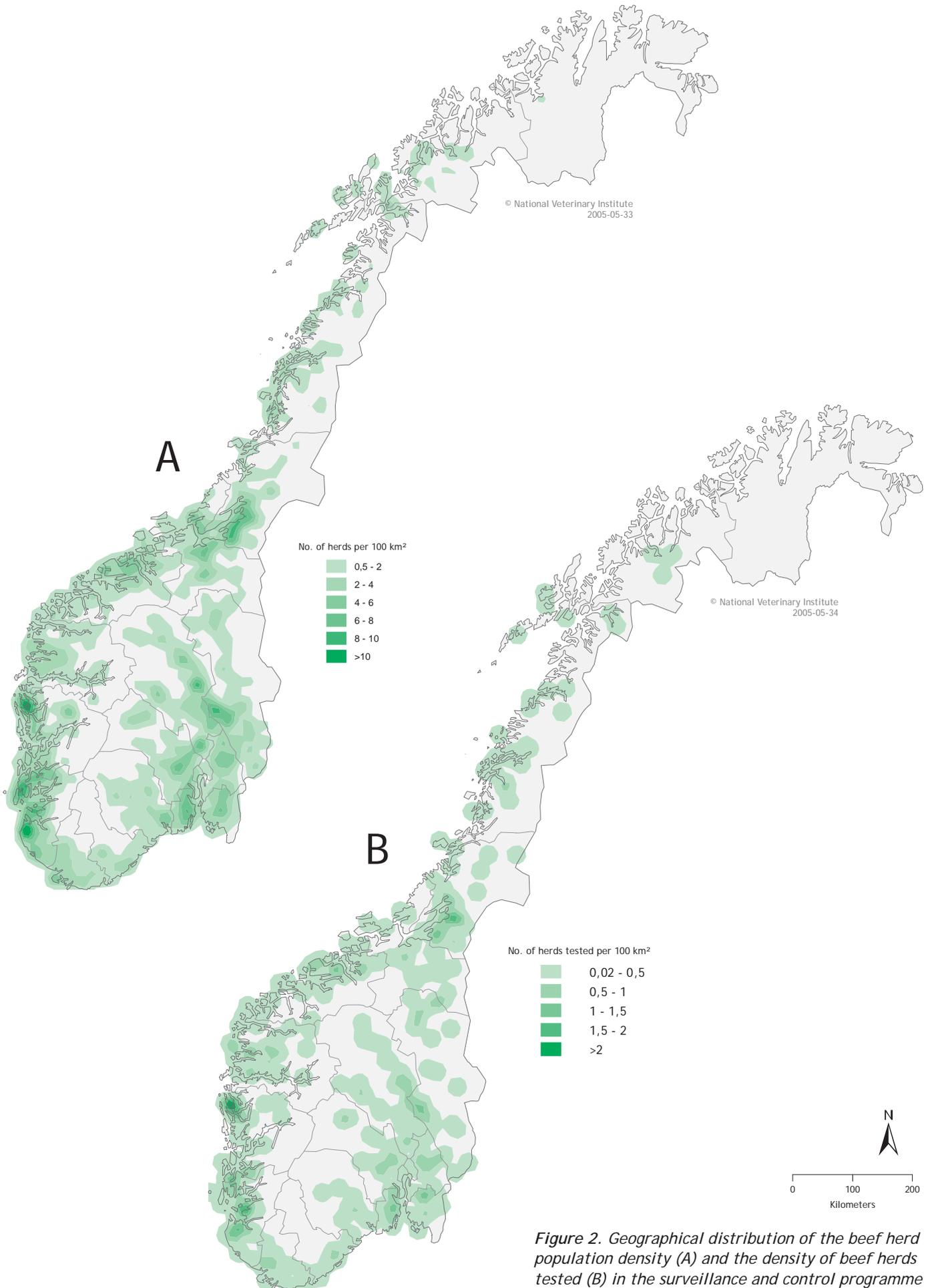
The results of the continuous testing since 1992/93 strongly indicate that the Norwegian cattle population is free from IBR/IPV-infection, and that the programme, combined with the additional guarantees and the testing procedures for imported cattle, are adequate means to discover new introduction of infection.

## References

1. Tharaldsen, J., Krogsrud, J., Ødegaard, Ø. 1993. Påvist besetningsinfeksjon med bovint herpesvirus 1 (BHV-1). *Nor. Vet. Tidsskr.* 105, 363-364.
2. Nylin, B., Strøger, U., Rønsholt, L., 2000. A retrospective evaluation of a bovine herpes virus-1 (BHV-1) antibody ELISA on bulk-tank milk samples for classification of the BHV-1 status of Danish dairy herds. *Prev. Vet. Med.* 47, 91-105.
3. Paisley, L.G., Tharaldsen, J., Jarp, J., 2001. A retrospective analysis of the infectious rhinotracheitis (bovine herpes virus-1) surveillance program using Monte Carlo simulation models. *Prev. Vet. Med.* 50, 109-125.
4. Nyberg, O., Jarp, J., Tharaldsen, J., 2002. The surveillance and control programme for infectious bovine rhinotracheitis (IBR)/infectious pustular vulvovaginitis (IPV) in Norway. In: Fredriksen, B., Mørk, T. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001.* National Veterinary Institute, Oslo, Norway, pp. 67-73.
5. Nyberg, O., Tharaldsen, J., 2003. The surveillance and control programme for infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) in Norway. In: Heier, B.T. (Ed.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002.* National Veterinary Institute, Oslo, Norway, pp. 57-63.
6. Nyberg, O., Tharaldsen, J., Heier, B.T., 2004. The surveillance and control programme for infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) in Norway. In: Mørk, T., Hellberg, H. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003.* National Veterinary Institute, Oslo, Norway, pp. 51-56.



*Figure 1. Geographical distribution of the dairy herd population density (A) and the density of dairy herds tested (B) in the surveillance and control programme for IBR/IPV in 2004.*



*Figure 2. Geographical distribution of the beef herd population density (A) and the density of beef herds tested (B) in the surveillance and control programme for IBR/IPV in 2004.*

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



Annual report 2004

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## Introduction

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

The Norwegian Food Safety Authority is responsible for carrying out the EBL surveillance and control programme. 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.

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 adult animals were positive.

## Aims

The intention of the EBL surveillance and control programme is to document freedom from this infection in Norway. Further, the intention is to apply for EBL free status according to the EEC-agreement (Council Directive 64/432/EEC of 26.06.64 as amended by Council Directives 97/12 of 17.03.97 and 98/46 of 24.06.98).

## Materials and methods

The surveillance 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 2004, bulk milk samples from 1,573 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 2003. A total of 3,364 individual blood samples from 402 beef herds were analysed in pools, with a maximum of 20 samples in each. The sampled herds represented approximately 9.7% of the Norwegian cattle herds.

The number of herds in the monitoring programme for EBL in 2004 is given in 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 from a maximum of 20 samples) were examined by an indirect ELISA (SVANOVA<sup>®</sup>) (3). For verification and for follow up of suspect cases, LACTELISA BLV Ab and SERELISA BLV Ab from SYNBIOTICS were used.

Table 1. Total number of dairy herds and beef herds within the frame of the Norwegian monitoring programme for EBL in 2004

Herd category	Total no. of cattle herds*	No. of herds tested	% tested of the total no. of herds
Dairy herds	16,611	1,573	9.5
Beef herds	3,793	402	10.6
<b>Total</b>	<b>20,404</b>	<b>1,975</b>	<b>9.7</b>

\* Based on data from the Register of production subsidies as of 31 July 2003.

## Results

A historic survey of the surveillance of BLV-antibodies 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.

Bulk milk samples from 1,573 dairy herds and 3,364 individual blood samples from 402 beef herds were tested for antibodies against BLV in 2004 (Table 1). All bulk milk samples and blood samples tested for antibodies against EBLV in 2004 were negative.

Table 2. Antibodies against BLV in the Norwegian bovine population during the period 1995-2004

Year	Dairy herds	Beef herds		No. of positive samples
	No. of bulk milk samples analysed	No. of beef herds sampled	No. of individuals analysed	
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

## Discussion

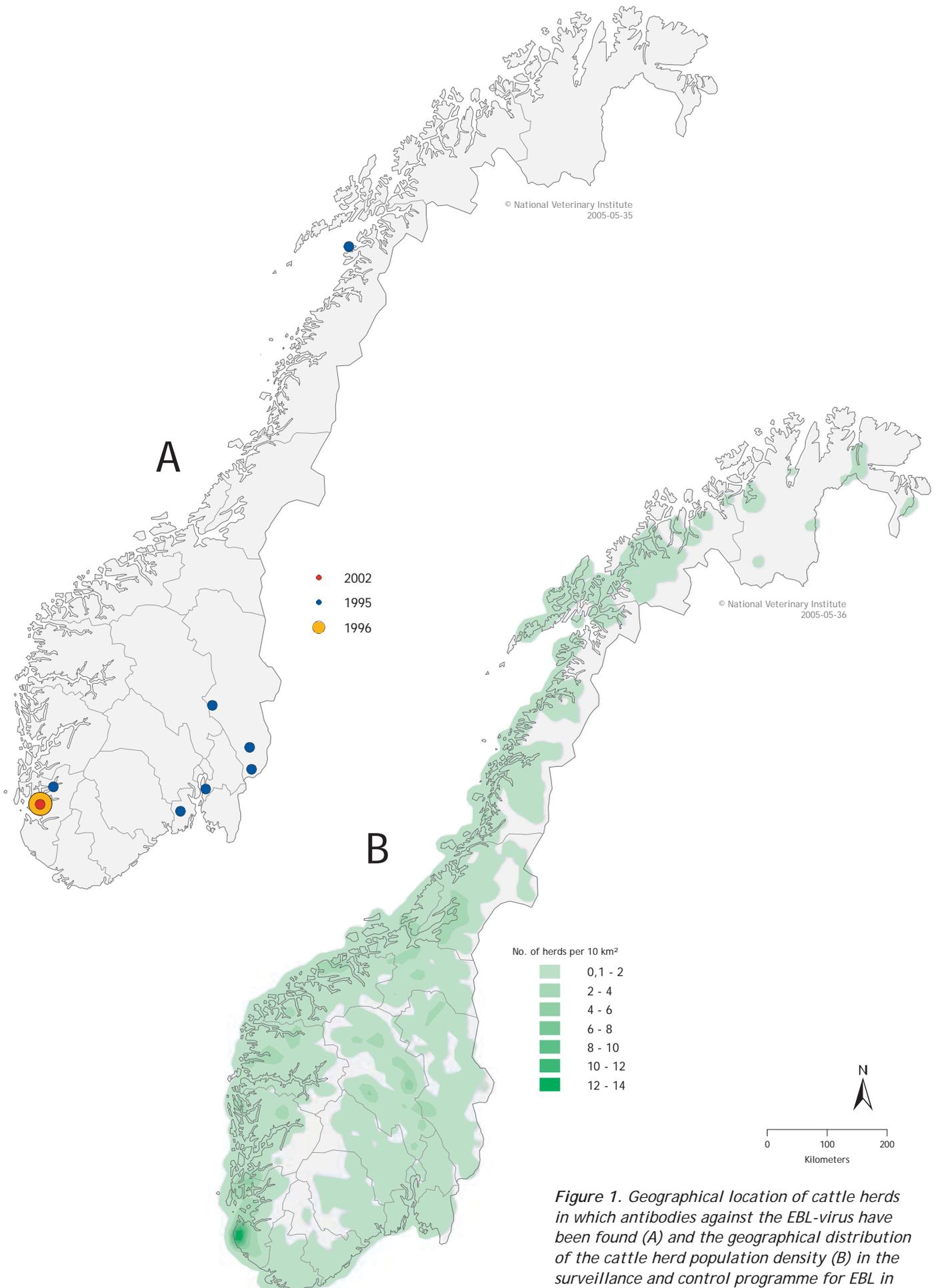
The requirement from the EU for granting an EBL free-status is that the prevalence must be lower than 0.2%, which represents 41 herds out of a total number of 20,404 herds. EBL had never been reported until the surveillance and control programme detected nine positive herds in 1995-96. These herds are now free of EBL, and no new herds tested positive during the period 1997-2001. From year 2000, only 10% of the herds are examined annually.

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 was healthy and had no clinical symptoms, she was slaughtered, and the pathological investigations gave no indication of leukosis. Further testing of individual blood samples of all cattle older than 24 months in the herd and six contact herds was negative. The conclusion is 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 (5, 6).

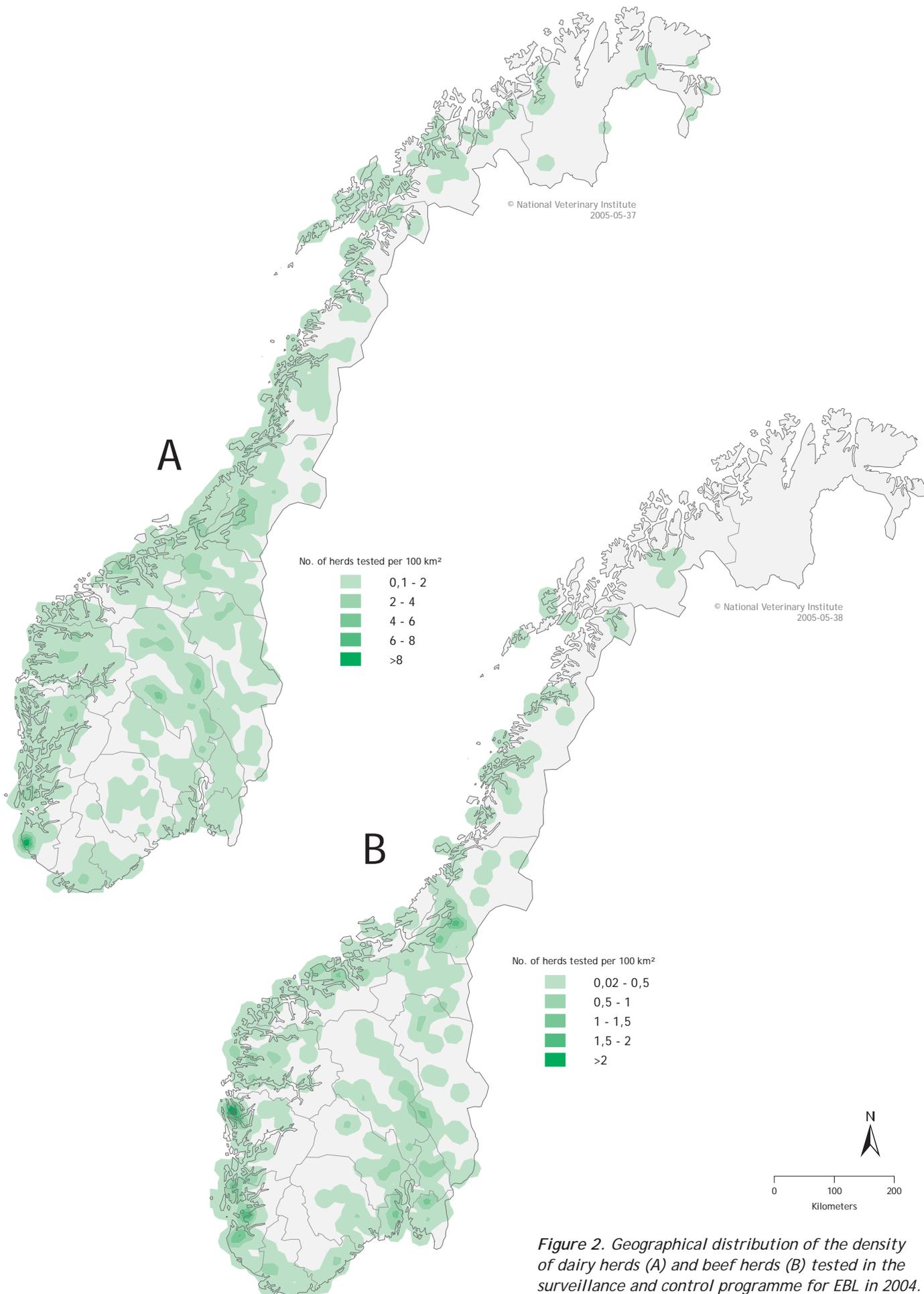
The results of the continuous surveillance since 1995 indicate that the Norwegian cattle population is free from EBL according to the EU requirements. Together with the possible isolation period of six months and the testing protocol for imported animals, this programme should be sufficient to discover introduction of new infection.

## References

- Norberg, H.S., 1978. Bovin leukose. Oversikt og redegjørelse for norske undersøkelser. (Bovine leukosis. Review of Norwegian investigations). *Nor. Vet. Tidsskr.* 90, 533-541.
- Tharaldsen, J., Ødegaard, Ø., Krogsrud, J., 1996. Smittsom storfeleukose diagnostisert i Norge. (Contagious bovine leukosis diagnosed in Norway). *Nor. Vet. Tidsskr.* 108, 550.
- Klintevall, K., Näslund, K., Svedlund, G., Hajdu, L., Linde, N., Klingeborn, B., 1991. Evaluation of an indirect ELISA for the detection of antibodies to bovine leukemia virus in milk and serum. *J. Virol. Methods.* 33, 319-333.
- Nyberg, O., Jarp, J., Tharaldsen, J., 2002. The surveillance and control programme for enzootic bovine leukosis (EBL) in Norway. In: Fredriksen, B., Mørk, T. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001.* National Veterinary Institute, Oslo, Norway, pp. 75-81.
- Nyberg, O., Tharaldsen, J., 2003. The surveillance and control programme for enzootic bovine leukosis (EBL) in Norway. In: Heier, B.T. (Ed.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002.* National Veterinary Institute, Oslo, Norway, pp. 65-71.
- Nyberg, O., Tharaldsen, J., Heier, B.T., 2004. The surveillance and control programme for enzootic bovine leukosis (EBL) in Norway. In: Mørk, T., Hellberg, H. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003.* National Veterinary Institute, Oslo, Norway, pp. 57-62.



*Figure 1. Geographical location of cattle herds in which antibodies against the EBL-virus have been found (A) and the geographical distribution of the cattle herd population density (B) in the surveillance and control programme for EBL in 2004.*



*Figure 2. Geographical distribution of the density of dairy herds (A) and beef herds (B) tested in the surveillance and control programme for EBL in 2004.*

# The surveillance and control programme for bovine brucellosis in Norway



Annual report 2004

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## Introduction

Eradication of bovine brucellosis in Norway was achieved in 1950 (2). Since 1994, EFTA Surveillance Authority (ESA) has recognised Norway as an "officially brucellosis free state" as described in ESA Decision 66/94/COL. In 2000, the Norwegian Animal Health Authority (from 2004: the Norwegian Food Safety Authority) launched a surveillance and control programme on bovine brucellosis 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 and 2003 (1, 2, 4). 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 probably were false positive reactions, most likely because of cross reactions (3).

## Aim

The purpose of the programme is to document freedom from bovine brucellosis according to demands in Directive 64/432/EEC with amendments and contribute to the maintenance of this favourable situation. The Food Safety Authority has implemented the programme while the National Veterinary Institute is responsible for planning, laboratory analyses and reporting.

## Material and methods

### Active surveillance

#### *Sampling of herds*

Dairy herds are selected from the total number of herds delivering milk to dairies during the sampling period, while beef herds are selected from all beef herds receiving subsidies the year before (2003). During 2004, 18.9% of the dairy herds and 21.4% of the beef herds were sampled (Table 2). The geographic distribution is given in Figure 1 and Figure 2.

Bulk-milk samples from the dairy herds are collected at the dairies, while individual blood samples are collected from all cattle older than 24 months in beef herds.

