



# The surveillance programme for bovine tuberculosis in Norway 2022



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## Summary

In 2022, organ samples from 70 cattle, one wild red deer and six alpacas were submitted to the Norwegian Veterinary Institute for the surveillance of bovine tuberculosis. One of the 70 samples were submitted following suspicion of bovine tuberculosis in a cow. *Mycobacterium bovis* was detected in multiple organ samples from one cow, suspected of infection with bovine tuberculosis while *M. caprae* and *M. tuberculosis* was not detected in any of the organ samples analysed in 2022. This is the first confirmed bovine tuberculosis case in cattle reported in Norway since 1986.

## Introduction

Apart from two single-herd outbreaks in Sogn og Fjordane county in 1984 and 1986, Norway has been considered free from bovine tuberculosis since 1963 (1). Since 1994, the EFTA (The European Free Trade Association) Surveillance Authority (ESA) has recognised Norway as officially free from bovine tuberculosis, as described in ESA Decision 032/21/COL.

In 2000, a surveillance programme for bovine tuberculosis in cattle was launched. Today the programme includes investigation of suspicious organ samples from cattle, llamas, alpacas, farmed and wild deer. From 2022 specific abattoirs were selected to submit a minimum number of tissue samples from lungs and lymph nodes with pathological lesions. Tuberculosis caused by three members of the *Mycobacterium tuberculosis* complex (MTC) group (*M. bovis*, *M. caprae* and *M. tuberculosis*) is classified as a list 2 disease in Norway. Bovine tuberculosis is listed by the World Organisation for Animal Health (WOAH) and is a category B, D and E disease in the EU.

## Aim

The aim of the surveillance programme is to document absence of bovine tuberculosis, according to demands in Regulation (EU) 2020/689, and to contribute to the maintenance of this favourable situation.

## Materials and methods

### Submission of organ samples from abattoirs and post mortem examinations

The Norwegian Food Safety Authority (NFSA) submits representative specimen from lung tissue, lymph nodes and other organs with suspicious pathological lesions compatible with tuberculosis during compulsory veterinary inspection of bovine carcasses at slaughter. The material is submitted to the Norwegian Veterinary Institute (NVI) for further examination. In cases where the NFSA strongly suspect bovine tuberculosis, samples are submitted as a suspicion of bovine tuberculosis, with specific routines in terms of notification and precautions taken before sample examination.

In addition, the NFSA, at specific abattoirs pre-selected by the NVI, submits samples from lungs and lymph nodes with pathological lesions from a minimum number of cattle. In 2022 the selected abattoirs were asked to submit 70 samples. These are samples with pathological lesions resembling tuberculous lesions (processes etc.), however, not the typical suspicious tuberculous lesions. Abattoirs that slaughter cattle from areas with a dense population of free ranging red deer are included. These areas are situated in the western part of Norway, from the county of Rogaland in the south to the county of Trøndelag in the north.

Farmed deer, llamas and alpacas are selected when the NFSA receive reports of dead or euthanized sick animals. Organ samples from wild deer are submitted by hunters through the wildlife surveillance programme (Vilt-HOP). The Norwegian Veterinary Institute performs most of the post mortem examinations of the farmed deer, llamas and alpacas. Some necropsies are performed in the field. If the result does not demonstrate any pathological tissue lesions consistent with tuberculosis, no further examination is performed.

### Gross examination

At NVI all macroscopic tissue examinations are performed by pathologists. The lesions are described in terms of their size, shape, colour, texture, numbers, etc.

### 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, sectioned at 3 µm and stained with haematoxylin and eosin (HE). Ziehl-Neelsen staining is used to demonstrate acid-fast bacteria in suspected cases of mycobacterial infection (granulomatous lesions).

### Molecular examination

Extracted nucleic acids from tissue samples are analysed by real-time PCR. The PCR test is based on the amplification of the insertion sequence segment IS6110 target, present within the members of the *Mycobacterium tuberculosis* complex (MTC).

### Bacteriological examination

Samples are examined as described in the WOAH manual (2). Samples are homogenised, decontaminated with 5% oxalic acid and centrifuged. The sediment is used for culturing and for microscopic examination for acid-fast bacilli after staining with Ziehl-Neelsen. The sediment is

inoculated onto slopes of Löwenstein Jensen medium, Stonebrink's medium, Middelbrook 7H10 medium with and without antibiotics supplement, and Dubos medium. The slopes are incubated under aerobic conditions at 37°C for two months and checked every week for growth of acid-fast bacilli, determined by the Ziehl-Neelsen method. If colonies of acid-fast bacilli are detected, molecular methods are used for species identification.

## Results and discussion

Table 1 shows the number of samples submitted and the number of positive samples since the programme started in 2000. In 2022, samples from 70 bovine, one wild red deer and six alpacas were submitted. One of the bovine samples were submitted following a strong suspicion of bovine tuberculosis after the finding of multiple granulomatous lesions in one cow at slaughter. Histopathological examination was performed on material from all submitted cases. Tissue samples from seven bovines and one wild red deer were further investigated by bacteriological culturing for the detection of *Mycobacteria*. The examinations confirmed that the lesions in the suspected bovine case was due to infection with *Mycobacterium bovis*. This is the first confirmed bovine tuberculosis case in cattle reported in Norway since 1986. The other samples from cattle were negative by culture.

The wild red deer had an infection with *Mycobacterium kansasii* which is an environmental bacterium that can cause opportunistic infections in animals and humans. Infections due to *M. kansasii* can resemble tuberculosis caused by MTC, both in the clinical presentation of disease and the pathologic lesions.

All animals in the herd, from which the positive cow originated, were humanely slaughtered and the outbreak was further investigated by the NFSA.

The low number of samples submitted due to gross pathological tissue lesions at post mortem inspection prior to 2022, indicates a low prevalence of suspicious lesions. In 2022, an effort was made to increase the number of samples by asking selected abattoirs to submit lungs and lymph nodes with pathological lesions from a minimum number of cattle; 69 organ samples were submitted. These pathological lesions were not highly suspicious for tuberculosis, and consisted mostly of pulmonary processes of various types, including regional lymph nodes. Infection due to *Mycobacterium* spp. was not detected in any of these samples.

In addition, one sample was submitted due to suspicion of bovine tuberculosis in a cow. *Mycobacterium bovis* was detected in the sample material. This shows the importance of continuous surveillance and vigilance by the veterinary meat inspection.

*Table 1: Samples submitted for testing of bovine tuberculosis from 2000 to 2022, and number of positive samples.*

Year*	No. of cattle samples	No. of cattle herds	No. of positive cattle samples	No. of camelid samples	No. of camelid herds	No. of positive camelid samples	No. of red deer samples	No. red deer herds	No. of positive red deer samples
2000	0	0	0						
2001	3	3	0						
2002	0	0	0						
2003	1	1	0						
2004	4	4	0						
2005	1	1	0						
2006	3	3	0						
2007	0	0	0						
2008	4	2	0						
2009	1	1	0						
2010	1	1	0						
2011	1	1	0						
2012	0	0	0						
2013	5	4	0						
2014	1	1	0	1	1	0			
2015	2	2	0	15	14	0			
2016	3	3	0	11	10	0			
2017	1	1	0	14	12	0			
2018	1	1	0	9	9	0			
2019	2	2	0	5	5	0			
2020	2	2	0	5	5	0			
2021	5	4	0	2	2	0	1	1	0
<b>2022</b>	<