Table 1. Number of bulk milk samples, blood samples and foetuses examined for brucellosis in the Norwegian cattle population in 2001, 2002 and 2003

Year	Material	Dairy cattle		Beef cattle		Total	
		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

Table 2. Total number of dairy herds and beef herds within the Norwegian monitoring programme for bovine brucellosis in 2004

Herd category	Total no. of herds*	No. of herds tested	% tested of the total no. of herds
Dairy herds	16,611	3,138	18.9
Beef herds	3,793	813	21.4
<b>Total</b>	<b>20,404</b>	<b>3,951</b>	<b>19.4</b>

\* Based on data from the Register of production subsidies as of 31 July 2003.

### 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.

Foetuses, foetal membranes and blood samples are collected by the official inspectors from the Food Safety Authority and submitted to the National Veterinary Institute, Oslo.

### Serology

All bulk milk samples and individual blood samples are tested for antibodies against *Brucella abortus* in an indirect ELISA (Svanova®). In the bulk milk testing, the volume of milk is 100 µl per well, and in the blood sample testing, 4 µl per well. The initial screening is performed using a single well per sample and doubtful or positive reactions were 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®).

### 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 formalin. The specimens are processed according to a standard routine protocol, sectioned at 5 µm and stained with haematoxylin and eosin (HE).

### 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 (MZN) staining. Samples are cultured on bovine blood agar containing 5% bovine blood, Skirrows medium and Tryptone Soy Agar (TSA) at 37°C in a 10% CO<sub>2</sub> 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.

## Results

A total of 3,138 bulk-milk samples from 3,138 dairy herds, 7,986 blood samples from 813 beef herds and individual blood samples related to abortions were analysed (Table 3).

All bulk milk samples and blood samples tested for antibodies against *Brucella abortus* in 2004 were negative.

Post mortem investigations on foetuses in 2004 did not reveal pathological changes indicative of brucellosis. All bacteriological investigations on *Brucella abortus* were negative in 2004.

## Discussion

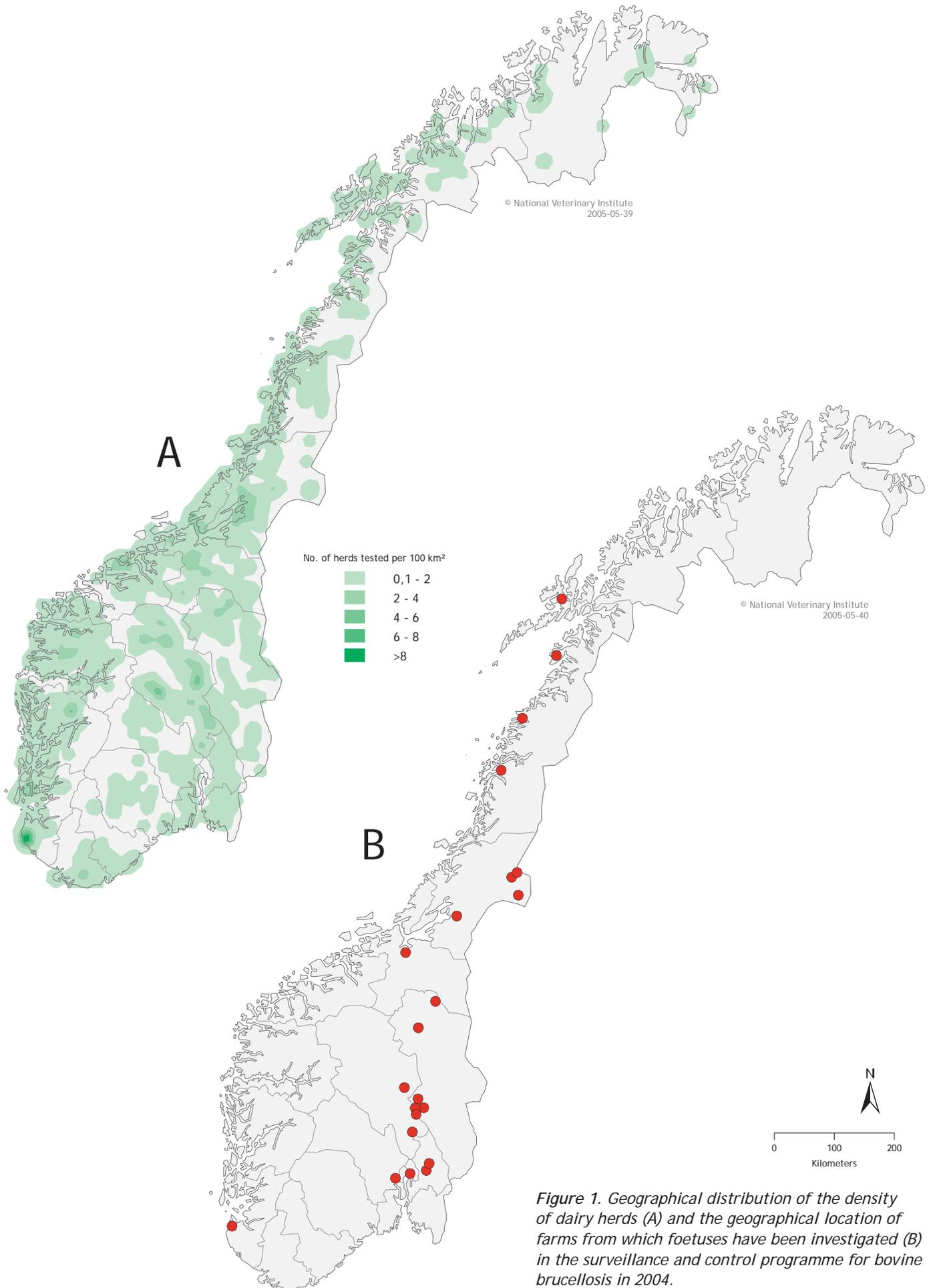
There was no detection of bovine brucellosis in 2004. With the exception of a single relapse in 1953, bovine brucellosis has not been detected in Norway since 1950 (1, 2, 3, 4).

Table 3. Number of bulk milk samples, blood samples and foetuses tested for bovine brucellosis in dairy herds and beef herds in 2004

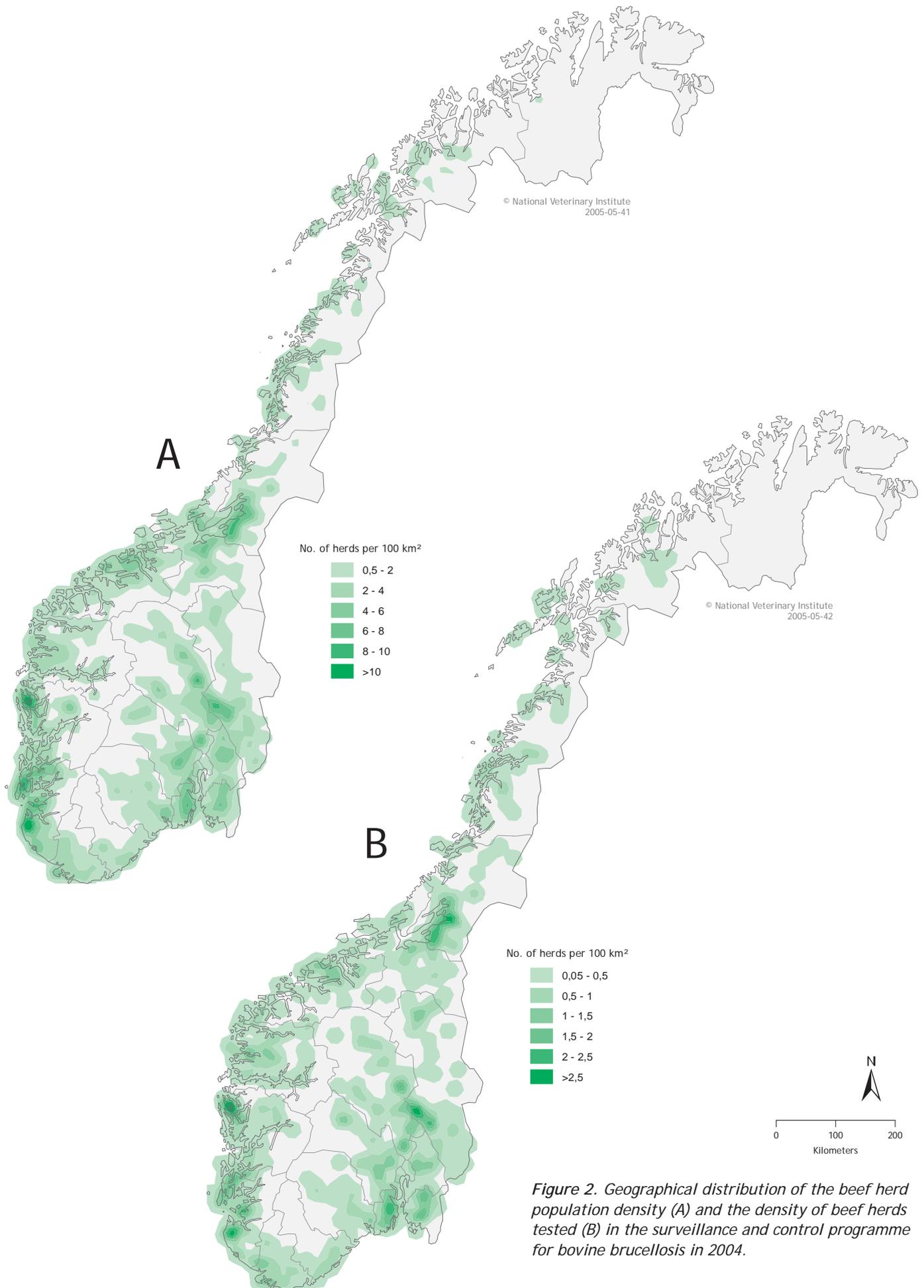
Material	Dairy cattle		Beef cattle		Total	
	Samples	Herds	Samples	Herds	Samples	Herds
Bulk-milk/blood	3,138	3,138	7,986	813	11,124	3,951
Foetuses	25	21	2	2	27	23
Blood samples related to abortions	28	19	2	2	30	21

## References

1. Mørk, T., Tharaldsen, J., Bratberg, B., Holstad, G., Jarp, J., 2001. Overvåkings- og kontrollprogrammet for brucellose i Norge 2000: Serologiske, Patoanatomiske og Bakteriologiske undersøkelser. In: Fredriksen, B., Mørk, T. (Eds.), Årsrapport 2000. Overvåkings- og kontrollprogram innen fiske- og dyrehelse. Veterinærinstituttet, Oslo, Norge, pp. 66-72.
2. Mørk, T., Tharaldsen, J., Bratberg, B., Holstad, G., Jarp, J., 2002. The surveillance and control programme for bovine brucellosis in Norway. In: Fredriksen, B., Mørk, T. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001. National Veterinary Institute, Oslo, Norway, pp. 75-81.
3. Nyberg, O., Tharaldsen, J., 2003. The surveillance and control programmes for bovine brucellosis in Norway. In: Heier, B.T. (Ed.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002. National Veterinary Institute, Oslo, Norway pp. 73-79.
4. Nyberg, O., Tharaldsen, J., Heier, B.T., 2004. The surveillance and control programme for bovine brucellosis in Norway. In: Mørk, T., Hellberg, H. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003. National Veterinary Institute, Oslo, Norway pp. 63-67.



*Figure 1. Geographical distribution of the density of dairy herds (A) and the geographical location of farms from which foetuses have been investigated (B) in the surveillance and control programme for bovine brucellosis in 2004.*



*Figure 2. Geographical distribution of the beef herd population density (A) and the density of beef herds tested (B) in the surveillance and control programme for bovine brucellosis in 2004.*



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



Annual report 2004

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## Introduction

Bovine virus diarrhoea (BVD) is a notifiable disease in Norway. From 1984 to 1986, preliminary investigations indicated that nearly 30% of the dairy herds contained animals with antibodies to BVDV (1). The high prevalence and cost of the disease made a surveillance and control programme urgent, and this was started in December 1992. The Animal Health Authority (from 2004: the Norwegian Food Safety Authority) was in charge of the programme and responsible for blood sampling and imposing control measures in positive herds. The National Veterinary Institute performed the laboratory analyses (2, 3, 4). The government and the industry financed the programme.

During the programme period, the number of restricted herds has decreased from 2,950 in 1994 to three in 2004 (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 from the beginning 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 step three 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 are tested more often in many herds. Tables 1-3 show the results of the tested herds in the programme during the period 1993-2003.

Table 1. Distribution of Norwegian dairy herds in relation to BVDV antibody level in bulk milk during the period 1993-2003

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

Table 2. Herds positive for antibodies against BVDV in pooled milk 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

\* Presentation of results from 1997 is omitted because data disappeared in the process of change of computer system in 1998.

Table 3. Pooled serum samples from young stock positive for antibodies against BVDV during the period 1993-2003

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

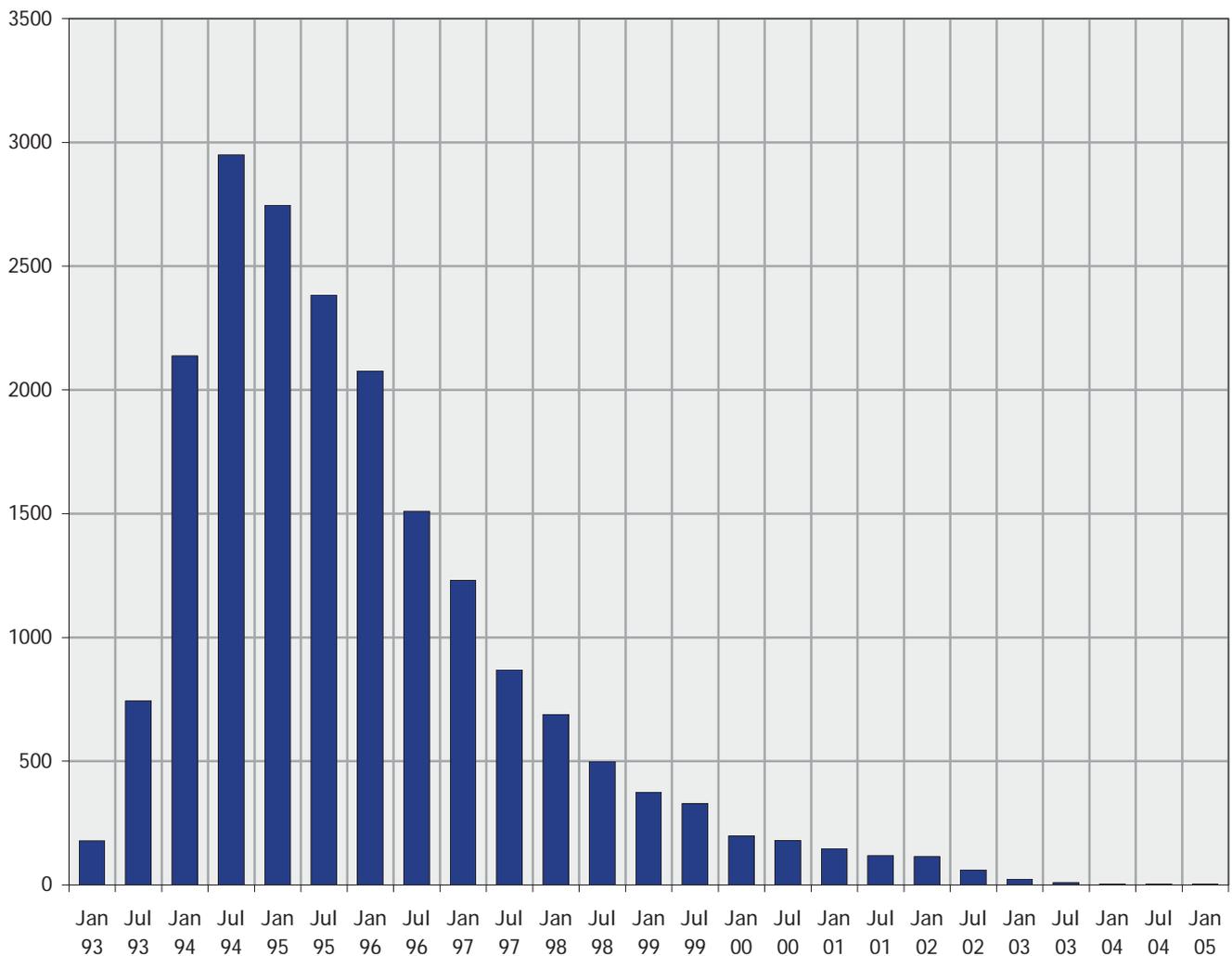


Figure 1. Number of herds with imposed restrictions because of BVDV infection during the period 1993-2004.

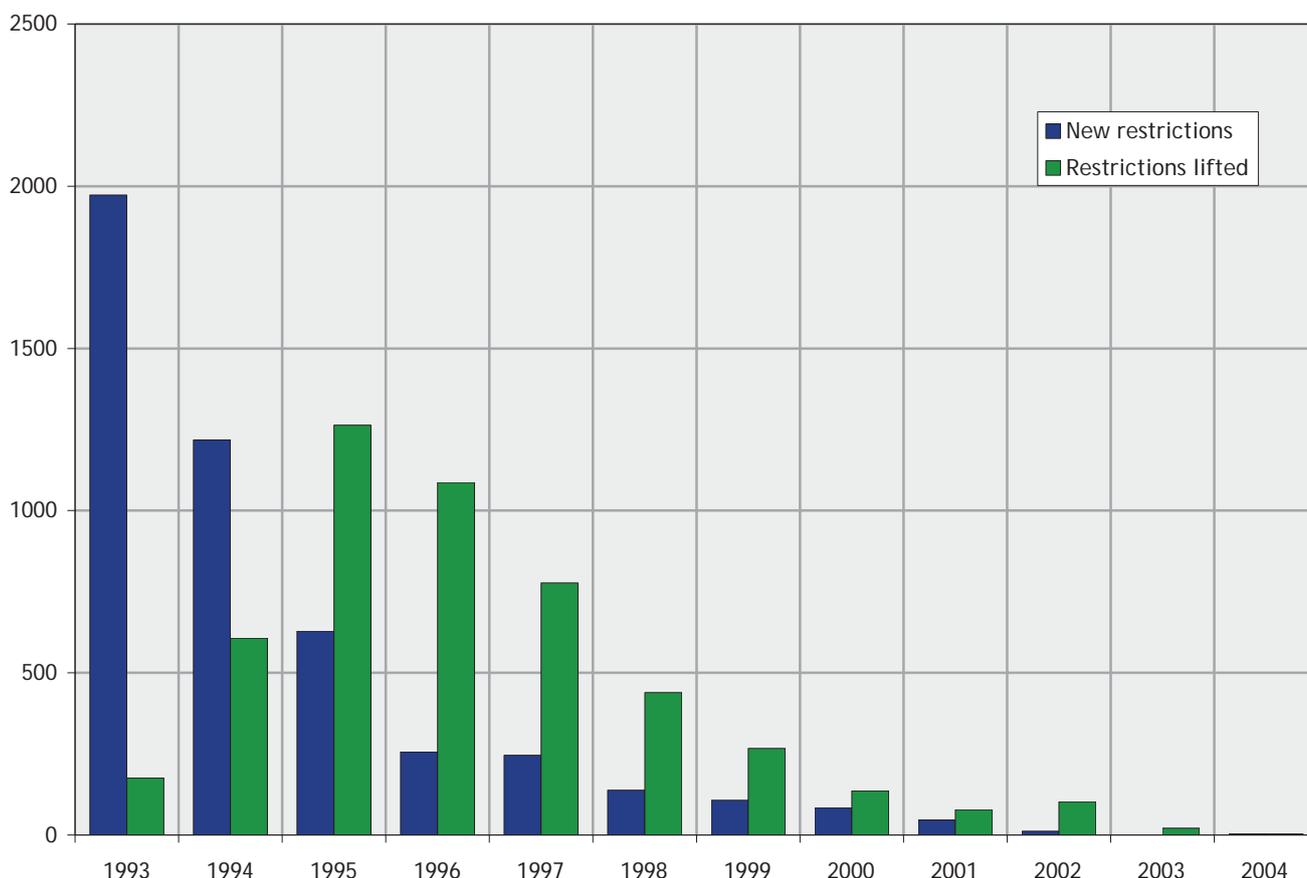


Figure 2. Number of new herds with restrictions imposed/restrictions lifted per year because of BVDV infection during the period 1993-2004.

## Aim

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

## Material and methods

An indirect ELISA test (SVANOVIR<sup>®</sup>, Svanova Biotech AB, Uppsala, Sweden) was used for measuring antibodies against BVDV in milk and blood (5). An antigen-capture ELISA test (Moredun Animal Health, Edinburgh, Scotland) was used for the detection of BVD virus up to 2003 (6, 7). After the need of virus tests declined to less than 1,000 a year, 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) (8).

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 1 January 1998 the cut off value between class 1 and 2 was set at S/P ratio=0.250. The border value was reduced to be able to discover newly infected herds at an early stage.

The group of dairy herds sampled in 2004 did not, for the first time, include all dairy herds in Norway. From the special zones implemented in 2001 (see discussion), all dairy herds were sampled twice. From the rest of the country, 25% of the herds were sampled. Totally 44% of the dairy herds were sampled.

Pooled milk samples from primiparous cows were not collected in 2004 (Table 2).

Table 5. Norwegian dairy herds classified according to BVDV antibody level in bulk milk in 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<0.15)	% of herds in class 2 (0.15≤S/P ratio<0.55)	% of herds in class 3 (S/P ratio≥0.55)
2004	7,365	95.8	2.8	1.3	0.1

Table 6. Antibodies against BVDV in pooled serum samples from young stock in 2004

Year	No. of herds examined	% AB positive samples
2004	1,351	1.4

Table 7. Examination of individual blood samples for BVDV antigen during the period 1998-2004

Year	No. of individual samples examined	No. of herds examined	Virus positive samples		Virus positive herds	
			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

Pooled serum samples from 1,351 different dairy (10%) and beef cattle herds (90%) were examined in 2004 (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 a herd could be persistently infected, therefore, restrictions are imposed on the farm. Identification of such animals must be done by testing blood samples from every individual in the herd for antibodies, and the presence of virus in antibody negative individuals. In 2004, a total of 1,017 animals from 84 herds were investigated.

In 2001, nearly all beef herds having 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 in beef herds and led to a reduced testing in such herds. In 2002, only 20% of the beef herds were tested in a few counties, which for more than one year had been free of herds with restrictions.

The number of counties with this reduced testing scheme in 2004 increased to 16 of a total of 18 counties.

## Results

A total of 7,365 dairy herds were tested for antibodies against BVDV in 2004, and nearly 96% of these were negative regarding antibodies against BVDV (Table 5).

Of a total of 1,382 pooled serum samples from 1,351 different dairy and beef cattle herds, 1.4% was antibody positive (Table 6).

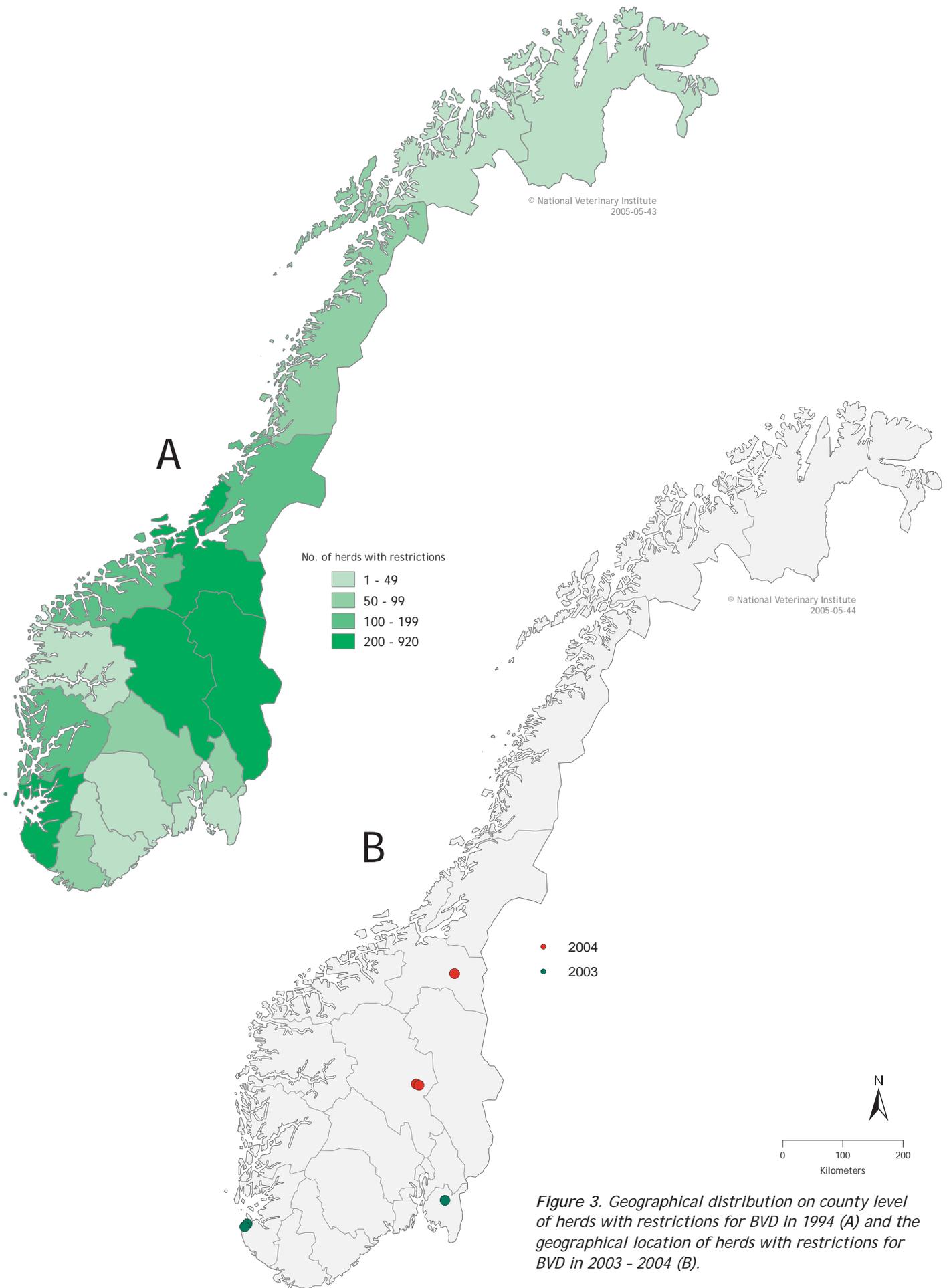
BVDV was found in 0.6% of the individual blood samples tested (Table 7).

## Discussion

Special zones were established in 2001 in areas with many remaining BVDV infected herds. In these zones, specific testing schemes were followed before animals could be sold or allowed access to common pastures. For two and a half years, from the beginning of 2001, a person was engaged specifically for more intensive follow up of infected herds. In addition, information to veterinarians, other advisors and farmers about the disease and how to act to avoid re-infection was enforced (9). 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 (10). The mopping-up in a few herds is still going on, three herds had restrictions at the end of 2003, and three other herds had restrictions at the end of 2004 (Figure 3). Totally four new herds got restrictions because of suspected BVDV infection in 2004 (Figure 2). So far, active infection was found only in one of the herds, and could be traced back to earlier infection in the neighbourhood and confusion with two heifers bearing ear tags with the same number in the same herd.

## References

1. Løken, T., Krogsrud, J., Larsen, I.L., 1991. Pestivirus infections in Norway. Serological investigations in cattle, sheep and pigs. *Acta. Vet. Scand.* 32, 27-34.
2. Nyberg, O., Lindheim, D., Gudmundsson, S., Eikenæs, O., 2002. The surveillance and control programme for bovine viral diarrhoea (BVD) in Norway. In: Fredriksen B., Mørk T. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001.* National Veterinary Institute, Oslo, Norway, pp. 93-101.
3. Nyberg, O., Lindheim, D., 2003. The surveillance and control programme for bovine viral diarrhoea (BVD) in Norway. In: Heier B.T. (Ed.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002.* National Veterinary Institute, Oslo, Norway, pp. 81-88.
4. Nyberg, O., Gudmundsson, S., Åkerstedt, J., 2004. The surveillance and control programme for bovine virus diarrhoea (BVD) in Norway. In: Mørk T., Hellberg H. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003.* National Veterinary Institute, Oslo, Norway, pp. 72-77.
5. Juntti, N., Larsson, B., Fossum, C., 1987. The use of monoclonal antibodies in enzyme linked immunosorbent assays for detection of antibodies to bovine viral diarrhoea virus. *J. Vet. Med. B.* 34, 356-363.
6. Fenton, A., Nettleton, P.F., Entrican, G., 1991. Identification of cattle infected with bovine virus diarrhoea virus using a monoclonal antibody capture ELISA. *Arch. Virol. (Suppl 3)* pp. 169-174.
7. Sandvik, T., Krogsrud, J., 1995. Evaluation of an antigen-capture ELISA for detection of bovine viral diarrhoea virus in cattle blood samples. *J. Vet. Diagn. Invest.* 7, 65-71.
8. Niskanen, R., 1993. Relationship between the levels of antibodies to bovine virus diarrhoea virus in bulk tank milk and the prevalence of cows exposed to the virus. *Vet. Rec.* 133, 341-344.
9. Lindheim, D., Nyberg, O., Plym Forshell, K., Nafstad, O., 2002. Completion of the Norwegian bovine virus diarrhoea (BVD) eradication programme - a demanding challenge. In: *Abstracts from the XXII World Buiatrics Congress, Hannover, Germany*, p. 53.
10. Nyberg, O., Østerås, O., Plym-Forshell, K., 2004. Eradication of BVDV-infection in Norwegian cattle 1992-2003 - a success story. In: *Abstracts from the Second European Symposium on BVDV Control, Porto, Portugal, 20-22 October*, p. 40.



*Figure 3. Geographical distribution on county level of herds with restrictions for BVD in 1994 (A) and the geographical location of herds with restrictions for BVD in 2003 - 2004 (B).*

# The surveillance and control programme for bovine tuberculosis in Norway



Annual report 2004

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## Introduction

Since 1994, the EFTA Surveillance Authority (ESA) has recognised Norway as an "officially bovine tuberculosis free state", as described in ESA Decision 66/94/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 slaughtering, a requirement that has been in force for decades, with submission of suspicious material to the National Veterinary Institute, Oslo.

## Aims

The purposes of the programme are to document freedom from 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

### Criteria for submission of material from slaughterhouses

Submission of lung tissue, lymph nodes and other organs with pathological lesions where bovine tuberculosis can not be excluded, are recommended.

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 µm. All samples are stained with haematoxylin and eosin (HE) and Ziehl Neelsen (ZN) (1).

### Bacteriological examination

Samples are examined as described in the OIE manual (1). 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 Petraghani 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 2004, four samples were submitted.

Low number of submitted samples from the slaughterhouses indicates a low prevalence of suspicious pathological lesions. With the exception of two single cases in 1984 and 1986, bovine tuberculosis has not been diagnosed in Norway since 1963 (2, 3, 4, 5).

Table 1. Number of samples tested for bovine tuberculosis during the period 2000-2003

Year	No. of samples	No. Of herds	No. of positive	
			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

## References

1. Anonymous, 2000. Manual of standards for diagnostic tests and vaccines. List A and list B diseases for mammals, birds and bees. 4<sup>th</sup> ed. Office International des Epizooties, Paris.
2. Mørk, T., Bratberg, B., Djønne, B., 2002. The surveillance and control programme for bovine tuberculosis in Norway. In: Fredriksen, B., Mørk, T. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001. National Veterinary Institute, Oslo, Norway, pp. 103-107.
3. Nyberg, O., 2003. The surveillance and control programme for bovine tuberculosis in Norway. In: Heier, B.T. (Ed.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002. National Veterinary Institute, Oslo, Norway, pp. 89-92.
4. Nyberg, O., 2004. The surveillance and control programme for bovine tuberculosis in Norway. In: Mørk, T., Hellberg, H. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003. National Veterinary Institute, Oslo, Norway, pp 79-81.
5. Sandvik, O., Næss, B., 1994. Animal Health Standards in Norway. The Royal Ministry of Agriculture, Oslo, Norway.



# The surveillance and control programme for maedi in Norway

Annual report 2004



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## Introduction

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 in other continents. Visna is caused by the same virus as maedi, but is a neuropathogenic manifestation of the disease (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 in the sheep population with imported Texel sheep in the 1960s. An overview of number of new affected flocks registered each year up to 2004 is given in Figure 1. 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). Movement of sheep across county borders was prohibited, and governmental restrictions concerning sale and purchase of sheep were imposed on both affected and contact flocks. In these flocks, all sheep more than one and a half year old were serologically tested annually during a five-year period. The flocks were not allowed to share breeding rams with other flocks during the mating season. Inspection of sheep lungs at the slaughterhouse during meat inspection was intensified nationwide. All affected flocks were slaughtered on a voluntary basis.

As no new infected flock was detected during the early 1990s, the restrictions were lifted in all flocks by the end of 1994, but in 1995, maedi was again diagnosed at slaughter in a ram from a flock in the Hordaland county. During the period 1995 to 1997, 29 infected flocks were detected in the counties of Rogaland and Hordaland in western Norway. Among these, 24 flocks were detected in 1995, four flocks in 1996, and one flock in the spring of 1997 (Figure 1).

## The old programme

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). The sampling frame for the flock selection was the governmental database for production subsidies in the two counties. Table 1 presents the target and study population in the programme. Animals older than one and a half year were considered high-risk and therefore selected for testing. An agar gel immunodiffusion test was used to screen sera for antibodies against maedi-visna virus. In the rest of the country the surveillance was limited to inspection at slaughter.

In November 2002, post mortem examinations of lungs from two diseased sheep from different farms in Nord-Trøndelag county showed histopathological changes consistent with maedi. The diagnoses were confirmed by serological tests of blood samples.

The prevalence of positive animals was high in both flocks (55% and 64%). In one flock there had been no contact with other flocks, whereas the other played a major role as a supplier of breeding animals to a large ram circle consisting of several smaller ones, in total approximately 130 flocks in 12 different municipalities. In addition, sheep from the original flock had been transferred to approximately 120 other flocks, including six flocks in the southern part of Norway. 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, among 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.

Table 1. The number of flocks and sheep tested in the old Norwegian surveillance and control programme for maedi during the period 1997 to 2003

Year	No. of flocks in the population	No. of flocks sampled	No. of animals tested	No. of positive flocks
1997	6,301	469	8,745	0*
1998	6,192	1,478	28,207	1
1999	6,161	1,459	27,990	0
2000	6,112	1,301	24,478	1
2001	6,037	642	11,714	0
2002	5,773	737	12,961	0
2003	5,378	386	5,678	0

\* One positive flock was diagnosed in 1997 before the programme started.

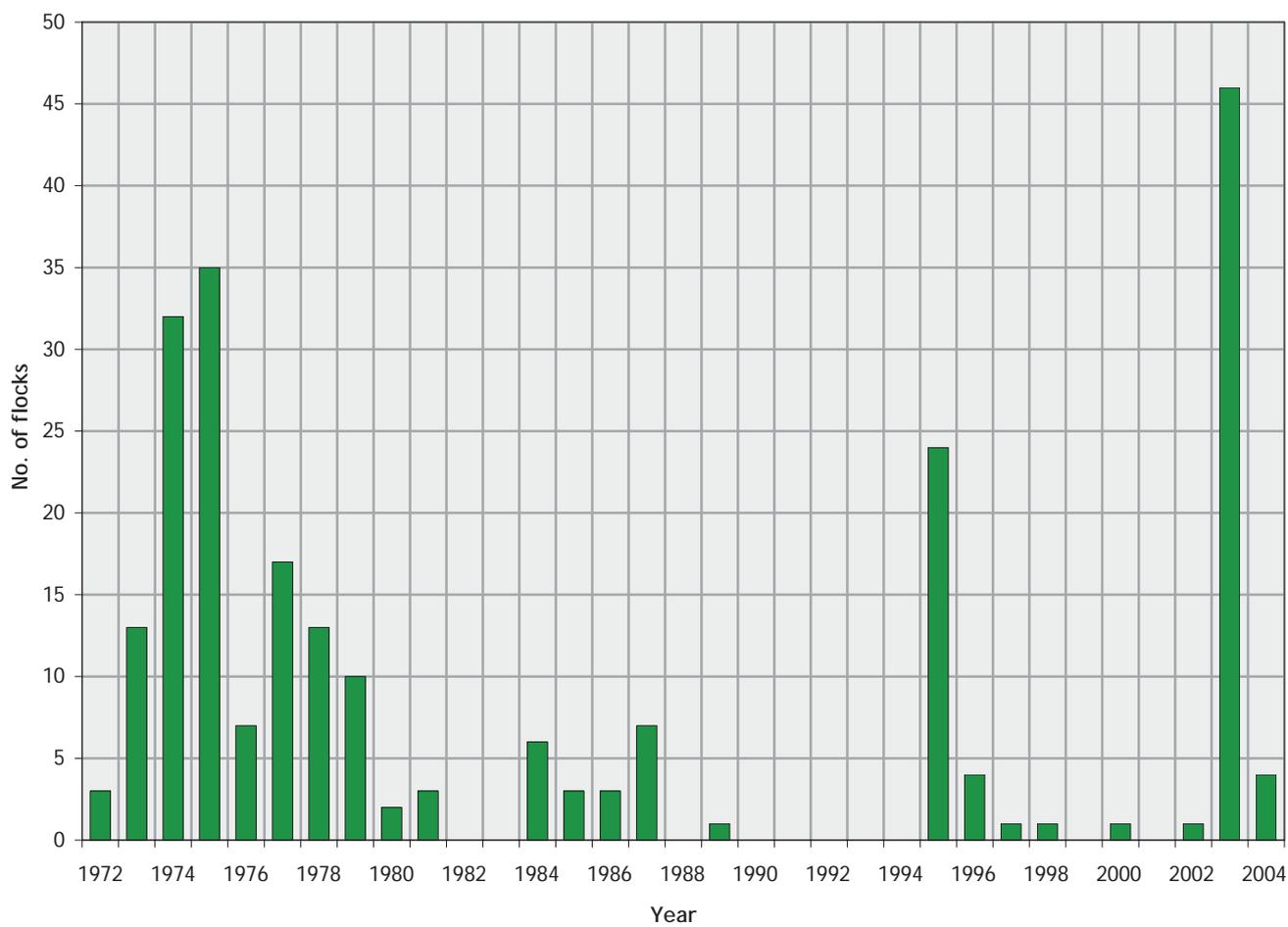


Figure 1. The number of new flocks infected with maedi registered during the period 1972 to 2004. The bars for 2003 and 2004 show both seropositive flocks detected through the investigations after the outbreak in Nord-Trøndelag county and seropositive flocks discovered in the programme.

### The new 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 very few rams used for breeding in the Norwegian sheep population are recruited from other flocks. Approximately 2,400 flocks were part of this breeding system in 2004, of a total of more than 17,000 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 Animal Health Authority, the Norwegian Sheep and Goat Breeders Association (NSG) and the Norwegian Sheep Health Service. The new programme started in November 2003.

### 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 1,030 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 per flock were tested in flocks with more than 200 animals. All rams and the oldest sheep among those more than one and a half year old were sampled in each flock.

The programme in 2004 was based on serological examination of blood samples from the selected sheep for antibodies against maedi-visna virus with the ELISA from Pourquier (ELISA CAEV/MAEDI-VISNA serum verification kit, Institut Pourquier, Montpellier, France). Seropositive 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 seropositive flocks which both sheep and goats were tested with a PCR developed at the National Veterinary Institute. The PCR 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.

## Results

Samples from a total of 1,230 flocks, constituting approximately 43% of the breeding flocks and 7% of the total Norwegian sheep flocks, were analysed in 2004. The geographical distribution of the Norwegian sheep population and the tested flocks is shown in Figure 2.

### The new programme

The new surveillance programme started at the end of November 2003, which means that the figures for 2003 in Table 2 represent less than two months of sampling. Four sheep in one flock comprising 120 sheep in the county of Hordaland were seropositive in 2003 (5).

In 2004, a sample from one sheep in a flock in Rogaland county was positive for antibodies against maedi-visna virus. Sheep from 16 farms with both sheep and goats or with close contact with goat herds also tested positive in the serological tests. Sheep from 12 of these herds were confirmed to be infected by CAEV by sequencing of the PCR product.

Table 2. The number of flocks and sheep tested in the new Norwegian surveillance and control programme for maedi from November 2003 to December 2004.

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

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

## Discussion

In the old Norwegian surveillance and control programme all the flocks in the counties of Rogaland and Hordaland were tested for the presence of antibodies against maedi-visna virus within a seven-year period. In the other parts of the country the sheep population was passively surveyed for maedi by the lung inspection carried out during the meat control and by veterinarians in clinical practice. The two specific counties were regarded as high-risk areas and it was considered most cost-efficient to restrict the testing to these counties relative to a nationwide programme.

Maedi is a progressive disease, and antibodies may not be detected in infected sheep until several years after infection. In some of the contact flocks previously tested, only 6% of the animals had been seropositive against maedi-visna virus. Supposing a 95% confidence level the number of animals tested with the previously used diagnostic test would not be sufficient to detect the infection in flocks with low prevalence. Based on the old surveillance and control programme one could not conclude that a tested flock was not infected, but the investigation indicated that infected animals occurred in very small numbers (4).

Besides being nationwide, the aim of the new programme 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.

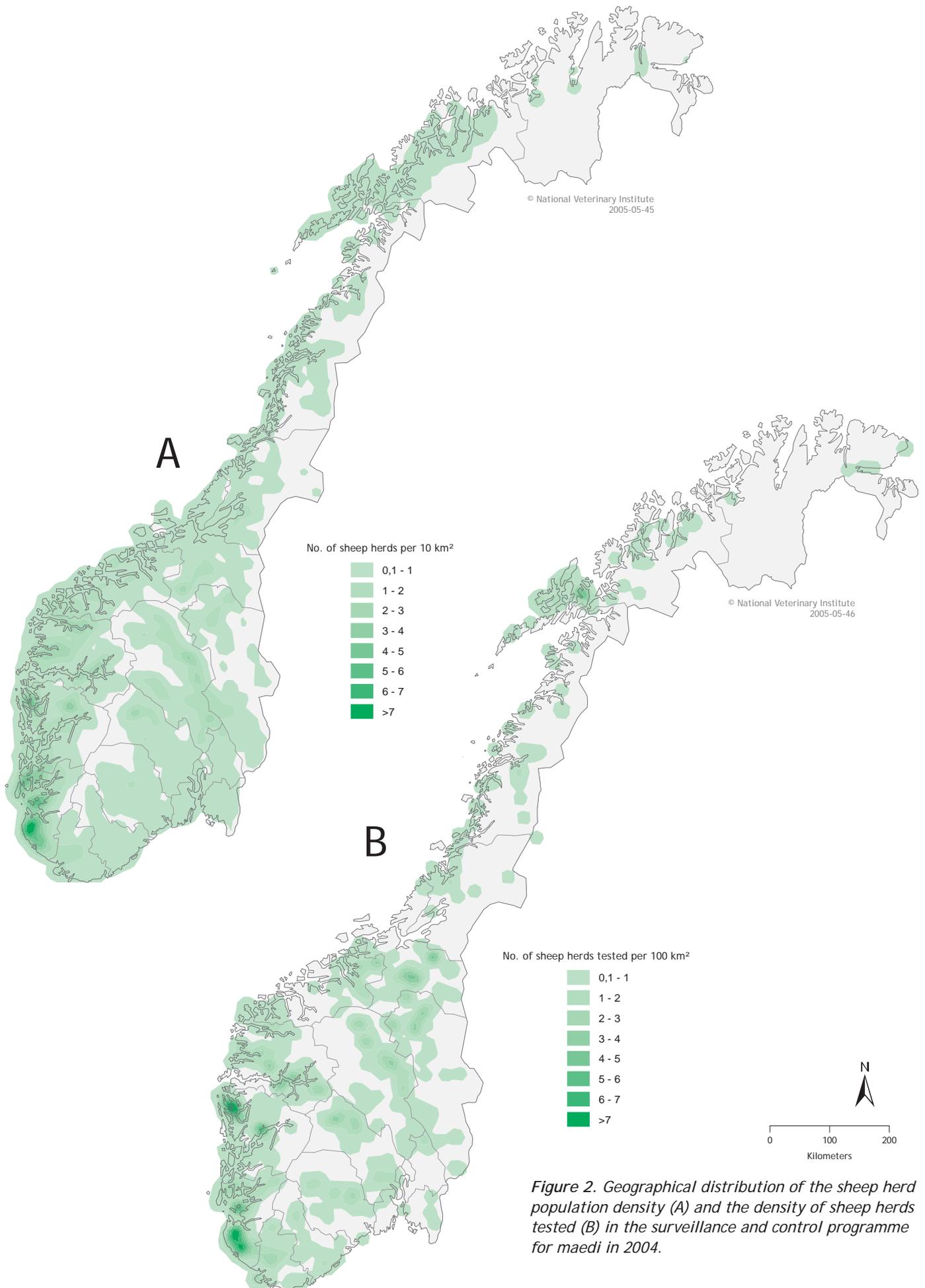
The sample size per flock was adjusted so that if none of the tested animals were seropositive, the prevalence of maedi-visna infected animals in a flock would be less than 6%, given a confidence level of 95% and a 100% test sensitivity.

The ELISA employed in the new 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, however, 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 this test-regimen implemented during the recent outbreak 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 2004, showed a preliminary prevalence of 0.1% positive flocks. 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.

## References

1. Pálsson, P.A., 1990. Maedi-visna. History and clinical description. In: Pétursson, G., Hoff-Jørgensen, R. (Eds.), *Maedi-visna and Related Diseases*. Kluwer Academic Publishers, Boston, USA, pp. 3-17.
2. Martin, W.B., Aitken, I.D., 2000. *Diseases of Sheep*, 3<sup>rd</sup> ed., Blackwell Scientific Publications, Oxford.
3. Krogsrud, J., Larsen, H.J.S., Rimstad, E., 1996. Mædi og lungeadenomatose. (Maedi and lung adenomatosis). *Nor. Vet. Tidsskr.* 108, 729-736.
4. Mork, J., Jarp, J., 2002. The surveillance and control programme for maedi in Norway. In: Fredriksen, B., Mørk, T. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001*. National Veterinary Institute, Oslo, Norway, pp. 109-115.
5. Sviland, S., Nyberg, O., Tharaldsen, J., Heier, B. T., Mork, J., 2004. The surveillance and control programme for maedi in Norway. In: Mørk, T., Hellberg, H. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003*. National Veterinary Institute, Oslo, Norway, pp. 89-95.



*Figure 2. Geographical distribution of the sheep herd population density (A) and the density of sheep herds tested (B) in the surveillance and control programme for maedi in 2004.*



# The surveillance and control programme for scrapie in Norway

Annual report 2004



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## Introduction

Scrapie was first diagnosed in indigenous Norwegian sheep in 1981. Increasing numbers of scrapie-infected flocks were identified in the 1990s, culminating with 31 detected flocks in 1996 (Figure 1). By the end of 2003, scrapie had been diagnosed in a total of 91 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 2004, the surveillance programme was adjusted according to the European Union Regulations, Regulation (EC) No. 999/2001 Annex III, as amended by Regulation (EC) No 2245/2003 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)
- 500 goats 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

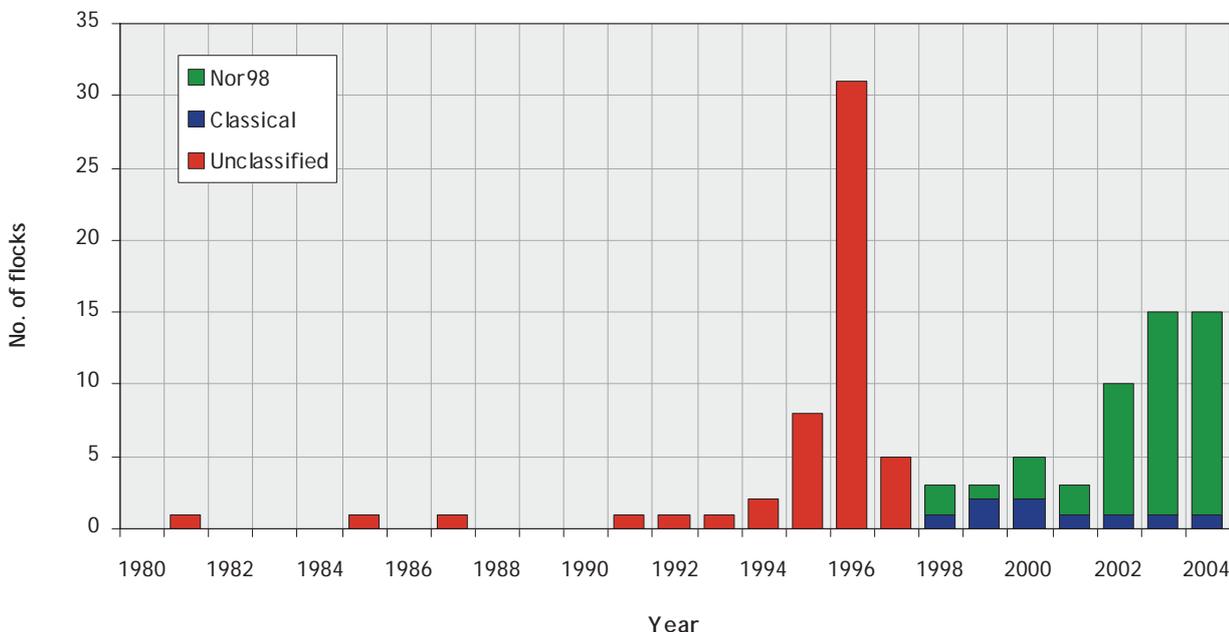


Figure 1. Annual number of sheep flocks diagnosed with classical scrapie and scrapie Nor98 during the time period 1980-2004. Before 1998 the cases were not classified according to type of scrapie, but the majority of the scrapie cases are supposed to be the classical type.

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 16 sheep and three goats 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 3,500 sheep and 180 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 unfixed *medulla oblongata* obtained through the *foramen magnum* using a metal spoon specially designed at the National Veterinary Institute, and retropharyngeal lymph nodes. Alternatively the samples consisted of formalin-fixed and unfixed brain halves and unfixed retropharyngeal lymph nodes. The samples were examined at the National Veterinary Institute in Oslo.

### Abattoir surveillance

Approximately 10,460 randomly collected brain samples from apparently healthy sheep and 132 randomly collected brain samples from apparently healthy goats older than 18 months were collected. The sheep samples were collected at 28 abattoirs, which process all the commercially slaughtered sheep in Norway.

The samples were obtained throughout the year, with approximately 55% 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 PrP<sup>Sc</sup> (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). Poly-morphisms 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 by 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.

## Results

Scrapie was diagnosed in 16 sheep. Three cases were reported because the sheep had shown clinical signs consistent with scrapie on the farm. Four scrapie cases were identified in fallen stock, and eight cases were apparently healthy animals slaughtered for human consumption. The last case was detected under scrapie eradication (Table 1). Scrapie was not diagnosed in goats (Table 1).

Table 1. Brain samples from sheep and goats submitted for examination for scrapie in 2004

Reason for submission to the laboratory	No. of samples	No. of rejected samples	Negative	Positive
<i>Sheep</i>				
Animals with clinical signs consistent with scrapie	16	0	13	3
Fallen stock	3,525	158	3,363	4
Healthy slaughtered animals	10,463 *	1	10,454 *	8
Animals killed under scrapie eradication	620	0	619	1
<b>Total sheep</b>	<b>14,624</b>	<b>159</b>	<b>14,449</b>	<b>16</b>
<i>Goats</i>				
Animals with clinical signs consistent with scrapie	3	0	3	0
Fallen stock	179	9	170	0
Healthy slaughtered animals	132 **	1	131	0
Animals killed under scrapie eradication	0	0	0	0
<b>Total goats</b>	<b>314</b>	<b>10</b>	<b>304</b>	<b>0</b>

\* 430 samples from unspecified small ruminants tested negative. These samples are included in the figures given for sheep.

\*\* The examined healthy slaughtered goats were not a part of the surveillance programme following the EU regulations.

**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 2004

Case nr	Year of birth	Reason for submission to laboratory examination <sup>1)</sup>	Breed <sup>2)</sup>	Prion Protein Genotype	Scrapie type
1	1996	Healthy slaughtered animals	Norwegian White Sheep	AHQ/ARQ	Nor98
2	1998	Healthy slaughtered animals	Dala Sheep	AHQ/AHQ	Nor98
3	1997	Suspect	Norwegian White Sheep	AHQ/ARR	Nor98
4	1996	Healthy slaughtered animals	Spæl Sheep	AHQ/ARQ	Nor98
5	2001	Suspect	Steigar Sheep	ARQ/VRQ	Classical
6	1997	Suspect	Steigar Sheep	AHQ/AHQ	Nor98
7	1999	Killed under scrapie eradication	Steigar Sheep	ARH/VRQ	Classical
8	1999	Fallen stock	Rygja Sheep	AHQ/ARR	Nor98
9	2000	Fallen stock	Norwegian White Sheep	AHQ/ARQ	Nor98
10	1998	Fallen stock	Norwegian White Sheep	ARQ/ARQ	Nor98
11	1996	Healthy slaughtered animals	Norwegian White Sheep	ARQ/ARR	Nor98
12	1998	Healthy slaughtered animals	Norwegian White Sheep	AHQ/ARQ	Nor98
13	1999	Healthy slaughtered animals	Norwegian White Sheep	ARQ/ARR	Nor98
14	1997	Healthy slaughtered animals	Steigar Sheep	ARR/ARR	Nor98
15	2001	Fallen stock	Spæl Sheep	AHQ/AHQ	Nor98
16	1999 (at least 5 years)	Healthy slaughtered animals	Spæl Sheep	AHQ/ARR	Nor98

<sup>1)</sup> Suspect: Clinical signs consistent with scrapie including animals showing clinical signs at ante-mortem inspection/monitoring of fallen stock/monitoring of healthy slaughtered animals/monitoring of animals killed under scrapie eradication measures.

<sup>2)</sup> Crossbred long-tailed breeds: Rygja Sheep, Steigar Sheep, Dala Sheep, Norwegian White Sheep; indigenous short-tailed breed: Spæl Sheep.

The individual age and breed were collected and the prion protein genotype was examined for all the 16 scrapie cases (Table 2). Fourteen of the 16 scrapie cases were diagnosed as scrapie Nor98, based on the unique Western blot profile (Table 2).

The identity of the flock was reported for 13,476 (92.1%) of the total of 14,624 samples from sheep. In the event of a positive sample from slaughtered animals, the flock identity of the remaining samples (7.9%) could be traced via the carcass number. The 13,476 samples were collected from 5,737 different sheep flocks. The mean number of animals tested per flock was 2.2 (range 1-36, flocks eradicated due to scrapie are excluded). From 1,523 flocks more than two samples were tested.

The identity of the herd was reported for 303 (96.5%) of the total of 314 samples from goats. In the event of a positive sample from slaughtered animals, the herd identity of the remaining samples (4.5%) could be traced via the carcass number. The 303 samples were collected from 188 different goat herds. The mean number of animals tested per herd was 1.6 (range 1-8).

The geographical distribution 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 0.14% (0.05-0.30%), (95% confidence interval [CI]), and the prevalence of scrapie in sheep slaughtered for human consumption was estimated to 0.08% (0.04-0.15%), (95% CI).

PrP genotyping was performed on 628 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 2004 grouped in accordance with the British National Scrapie Plan (NSP)

Genotype category	Number	%
NSP1, genetically most resistant, ARR/ARR	74	11.8
NSP2, genetically resistant, ARR/ARQ, ARR/ARH, ARR/AHQ, VRR/ARQ	252	40.1
NSP3, genetically little resistant, ARQ/ARQ	108	17.2
NSP3, genetically little resistant, AHQ/AHQ, ARH/ARH, ARH/ARQ, AHQ/ARH, AHQ/ARQ	79	12.6
NSP4, genetically susceptible, ARR/VRQ	0	0
NSP5, genetically highly susceptible, ARQ/VRQ, ARH/VRQ, AHQ/VRQ, VRQ/VRQ	115	18.3
<b>Total</b>	<b>628</b>	<b>100.0</b>

## Discussion

Scrapie Nor98 was diagnosed in 14 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), except that for the first time a scrapie Nor98 case was diagnosed in a Norwegian sheep with the prion protein genotype ARR/ARR, the most resistant genotype (NSP1), (Table 2). Atypical scrapie has previously been described in sheep with this PrP genotype in Germany and Portugal (9, 10). There were five scrapie Nor98 cases in genetically resistant (NSP2), while eight cases appeared among genetically little resistant sheep (NSP3). 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 the sheep in the 14 scrapie Nor98 flocks were genotyped. Animals with a VRQ allele and animals without at least one ARR allele were killed, and about 600 animals older than 18 months were examined for PrPSc, but no additional animal 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.

As in previous years, scrapie Nor98 was diagnosed in several different breeds. The sheep were between three and eight years old, 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 2004 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 0.14% (0.05-0.30%), (95% CI), which was lower, but not significantly different from the estimated prevalence in 2003 (0.28% [0.14-0.43%], [95% CI]). The prevalence of scrapie Nor98 in sheep slaughtered for human consumption was estimated to 0.08% (0.04-0.15%) (95% CI), which was higher, and significantly different from the estimated prevalence for 2003 (0.02% [0.008-0.031%], [95% CI]) (8). This result may indicate an increase in the prevalence of scrapie Nor98 in the slaughtered population, but does not necessary reflect an increase in the Norwegian sheep population.

The prevalences of scrapie Nor98 in fallen stock and in the slaughtered population are not significantly different, and may raise the question about how strong the association is between scrapie Nor98 and clinical signs and mortality. Only two of the 14 animals with a scrapie Nor98 diagnosis were reported to show clinical signs on the farm, while the remaining 12 scrapie Nor98 cases were detected through the surveillance of fallen stock and through the surveillance of apparently healthy slaughtered sheep. This result strengthens the impression that sheep with scrapie Nor98 rarely show severe clinical signs associated with disease affecting the central nervous system. However, it is reported that three of the eight animals with scrapie Nor98 diagnosed in the slaughtered population had shown mild ataxia or reduced body condition.

Classical scrapie was diagnosed in two animals within one flock located in the same area as the flocks with classical scrapie detected in 2002 and 2003. When the classical form of scrapie is detected, the whole flock is killed. Classical scrapie has not been detected in the active surveillance despite examination of about 66,000 animals during the last three years, a result that strengthens the opinion of a very low prevalence of this scrapie type.

The difference between the number of examined sheep from fallen stock (3, 367) 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.

In the monitoring of sheep between one and 36 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 50 breeding sheep older than 12 months. Sheep from 5,737 of the total of approximately 17,400 flocks have been examined.

The PrP genotyping of the slaughtered sheep showed that about 50% of the animals were most resistant or resistant to classical scrapie (NSP1 and NSP2). Both the diagnosed classical scrapie cases were in the category genetically highly susceptible (NSP5) which constituted approximately 18% of the examined sheep. Six of the Nor98 cases appeared in NSP1 and NSP2 and shows that breeding for these genotypes may reduce, but not eliminate the occurrence of scrapie Nor98.

## 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.

## References

1. Hopp, P., Bratberg, B., Ulvund, M.J., 2000. Skrape-sjuke hos sau i Norge (Scrapie in sheep in Norway), *Nor. Vet. Tidsskr.* 112, 368-375.
2. Thorud, K., Hagen, G., 2001. Die Scrapiebekämpfung auf nationaler Ebene (National eradication of scrapie). In: *Prionen und Prionkrankheiten/hrsg.*, Hörnlimann, Beat von (Ed.), Walter de Gruyter, Berlin, pp. 506-509.
3. Benestad, S.L., Sarradin, P., Thu, B., Schönheit, J., Tranulis, M.A., Bratberg, B., 2003. Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. *Vet. Rec.* 153, 202-208.
4. Moum T., Olsaker I., Hopp P., Moldal T., Valheim M., Moum T., Benestad S.L., 2005. Polymorphisms at codons 141 and 154 in the ovine prion protein gene are associated with scrapie Nor98 cases. *J. Gen. Virol.* 86, 231-235.
5. O'Rourke, K.I., Baszler, T.V., Miller, J.M., Spraker, T.R., Sadler-Riggleman, I., Knowles, D.P., 1998. Monoclonal antibody F89/160.1.5 defines a conserved epitope on the ruminant prion protein. *J. Clin. Microbiol.* 36, 1750-1755.
6. Valheim, M., Benestad, S.L., Hopp, P., Bratberg, B., 2002. The surveillance and control programme for scrapie in Norway. In: Fredriksen, B., Mørk, T. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001.* National Veterinary Institute, Oslo, Norway, pp. 117-125.
7. Valheim, M., Benestad, S.L., Hopp, P., Bratberg, B., 2003. The surveillance and control programme for scrapie in Norway. In: Heier, B.T. (Ed.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002.* National Veterinary Institute, Oslo, Norway, pp. 99-107.
8. Valheim, M., Benestad S.L., Bratbeg B., Eikenæs O., Hopp P., Moldal T., Mork J., 2004. The surveillance and control programme for scrapie in Norway. In: Mørk T., Hellberg H. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003.* National Veterinary Institute, Oslo, Norway, pp. 97-105.
9. Buschmann A., Lühken G., Schultz J., Erhardt G., Groschup M.H., 2004. Neuronal accumulation of abnormal prion protein in sheep carrying a scrapie-resistant genotype (PrP<sup>ARR/ARR</sup>). *J. Gen. Virol.* 85, 2727-2733.
10. Orge L., Galo A., Machado C., Lima C., Ochoa C., Silva J., Ramos M., Simas J.P., 2004. Identification of putative atypical scrapie in sheep in Portugal. *J. Gen. Virol.* 85, 3487-3491.

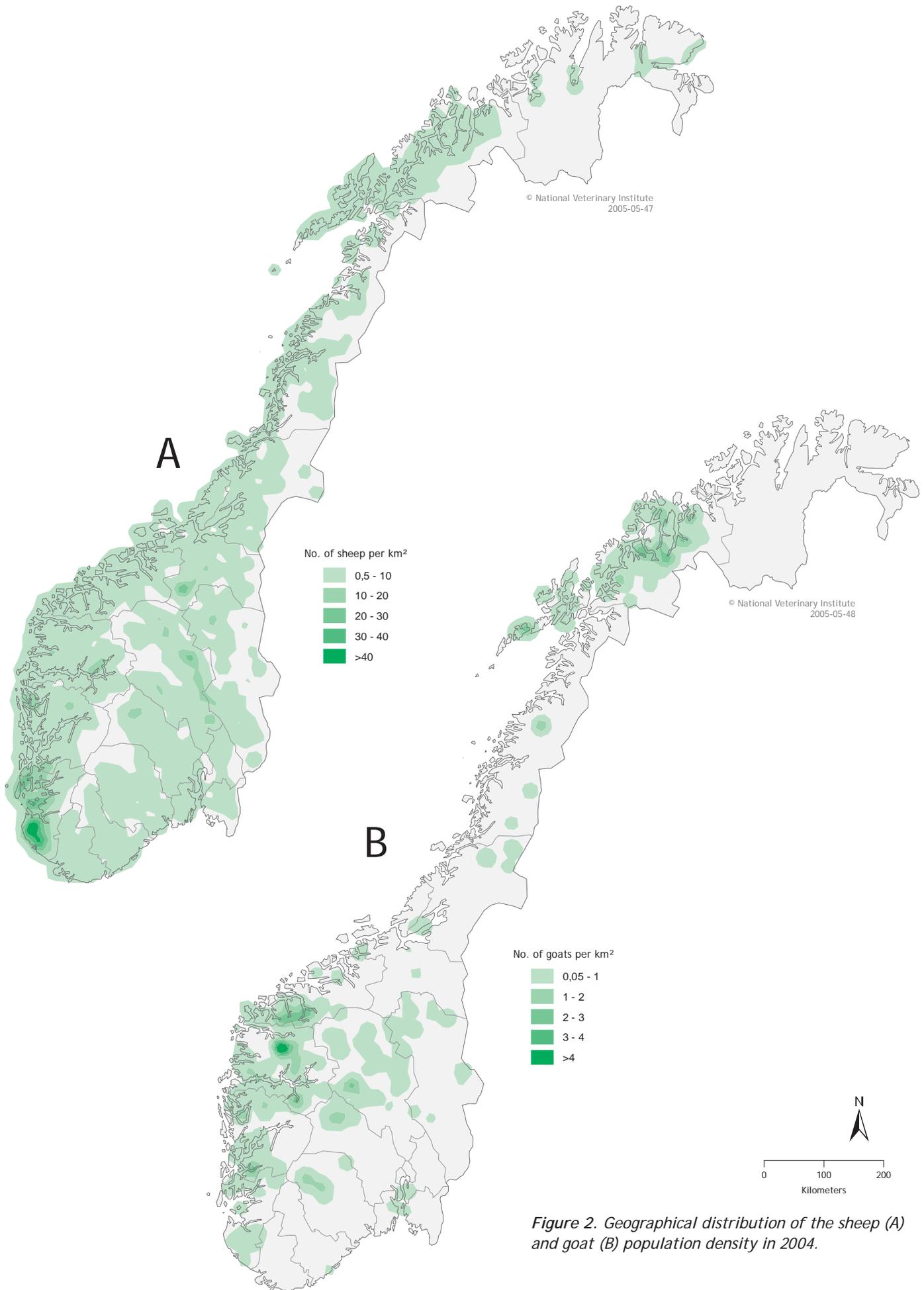
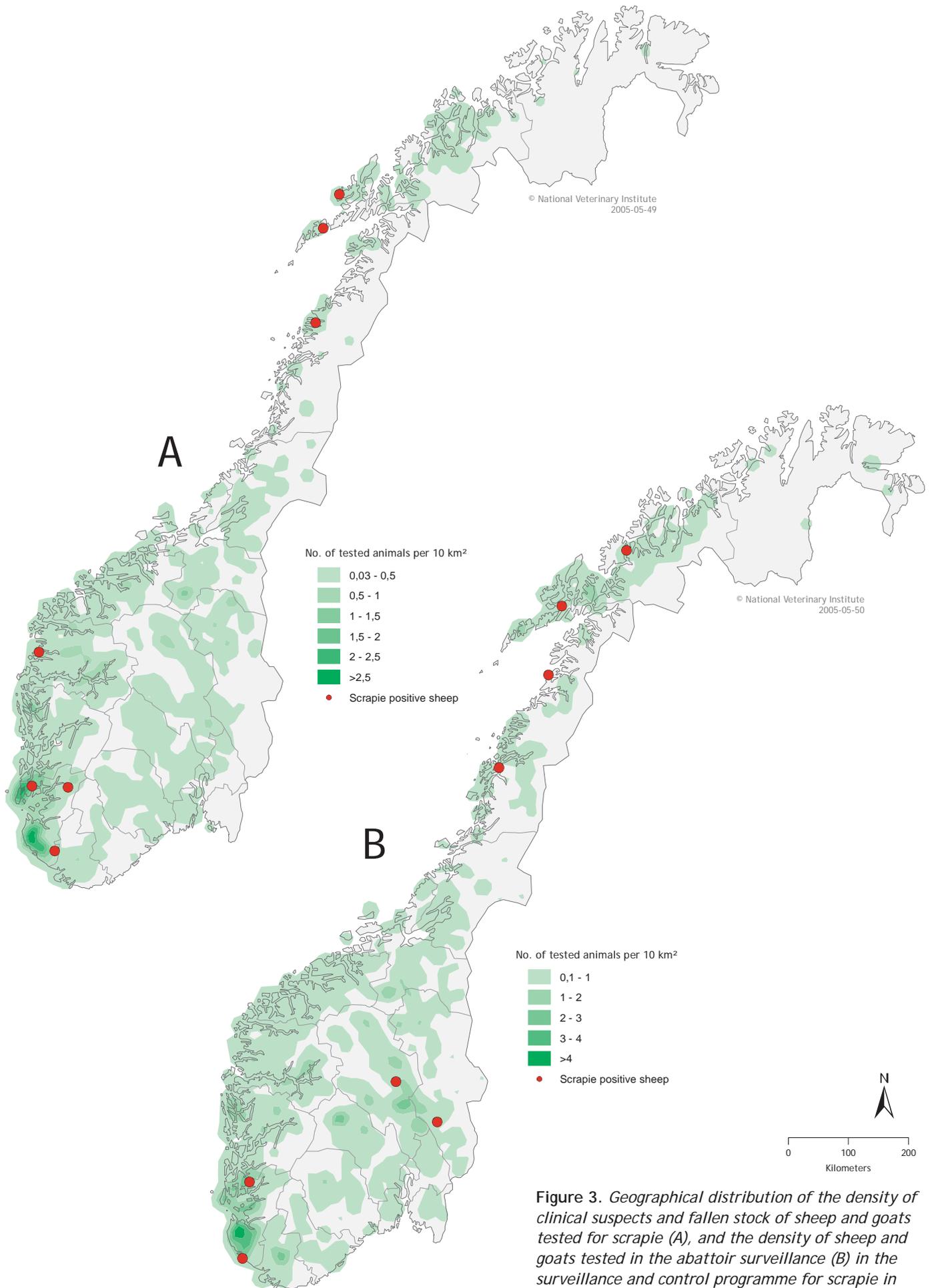


Figure 2. Geographical distribution of the sheep (A) and goat (B) population density in 2004.



**Figure 3.** Geographical distribution of the density of clinical suspects and fallen stock of sheep and goats tested for scrapie (A), and the density of sheep and goats tested in the abattoir surveillance (B) in the surveillance and control programme for scrapie in 2004.

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

Annual report 2004



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## Introduction

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

After the agreement on the European Economic Area in 1994, Norway achieved a 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* infection in sheep was established in 2004.

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

## Aims

The purposes of the programme are to document freedom from *Brucella melitensis* infection in sheep according to the demands in EU Directive 1991/68/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* infection in sheep, the Norwegian Sheep and Goat Breeders Association's register of ram circles and their member flocks constituted the basis population for the programme. The ram circles represent the top of the breeding system, and very few rams used for breeding in the Norwegian sheep population are recruited from other flocks. Approximately 2,400 flocks were part of this breeding system in 2004, of a total of more than 17,000 sheep flocks. In addition, sheep from 200 randomly selected flocks not belonging to any ram circle were included in the programme.

In flocks of less than 30 animals, all individuals were sampled, while 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* infection in sheep in 2004 is given in Table 1. The geographic distribution of the total number and the number of tested sheep flocks are shown in Figure 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.

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

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 flocks
2004	17,439	918,500	1,655	50,501	0

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

## Results

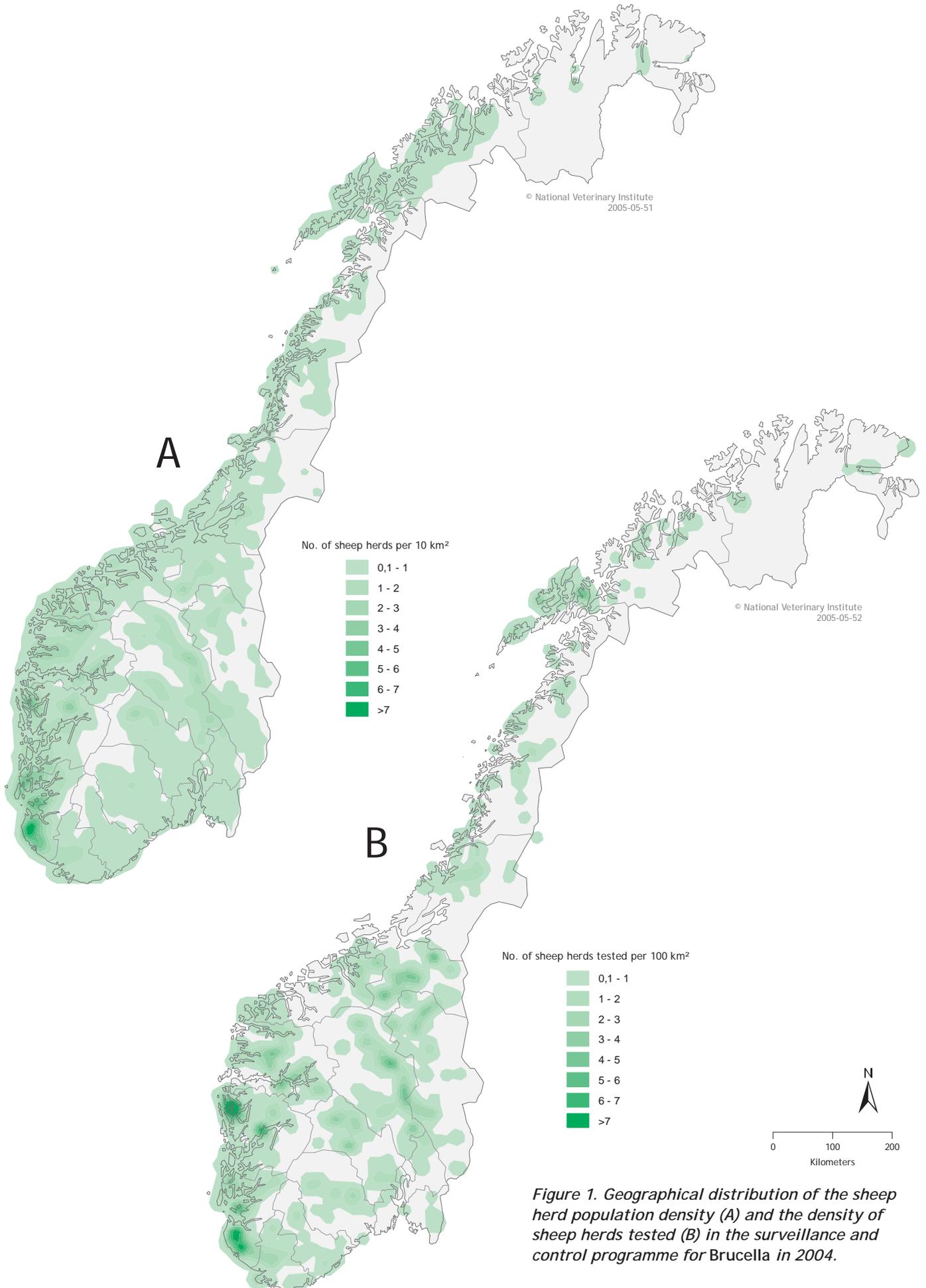
A total of 50,501 samples from 1,655 sheep flocks were analysed in 2004. All samples tested for antibodies against *Brucella melitensis* in 2004 were negative.

## Discussion

Norway has been regarded as free from *Brucella melitensis* infection in small ruminants on a historical basis 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 during 2004. The 50,501 samples analysed represented 5% of the total Norwegian population of sheep more than one year of age. The flocks tested constituted approximately 60% of the breeding flocks and 9% of the total number of Norwegian sheep flocks.

## References

1. Martin, W.B., Aitken, I.D., 2000. Diseases of Sheep, 3<sup>rd</sup> ed., Blackwell Scientific Publications, Oxford.
2. Timoney, J.F., Gillespie, J.H., Scott, F.W., Barlough, J.E., 1988. Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals, 8<sup>th</sup> ed., Comstock Publishing Associates, Ithaca.
3. Ødegaard, Ø., 2000. Brucellosis - Nordic perspective. In: Proceedings from the Course in Serious Contagious Diseases in Animals, Oslo, Norway.



**Figure 1. Geographical distribution of the sheep herd population density (A) and the density of sheep herds tested (B) in the surveillance and control programme for Brucella in 2004.**



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

Annual report 2004



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## Introduction

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, also porcine epidemic diarrhoea (PED) was included.

The EFTA Surveillance Authority (ESA) has recognised the swine population in Norway as free from AD since 1 July 1994, and has defined certain additional guarantees to protect the swine health status in Norway. Decisions concerning 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.

AD, PRRS, TGE and PRCV have never been detected in Norwegian pigs. Antibodies against Swine influenza (SI, H<sub>3</sub>N<sub>2</sub>) were detected once in 1998 in pigs in a multiplier herd tested in the National surveillance programme. No clinical signs of the disease were observed.

## Aims

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

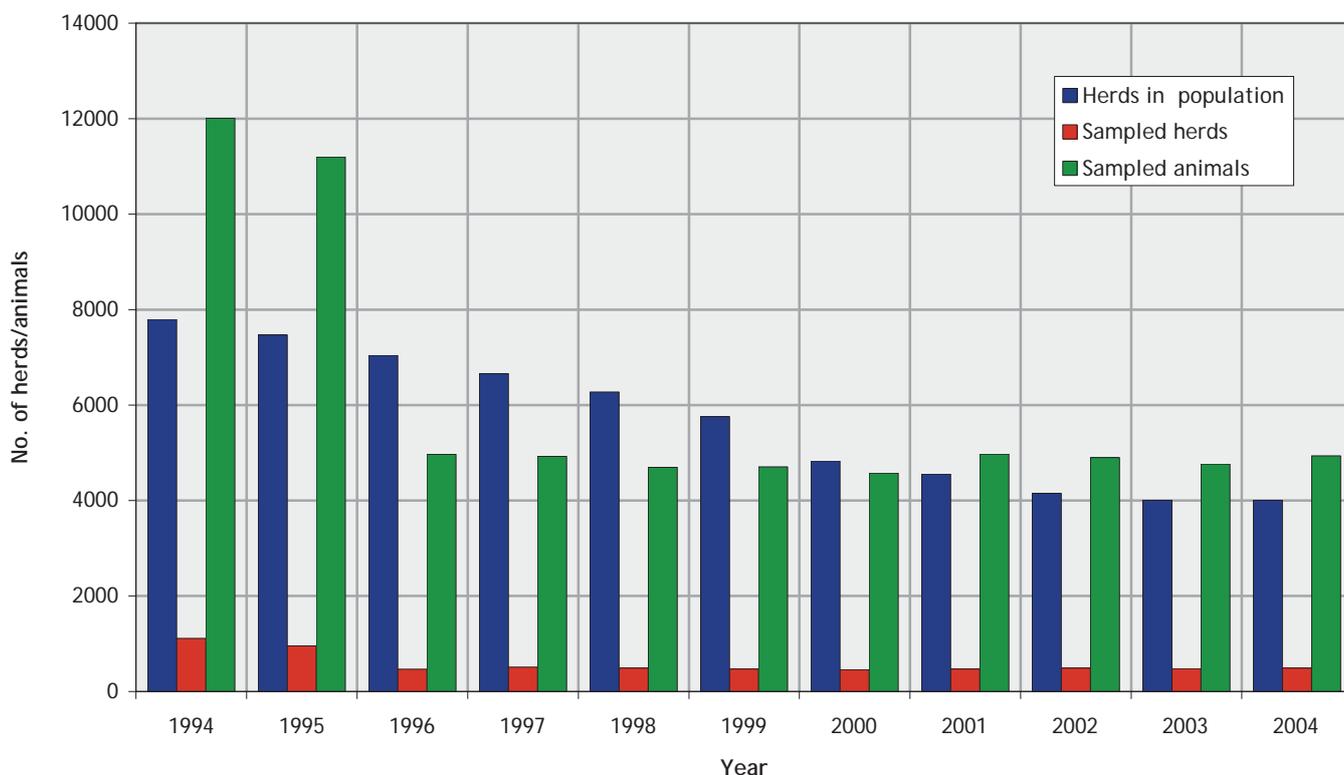


Figure 1. The size of the sampling frame and the number of sampled herds and animals in the Norwegian surveillance programme for specific virus infections in swine during the time period 1994-2004.

## Material

The surveillance of swine herds is focused on the breeding population of conventional swine. All elite breeding herds and multiplying herds were tested. In addition, the nucleus units of the sow pools and a random selection of the remaining breeding population and production animals 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 2003. The register included a total of 4,006 commercial swine herds. Based on this, the sampling plan specified 176 elite breeding and multi-plying herds, 7 sow pools, 280 integrated and piglet producing herds, and 60 fattening herds. Samples from elite breeding 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 five 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™). The test detects antibodies against glycoprotein I on the surface of the virus, and the test discriminates between infected and vaccinated animals.

### Transmissible gastroenteritis virus and porcine respiratory coronavirus

A combined blocking ELISA (SVANOVIR™) 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 H<sub>1</sub>N<sub>1</sub> and H<sub>3</sub>N<sub>2</sub> in the hemagglutination inhibition test (HI). The reagents were produced at the National Veterinary Institute in Oslo.

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

## Results

All serum samples were negative in all analyses.

Blood samples from 4,935 individual animals were submitted to the National Veterinary Institute. The number of negative and rejected samples for AD, SI, and PRRS, PRCV and TGE, respectively, is presented in Table 1. 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 10.1 (range 4 - 20).

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

Disease	Received	Rejected	Negative	Positive
AD	4,935	9	4,926	0
SI	4,935	14	4,921	0
PRRS	4,935	9	4,926	0
TGE/PRCV	4,935	27	4,908	0

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 2004

Category	No. of herds tested	% of herds tested	Total no. of individual samples collected	% of individual samples collected
Elite breeding herds and multiplying herds	168	34.1	1,718	34.8
Sow pools	7	1.4	70	1.4
Integrated and piglet producing herds	262	53.3	2,596	52.6
Fattening herds	55	11.2	551	11.2
<b>Total</b>	<b>492</b>		<b>4,935</b>	

## Discussion

The results from the surveillance programme give additional documentation of freedom from specific virus infections in the Norwegian swine population except pet pigs and farmed wild pigs. Antibodies against any of the specified viruses have been detected only once since the start in 1994, when a low level of antibodies against swine influenza (H<sub>3</sub>N<sub>2</sub>) was detected in samples from pigs in one herd in 1998. To date, there have been no clinical recordings indicating the presence of any of the viral infections included in this surveillance and control programme (1-5).

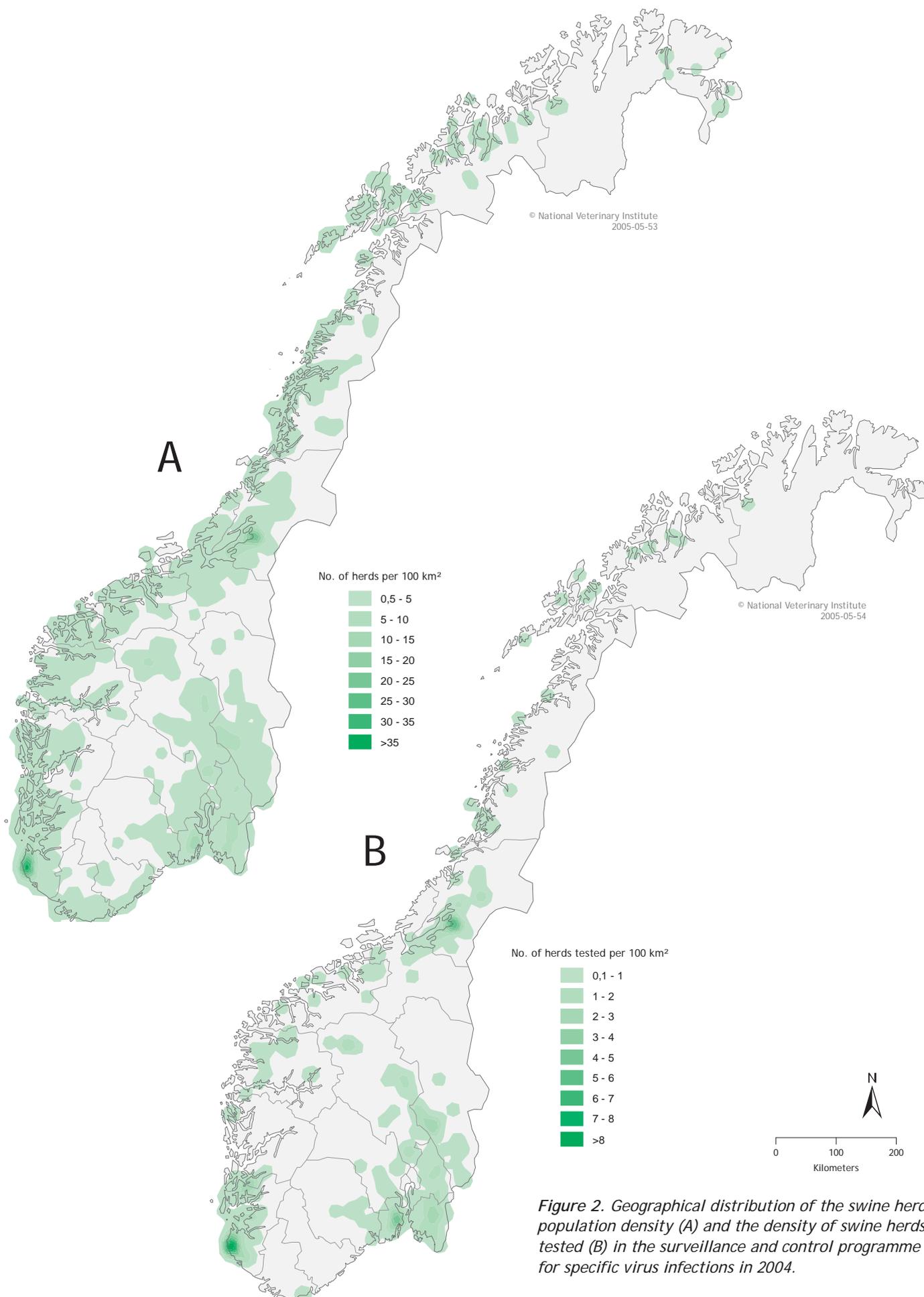
The Norwegian swine industry has been structurally changed during the last ten years. The number of herds has declined and the average herd size increased, while the produced tonnage of pork meat has been relatively stable. The number of sampled herds and animals was reduced in 1996 due to a modification of the 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 12.3% in 1994 and 2004, respectively. No wild swine population is registered in Norway. This is perhaps due to the cold winter climate, although in neighbouring Sweden, the wild swine population is growing. The geographical distribution of investigated farms is in accordance with the spatial distribution of the total swine herd population (Figure 2).

Due to low import of live swine and swine products, the Norwegian swine population is relatively isolated. In 2004, 200 doses of swine semen were imported from Finland and there was no import of live pigs. 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 breeding material from Norsvin International. The surveillance programme provides solid documentation of the favourable health situation in the Norwegian swine population in general and the breeding herds in particular, making such trade possible.

## References

1. Fredriksen, B., Tharaldsen, J., Krogsrud, J., 2000. Rapport for overvåkings- og kontrollprogrammet for gris i Norge 1999. (The surveillance and control programme for swine in Norway in 1999). National Veterinary Institute, Oslo, Norway.
2. Fredriksen, B., Tharaldsen, J., 2001. The surveillance and control programme on specific virus infections in Norwegian swine herds in 2000. National Veterinary Institute, Oslo, Norway.
3. Fredriksen, B., Tharaldsen, J., 2002. The surveillance and control programme for specific virus infections in swine herds in Norway. In: Fredriksen, B., Mørk, T. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001. National Veterinary Institute, Oslo, Norway, pp. 127-134.
4. Jarp, J., Tharaldsen, J., 2003. The surveillance and control programme for specific virus infections in swine herds in Norway. In: Heier, B.T. (Ed.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002. National Veterinary Institute, Oslo, Norway, pp. 109-115.
5. Hopp, P., Tharaldsen, J., 2004. The surveillance and control programme for specific virus infections in swine herds in Norway. In: Mørk, T., Hellberg, H. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003. National Veterinary Institute, Oslo, Norway, pp. 107-112.



*Figure 2. Geographical distribution of the swine herd population density (A) and the density of swine herds tested (B) in the surveillance and control programme for specific virus infections in 2004.*

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

Annual report 2004



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## Introduction

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 April 2003, ART was diagnosed in one broiler breeder farm in Rogaland with two flocks (2). Both flocks were stamped out. Other poultry flocks in the area were tested serologically, according to the control programme, but no other positive flocks were found. New flocks in the same houses have been tested in 2004 without positive results.

## 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 1998-2004 (from 2004: 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.

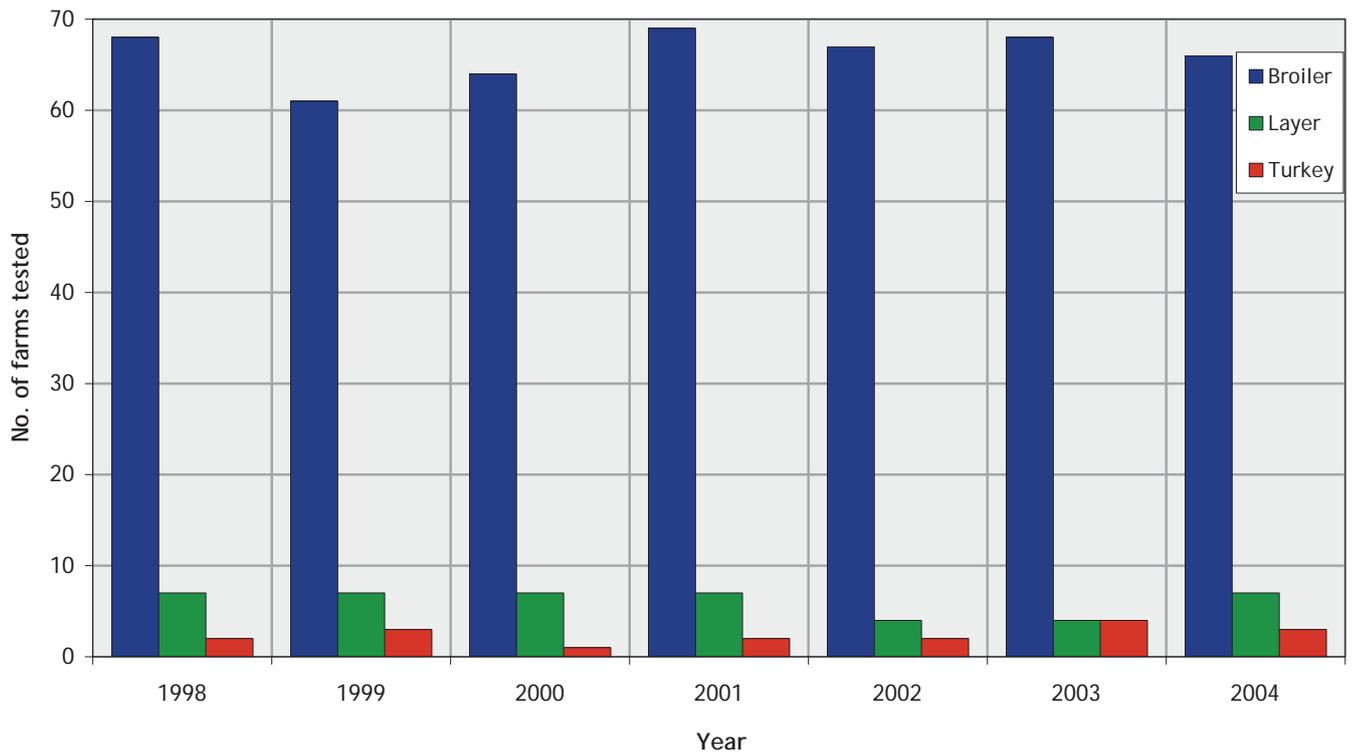


Figure 1. The number of farms tested in the surveillance and control programmes for infectious laryngotracheitis (ILT) and avian rhinotracheitis (ART) in poultry flocks in Norway during the time period 1998-2004.

Table 1. Number of farms, flocks and birds tested in the surveillance and control programmes for poultry in 2004

Production	No. of farms tested	No. of flocks tested	No. of birds tested per flock	Total no. of birds tested	Infection
Broiler	66	92	30	2,760	ILT, ART
Layer	7	16	30	480	ILT, ART
Turkey	3	4	30	120	ART
Total	76	112		3,360	

### ILT

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

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.

### ART

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

## Results

Table 1 shows the number of farms, flocks and birds tested in the different poultry production types in the national surveillance and control programmes in 2004.

### ART

Of the total 3,360 samples analysed for antibodies against APV in the surveillance programme, samples from two separate flocks from one large layer breeder company tested positive in August and December, respectively. No clinical symptoms were seen in any of these flocks. The company situated in Rogaland has its own and contract production in several houses spread over an area of approximately five kilometres in radius. At the first sampling occasion of the firstly recognised infected flock in August, only one out of 30 samples was seropositive, and the same prevalence of positives was seen three weeks later (two out of 60 were positive). However, six weeks after the first sampling occasion, 13 of 60 samples were seropositive. 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 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 held 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 sent to Veterinary Laboratories Agency, Weybridge UK for virus detection by RT-PCR and propagation in cell culture, but all attempts to identify the agent responsible for the positive serology were negative.

In December 2004, the next layer breeder flock from the same rearing house also tested positive for ART. The company with the infected flocks were still under restrictions and follow-up by the Norwegian Food Safety Authority at the end of the year.

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

### ILT

All the 3,240 blood samples were negative for antibodies against ILTV.

## Discussion

ART had never been diagnosed in Norwegian poultry before the demonstration of antibodies against APV in 2003 and 2004. 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 attempts, the infectious agent causing the seroconversion has not yet been isolated and identified. The diagnosis has thus been based on serology only, as for ART in many other countries (1). The main causes for the difficulties in virus identification are supposed to be a) timing of sampling due to no clinical symptoms b) probably limited virus excretion in subclinically infected birds, and c) the possibility that the infective agent is a pneumovirus variant not detectable by the present laboratory methods.

Despite close follow-up, in both local and more distantly located contact flocks, no other commercial flocks have tested positive indicating no further spread of the infection from the infected farms during 2004. The investigations on ART-status of the other flocks belonging to the large layer breeder company were not completed at the end of 2004. However, the strategy at the end of 2004 was as in 2003 to eliminate the infection by stamping out infected flocks.

## References

1. Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R., Swayne, D.E. (Eds.), 2003. Diseases of poultry, 11<sup>th</sup> ed. Iowa State University Press, Ames, Iowa.
2. Heier, B.T., Tharaldsen, J., 2004. The surveillance and control programmes for infectious laryngotracheitis (ILT) and avian rhinotracheitis (ART) in poultry flocks in Norway. In: Mørk T. and Hellberg H. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003. National Veterinary Institute, Oslo, Norway, pp. 113-117.
3. Jordan, F.T.W., Pattison, M., Alexander, D., Faragher, T., 2001. Poultry diseases, 5<sup>th</sup> ed. W.B. Saunders Company LTD, London.



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

Annual report 2004



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## Introduction

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. For close to 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 undisinfected water, eating at barbecues, occupational exposure to animals, and eating under-cooked pork (1).

The action plan regarding *Campylobacter* in Norwegian broilers was implemented in the spring of 2001. The objective is to reduce the 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 dissemination 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](http://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 ten swabs from fresh faecal droppings. The ten swabs are pooled into two samples and submitted in transport media to the National Veterinary Institute's laboratory in Trondheim, where the samples are analysed. At the onset of the surveillance period, the samples were taken ten to six days before slaughter. From September 2001 onwards sampling has been conducted eight to four days before slaughter.

Positive flocks are slaughtered at the end of the day, and the carcasses from these 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 ten whole caecae (before 1 May 2004 ten cloacal swabs) per flock at the slaughter line. Contents from the ten 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 3,626 flocks from 501 broiler farms were tested. These flocks were slaughtered in 3,842 batches (a batch includes all chickens from one flock slaughtered on the same day). A total of 200 flocks were slaughtered split into more than one batch. Most of these were slaughtered in two batches, a few were slaughtered in three or four batches.

Overall, 118 (3.3%) flocks (120 (3.1%) batches) were positive for *Campylobacter* sp. either pre-slaughter, at slaughter, or at both sampling times.

Of the 118 positive flocks, 60 (50.8%) tested positive pre-slaughter and were subject to sanitary measures at slaughter in order to prevent contaminated poultry from reaching the general market as fresh broiler meat. Three flocks tested positive at pre-slaughter only.

The positive flocks came from 89 (17.8%) of the farms. Of these 89 positive farms, 73 (82.0%) had only one positive incidence during 2004 (a positive incidence is defined as one positive flock or as several parallel positive flocks from different houses) and these produced 79 (66.9%) of the positive flocks. A total of 12 (13.5%) farms had two positive incidences (producing 24 (20.3%) of the positive flocks), two (2.2%) had three, one (1.1%) had four and one (1.1%) had five positive incidences. The four farms with more than two positive incidences in 2004 (equals 4.5% of positive farms and 0.8% of all farms) produced 15 positive flocks, which equal 12.7% 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 tests positive at the slaughterhouse (Figure 1).

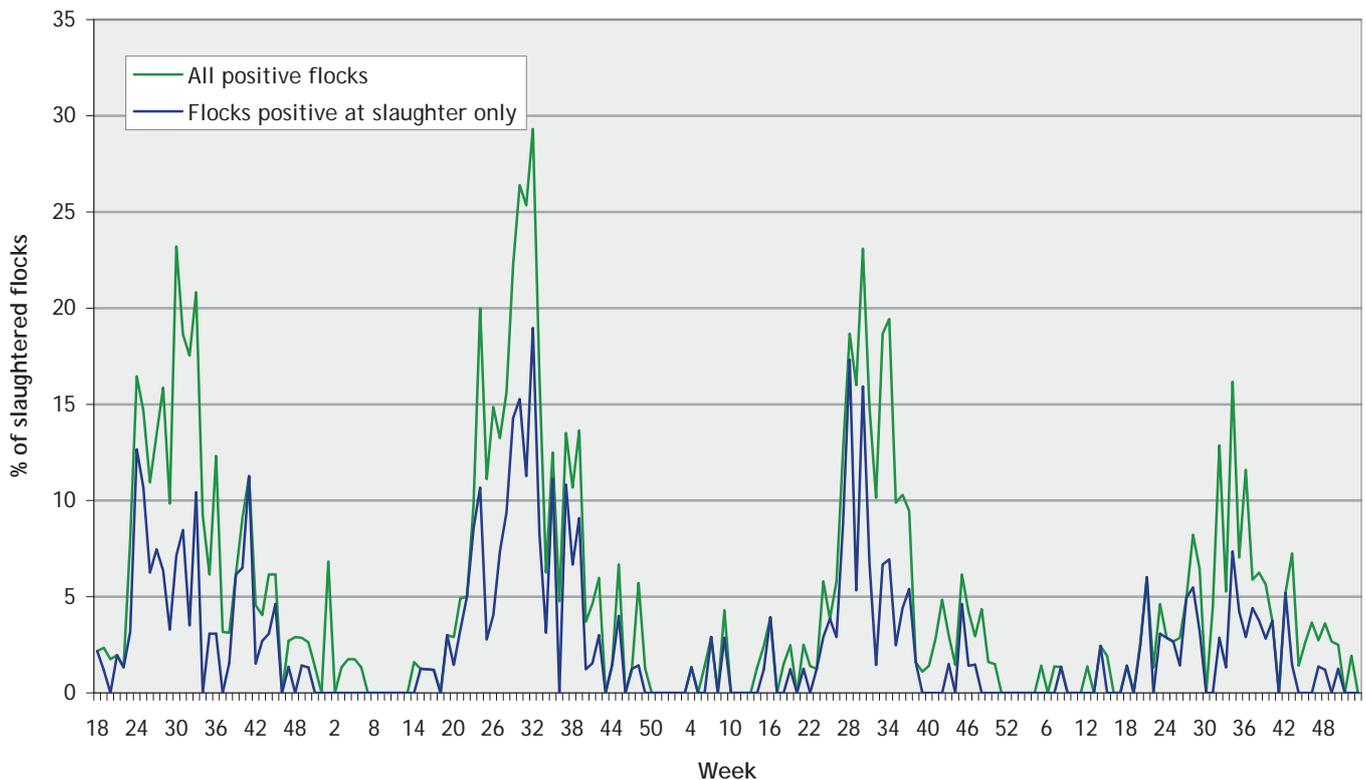


Figure 1. Weekly incidence of *Campylobacter* sp. in slaughtered Norwegian broiler flocks from week 18 in 2001 throughout 2004.

Many *Campylobacter* positive flocks fail to test positive pre-slaughter and are first discovered when the slaughterhouse samples are analysed (49.2% in 2004). A possible explanation may be that the pre-slaughter sample is taken approximately one week before slaughter. As most of the broilers are slaughtered at four - five weeks of age, a large part of their life still remains at pre-slaughter sampling. The possibility to be infected during this last week of life is therefore significant, and the pre-slaughter sample should therefore be taken as close to slaughter as possible. In that way, as many positive flocks as possible are identified before slaughter, and actions can be put in place to prevent exposure of positive products to the consumers.

Previously there have been problems with cross contamination between flocks, either during transport, at the slaughterhouse or at the laboratory. After changing the type of slaughterhouse samples to whole caecae from 1 May 2004 onwards, the problem seems to have disappeared.

For the positive pre-slaughter samples, *C. jejuni* was isolated from 95%, *C. coli* from 3% and *C. lari* from 2% of the samples. For those slaughterhouse samples where the reference laboratory confirmed *Campylobacter* sp.,

*C. jejuni* was isolated from 92%, *C. coli* from 6% and *C. lari* from 1% of the samples.

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

Most farmers follow the guidelines regarding time of pre-slaughter sampling, i.e. eight to four days before slaughter. For 2004, a total of 158 (4.4%) flocks were sampled earlier than eight days before slaughter, mostly in connection with holidays. In total, less than 0.5% of the flocks were not sampled according to the action plan (i.e. sampled only once).

Table 1. *Campylobacter* positive farms and flocks by county in Norway 2004

County	Farms		Flocks	
	N	No. positive (%)	N	No. positive (%)
Østfold	81	9 (11)	612	11 (2)
Akershus	15	2 (13)	111	2 (2)
Hedmark	112	21 (19)	823	33 (4)
Oppland	10	0 (0)	54	0 (0)
Buskerud	11	1 (9)	66	1 (2)
Vestfold	38	3 (8)	255	5 (2)
Telemark	4	1 (25)	26	1 (4)
Aust-Agder	4	0 (0)	24	0 (0)
Vest-Agder	5	1 (20)	35	1 (3)
Rogaland	86	25 (29)	673	30 (4)
Hordaland	16	0 (0)	102	0 (0)
Sogn og Fjordane	1	1 (100)	8	1 (13)
Møre og Romsdal	3	1 (33)	24	1 (4)
Sør-Trøndelag	56	13 (23)	365	16 (4)
Nord-Trøndelag	59	11 (19)	448	16 (4)
<b>Total</b>	<b>501</b>	<b>89 (17.8)</b>	<b>3,626</b>	<b>118 (3.3)</b>

## References

1. Kapperud, G., Espeland G., Wahl E., Walde A., Herikstad H., Gustavsen S., Tveit I., Natas O., Bevanger L., Digranes A., 2003. Factors associated with increased and decreased risk for *Campylobacter* infection. A prospective case-control study in Norway. *Am. J. Epidemiol.* 158, 234-242.
2. Hofshagen, M., Kruse, H., Opheim, M., 2002. The surveillance and control programme for *Campylobacter* in broiler flocks in Norway. In: Fredriksen, B., Mørk, T. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001.* National Veterinary Institute, Oslo, Norway, pp. 143-146.
3. Hofshagen, M., Kruse, H., Opheim, M., 2003. The surveillance and control programme for *Campylobacter* in broiler flocks in Norway. In: Heier, B.T. (Ed.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002.* National Veterinary Institute, Oslo, Norway, pp. 123-128.
4. Hofshagen, M., Bruheim, T., 2004. The surveillance and control programme for *Campylobacter* in broiler flocks in Norway. In: Mørk, T., Hellberg, H. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003.* National Veterinary Institute, Oslo, Norway, pp. 121-123.

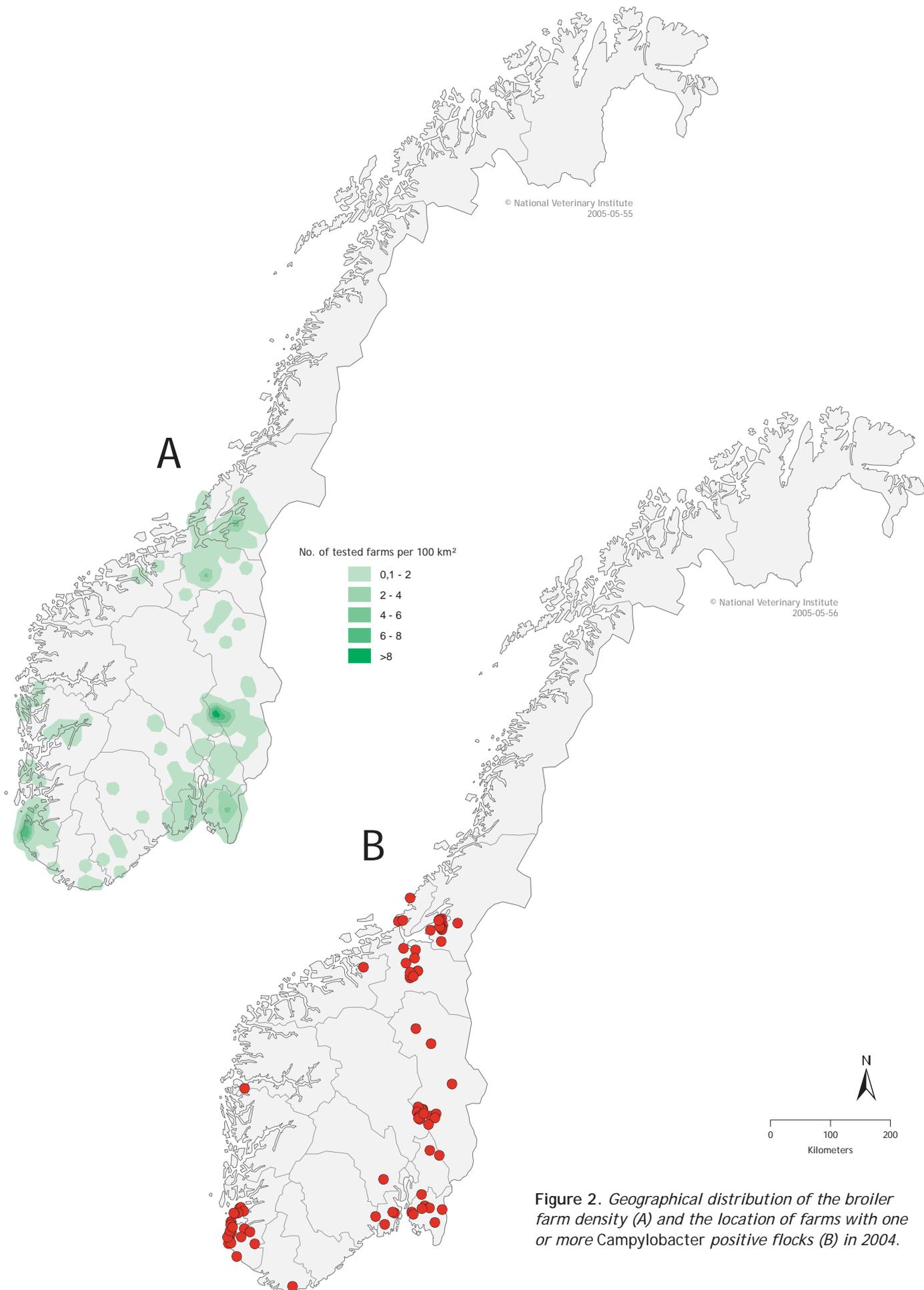


Figure 2. Geographical distribution of the broiler farm density (A) and the location of farms with one or more *Campylobacter* positive flocks (B) in 2004.

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

Annual report 2004

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## Introduction

Viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) are two important infections in salmonids caused by rhabdoviruses. The surveillance and control programme for these two diseases in Norway started in the autumn of 1994. The programme is formally run by the Norwegian Food Safety Authority which is also directly responsible for inspection and sampling. The National Veterinary Institute performs the laboratory procedures in accordance with Commission Decision 2001/183/EC (1) and prepares the report.

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 has caused disease in Pacific cod (*Gadus macrocephalus* (Tilesius)) and Pacific herring (*Clupea harengus pallasii* (Valenciennes)) (2, 3, 4). This strain is not pathogenic to rainbow trout (*Oncorhynchus mykiss* (Walbaum)). VHS virus has been isolated from several different species of marine fish in North European coastal waters (the English Channel, the Baltic Sea, the North Sea, the Norwegian Sea, Skagerak) (2). VHS was reported for the first time in Norway in 1964 and until 1974, several clinical disease outbreaks were diagnosed.

IHN 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.

## Aim

The purpose of the surveillance and control programme is to maintain Norway's status as free from viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN).

For more detailed information on VHS and IHN, reference is made to previous reports of the surveillance and control programmes (6, 7).

## Materials and methods

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 1994 (5).

According to Directive 91/67/EEC (5) and Decision 2001/183/EC (1), virological examinations must be carried out in 50% of all fish farms in which species susceptible to VHS and IHN infection are kept. The samples to be examined for maintenance of VHS/IHN free status, shall contain spleen, anterior kidney, and in addition, either heart or encephalon. Under certain circumstances, ovarian fluid must be examined (brood fish). For fry (<4 cm) the entire fish excluding the body behind the vent shall be examined. According to Decision 2001/183/EC, organ material from 30 fish from each farm 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.

Sampling and inspection is carried out by the District Offices of the Norwegian Food Safety Authority in accordance with approved yearly sampling schedules. The required material is sent to the National Veterinary Institute for analysis.

EU Decision 2001/183/EC (1) gives detailed information on how to carry out the virological examinations and the type of cells to be used in cell culture (BF-2 and EPC or other alternatives given by the EU reference laboratory in Århus (Danish Veterinary Institute)). Furthermore, 2001/183/EC advises on identification of the virus, should a cytopathogenic effect develop from a given sample. Since IPN virus is ubiquitous 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.

## Results

In 2004, material from 375 farms was examined. Table 1 gives an overview of the distribution of sites and species examined in 2004. Table 2 shows the number of farms examined in previous years of the surveillance and control programme. Figures 1 and 2 show the geographical distribution and number of farms by the different species examined in 2004.

No cases of VHS or IHN virus were detected in 2004.

Table 1. Different categories of fish examined for VHS/IHN in 2004\*

	Fry - smolt		Fish for consumption		Brood fish		Total	
	No. sites	No. of samples	No. sites	No. of samples	No. sites	No. of samples	No. sites	No. of samples
Atlantic salmon	31	1040	258	7,710	6	180	295	8,930
Rainbow trout	3	90	44	1,260	1	30	48	1,380
Brown trout	19	740	1	30	1	30	21	800
Arctic char	3	70	2	60			5	130
Turbot			1	40			1	40
Sea trout	1	20			1	30	2	50
Brook trout	2	50					2	50
Relict Atlantic salmon	1	30					1	30
<b>Total</b>	<b>60</b>	<b>2040</b>	<b>306</b>	<b>9,100</b>	<b>9</b>	<b>270</b>	<b>375</b>	<b>11,410</b>

\* Samples received, but deemed unsuitable for analysis, are not included in the table. In total, 450 samples from 15 farms were found unsuitable in 2004 and new samples had to be taken.

Table 2. Number of farms examined for VHS/IHN during the time period 1995-2004

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

## Discussion

In 2004, 11,410 samples from 375 fish farms were sampled compared to 15,150 samples from 498 fish farms in 2003 (8). The Norwegian Food Safety Authority is responsible for selection of sites and sampling.

In 2004, 450 samples from 15 farms were rejected and new samples had to be collected. According to the specifications of Decision 2001/183/EC, the samples must be kept cool during transport; the temperature shall not exceed 10°C.

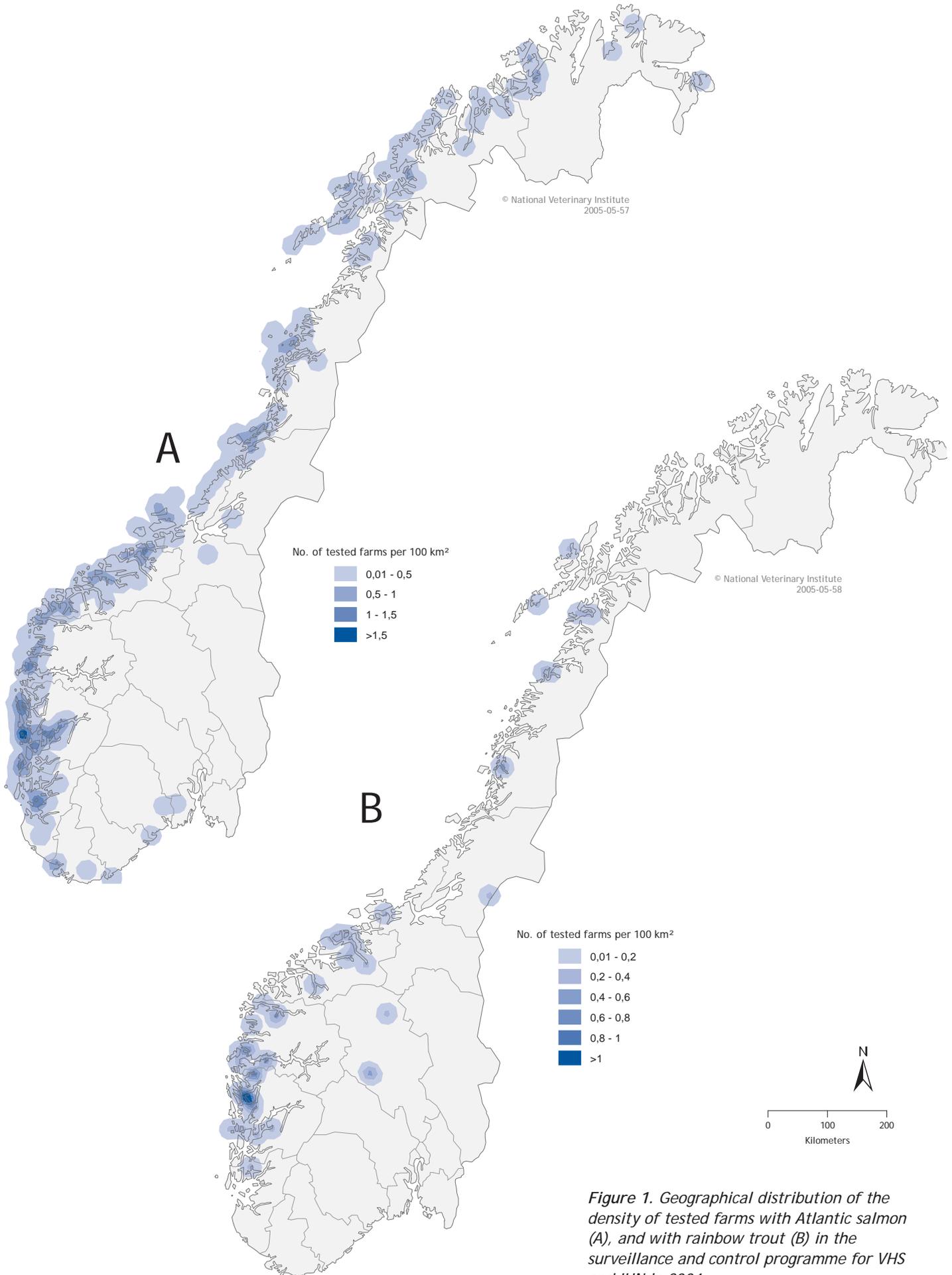
Temperatures exceeding 10°C due to the use of unsuitable transport boxes or delays in postal service were the main reasons for rejection of samples. Sampling instructions will be revised in co-operation with The Norwegian Food Safety Authority to ensure proper cooling and transport of future samples.

## Conclusion

No suspected or confirmed cases of VHS virus or IHN virus have been registered in Norwegian fish farms in 2004, based on the examinations carried out in the surveillance and control programme for VHS and IHN at the National Veterinary Institute.

## References

1. Commission Decision 2001/183/EC of 22 February 2001 laying down the sampling plans and diagnostic methods for the detection and confirmation of certain fish diseases repealing Decision 96/532/EEC.
2. Anonymous, 2003. Diseases of Fish. In: Manual of diagnostic tests for aquatic animals. 5<sup>th</sup> ed. Office International des Epizooties, Paris, part 2.
3. Kocan, R., Bradley, M., Elder, N., Meyers, T., Batts, W., Winton, J., 1995. North American strain of viral haemorrhagic septicaemia virus is highly pathogenic for laboratory reared Pacific herring. *J. Aquat. Animal Health* 9, 279-290.
4. Meyers, T., Winton, J., 1995. Viral haemorrhagic septicaemia virus in North America. *Annual Review of Fish Diseases* 5, 3-24.
5. Council Directive 91/67/EEC concerning the health conditions governing the placing on the market of aquaculture animals and products.
6. Håstein, T., Dannevig, B., Heier, B.T., 2002. The surveillance and control programme for viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) in Norway. In: Fredriksen, B., Mørk, T. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001*. National Veterinary Institute, Oslo, Norway, pp. 147-154.
7. Håstein, T., Dannevig, B., 2003. The surveillance and control programme for viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) in Norway. In: Heier, B.T. (Ed.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002*. National Veterinary Institute, Oslo, Norway, pp. 129-136.
8. Håstein, T., Dannevig, B., Hellberg, H., 2004. The surveillance and control programme for viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) in Norway. In: Mørk, T., Hellberg, H. (Eds.), *Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003*. National Veterinary Institute, Oslo, Norway, pp. 125-132.



**Figure 1.** Geographical distribution of the density of tested farms with Atlantic salmon (A), and with rainbow trout (B) in the surveillance and control programme for VHS and IHN in 2004.

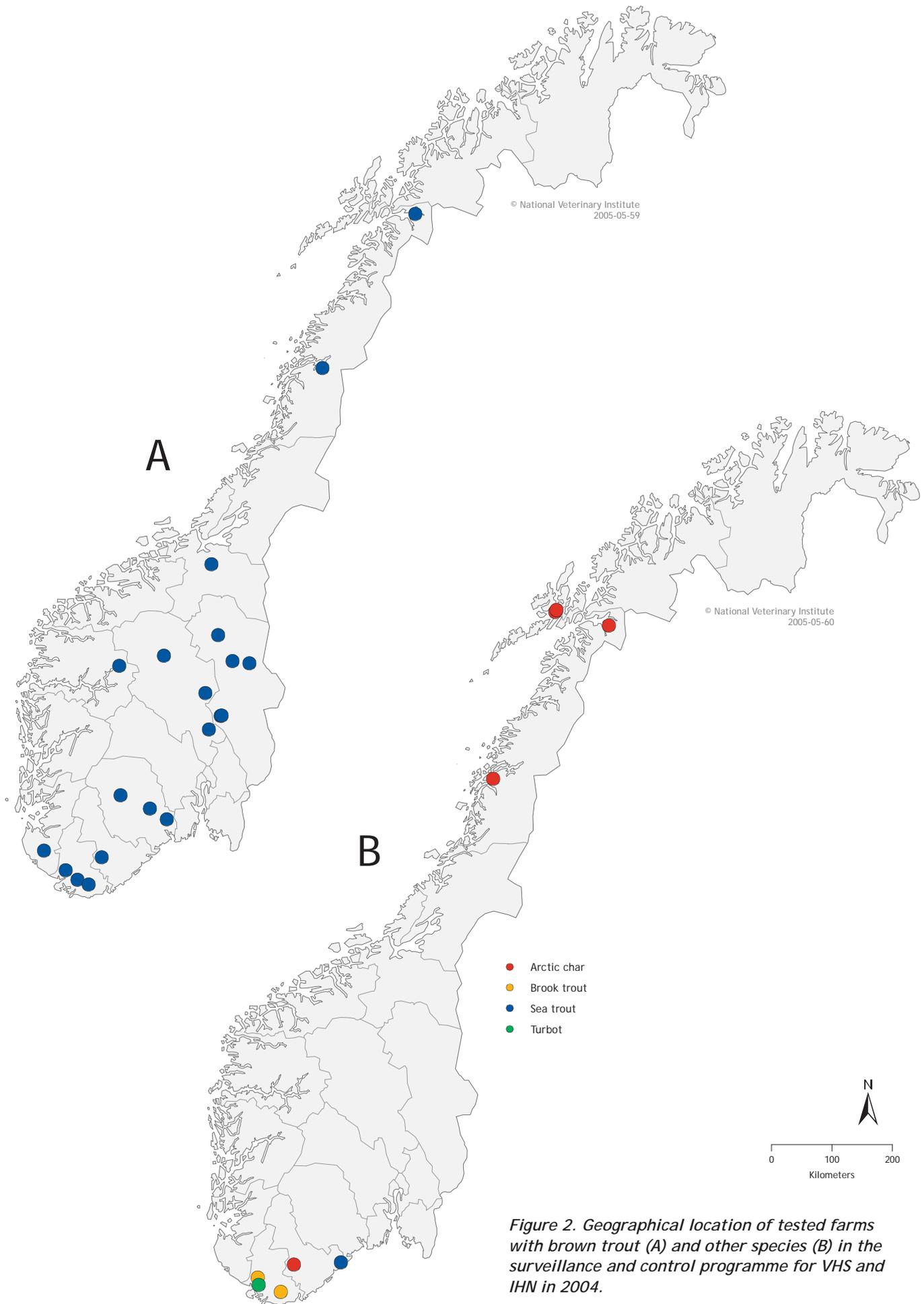


Figure 2. Geographical location of tested farms with brown trout (A) and other species (B) in the surveillance and control programme for VHS and IHN in 2004.



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

Annual report 2004

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## Introduction

*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/smолts and 26 hatcheries/farms with rainbow trout (*Oncorhynchus mykiss*) during the period 1975 to 2004. 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 2004, *G. salaris* was confirmed eradicated from 15 rivers and from all hatcheries/fish farms. For eleven additional rivers the result of the eradication procedure has not yet been confirmed. The parasite is known to be present in 19 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 (1, 2, 3) (from 2004: the Norwegian Food Safety Authority). In 2004 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/smолts 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, 4,509 fish specimens from 120 rivers were examined in 2004.

Table 1. Rivers examined for *Gyrodactylus salaris* in 2004

County	Rivers	Species	No. of fish examined	Detections
Finnmark	9	Atlantic salmon	373	0
Troms	10	Atlantic salmon	291	0
Nordland	22	Atlantic salmon	1251	2 <sup>1</sup>
Nord-Trøndelag	19	Atlantic salmon	807	0
Sør-Trøndelag	5	Atlantic salmon	153	0
Møre og Romsdal	17	Atlantic salmon	457	0
Sogn og Fjordane	17	Atlantic salmon	485	0
Hordaland	6	Atlantic salmon	180	0
Rogaland	2	Atlantic salmon	61	0
Vest-Agder	2	Atlantic salmon	77	0
Aust-Agder	1	Atlantic salmon	30	0
Telemark	0			
Vestfold	3	Atlantic salmon	128	0
Buskerud	1	Atlantic salmon	30	0
Akershus	5	Atlantic salmon	155	0
Oslo	0			
Østfold	1	Atlantic salmon	31	0
<b>Total</b>	<b>120</b>		<b>4,509</b>	<b>2</b>

<sup>1</sup> Reappearance after rotenone treatment.

*G. salaris* re-appeared in two rivers; Leirelva and Halsanelva, in Nordland County. Leirelva was rotenone treated in 1996 and declared free from the parasite in 2003.

Halsanelva was rotenone treated in 2003. Altogether, 1,017 fish specimens from 34 fish farms were examined in 2004 without any observation of *G. salaris*.

Table 2. Fish farms examined for *Gyrodactylus salaris* in 2004

County	Farms	Species	No. of fish examined	Detections
Finnmark	1	Atlantic salmon	30	0
Troms	3	Atlantic salmon	90	0
Nordland	4	Atlantic salmon, rainbow trout	125	0
Nord-Trøndelag	0			
Sør-Trøndelag	0			
Møre og Romsdal	6	Atlantic salmon	180	0
Sogn og Fjordane	6	Atlantic salmon, rainbow trout	140	0
Hordaland	6	Atlantic salmon	179	0
Rogaland	1	Atlantic salmon	55	0
Vest-Agder	2	Atlantic salmon	65	0
Aust-Agder	0			
Telemark	2	Atlantic salmon	62	0
Vestfold	1	Atlantic salmon	31	0
Buskerud	0			
Akershus	0			
Oslo	0			
Østfold	0			
Oppland	2	Rainbow trout	60	0
Hedmark	0			
<b>Total</b>	<b>34</b>		<b>1,017</b>	<b>0</b>

## Conclusion

In 2004, *Gyrodactylus salaris* was detected in Atlantic salmon parr in two rivers (Leirelva and Halsanelva, Nordland county), but not in fish farms. Both rivers had been rotenone treated to eradicate the parasite. Leirelva was rotenone treated in 1996 and declared free from the parasite in 2003, while Halsanelva was rotenone treated in 2003.

## References

1. Mo, T.A., Nordheim, K., 2002. The surveillance and control programme for *Gyrodactylus salaris* in Atlantic salmon and rainbow trout in Norway. In: Fredriksen, B., Mørk, T. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2001. National Veterinary Institute, Oslo, Norway, pp. 155-159.
2. Mo, T.A., Nordheim, K., 2003. The surveillance and control programme for *Gyrodactylus salaris* in Atlantic salmon and rainbow trout in Norway. In: Heier, B.T. (Ed.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2002. National Veterinary Institute, Oslo, Norway, pp. 137-141.
3. Mo, T.A., Nordheim, K., 2004. The surveillance and control programme for *Gyrodactylus salaris* in Atlantic salmon and rainbow trout in Norway. In: Mørk, T., Hellberg, H. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003. National Veterinary Institute, Oslo, Norway, pp. 135-137.



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

Annual report 2004

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## Introduction

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 6 November 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. Eight sites along the Norwegian coast have been sampled for several years; however, the wild population in Oslofjorden was not included in 2004 due to declining numbers of flat oysters (Figure 1). Selection of sampling sites was based on the geographical distribution and size of wild populations, and the structure of the oyster industry.

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. A total of 4,810 oysters were examined during the initial two-year control period 1995-1997. *Bonamia* sp. or *Marteilia refringens* were not observed. During the following years until 31 December 2003, a total of 3,330 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 Haematoxylin-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 2004, the National Veterinary Institute in Bergen received a total of 420 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 and marteiliosis in 2004

Sample site	Spring 2004	Autumn 2004	Total 2004
1	*	*	0
3	30	30	60
4	30	30	60
5	30	30	60
6	30	30	60
7	30	30	60
8	30	30	60
9	30	30	60
<b>Total: 8</b>	<b>210</b>	<b>210</b>	<b>420</b>

\* Oslofjorden. Not sampled

## 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.

## References

1. Mortensen, S.H., 1993. A health survey of selected stocks of commercially exploited Norwegian bivalve molluscs. *Dis. Aquat. Organ.* 16, 149-156.
2. Hellberg, H., Mortensen, S.H., Hernar, I., Knutsen, G.H., 2002. Resultater fra overvåknings- og kontrollprogrammet for parasittene *Bonamia ostreae* og *Marteilia refringens* i norske bestander av europeisk flatøsters (*Ostrea edulis* L.) (Results from the surveillance and control programme for the parasites *Bonamia ostreae* and *Marteilia refringens* in Norwegian populations of European flat oysters (*Ostrea edulis* L.)). *Nor. Vet. Tidsskr.* 114, 13-17.
3. Steins, N.A., 1997. *Ostrea edulis* in crisis. ESSFiN Concerted Action Workshop 2 Northern Waters, Aarhus, Denmark.
4. Grizel, H., 1979. *Marteilia refringens* and oyster disease - recent observations. *Mar. Fish. Rev.* 41, 38-39.
5. Pichot, Y., Comps, M., Tige, G., Grizel H, Rabouin, M-A., 1981. Recherches sur *Bonamia ostreae* gen. n., sp. n., parasite nouveau de l'huitre plate *Ostrea edulis* (Studies on *Bonamia ostreae* gen. n., sp. n., a new parasite of the European flat oyster, *Ostrea edulis* L.) *Rev. Trav. Inst. Pêch. Marit.* 43, 131-140.
6. EFTA Surveillance Authority Decision No. 225/04/COL of 9 September 2004.
7. Commission Decision 2002/878/EC of 6 November 2002 establishing the sampling plans and diagnostic methods for the detection and confirmation of the mollusc diseases Bonamiosis (*Bonamia ostreae*) and Marteiliiosis (*Marteilia refringens*).
8. Anonymous, 2003. Diseases of Molluscs. In: Manual of Diagnostic Tests for Aquatic Animals. 5<sup>th</sup> ed. Office International des Epizooties, Paris, part 3.
9. Hellberg, H., 2004. The surveillance and control programme for bonamiosis and marteiliosis in European flat oysters (*Ostrea edulis* L.) in Norway. In: Mørk, T., Hellberg, H. (Eds.), Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2003. National Veterinary Institute, Oslo, Norway, pp. 149-153.

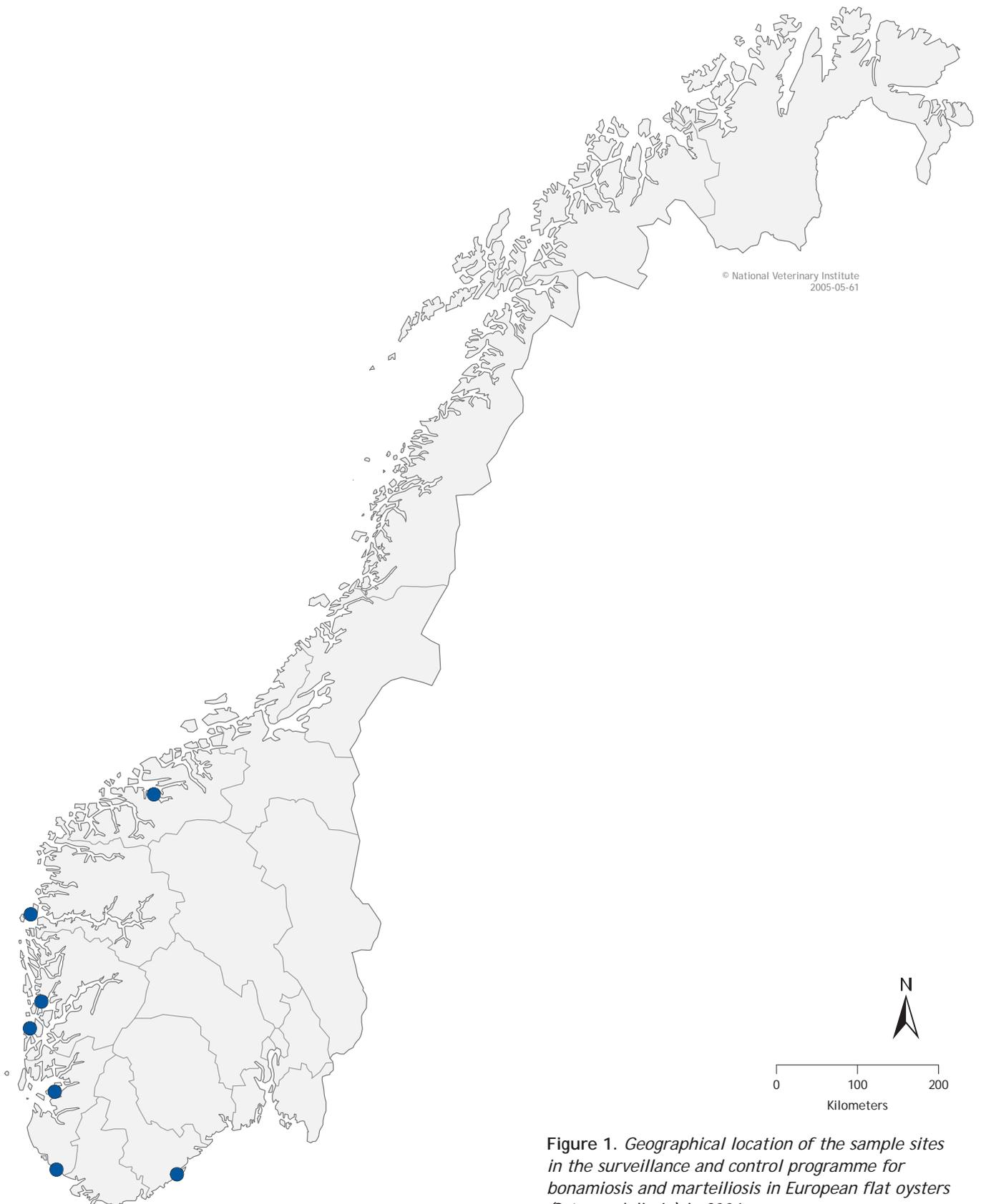


Figure 1. Geographical location of the sample sites in the surveillance and control programme for bonamiosis and marteiliosis in European flat oysters (*Ostrea edulis* L.) in 2004.

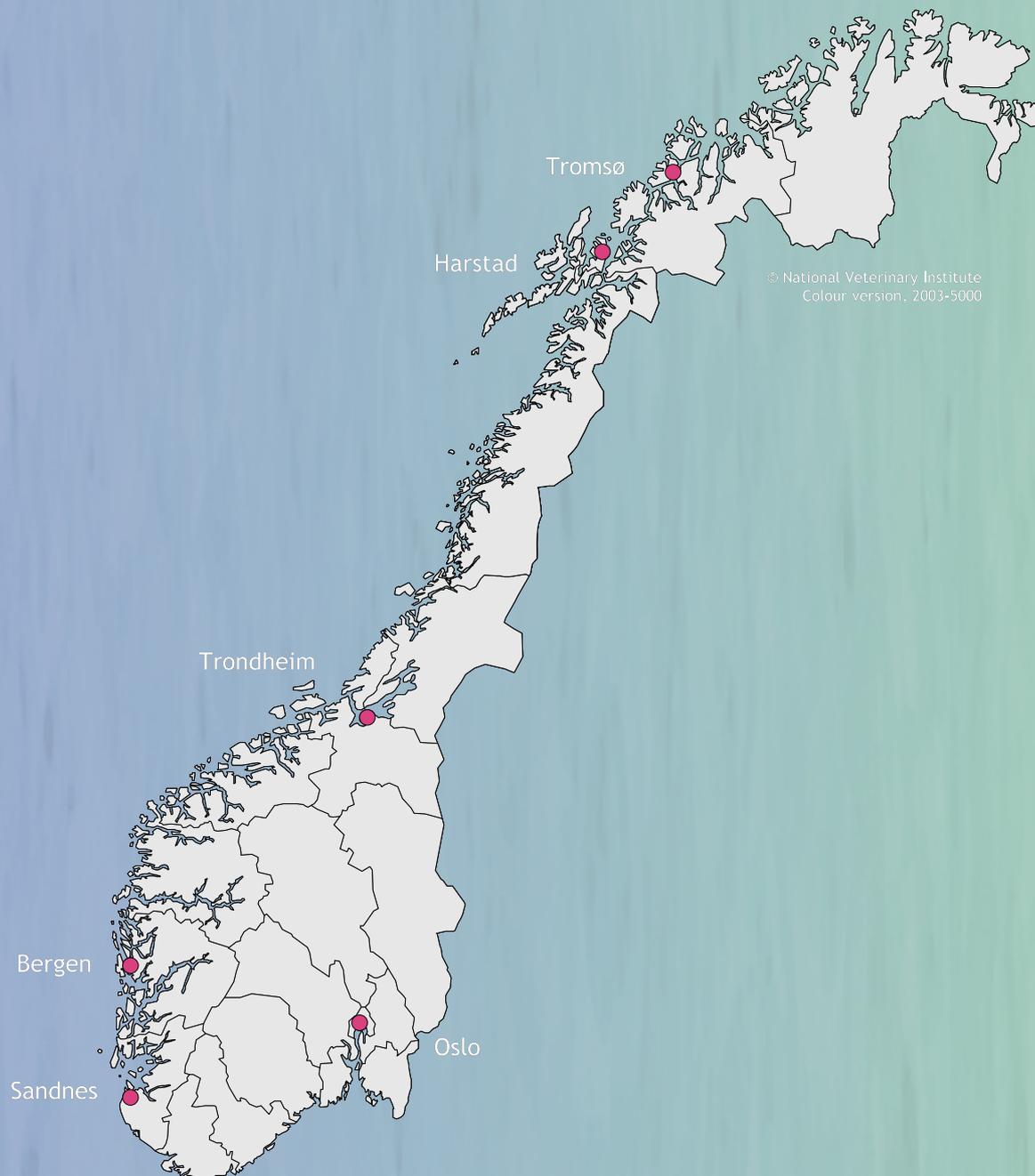




The National Veterinary Institute is a government research institution, which provides scientifically based advice to the authorities on food and feed safety and animal, fish and shellfish health. The institute performs surveillance, offers diagnostic services and maintains preparedness to deal with emergency disease situations and other important matters related to health and environment.

The institution is comprised of the central laboratory and administration located in Oslo and the regional laboratories in Sandnes, Bergen, Trondheim, Harstad and Tromsø.

The Norwegian Zoonosis Centre is organised within the National Veterinary Institute in cooperation with the National Institute of Public Health.



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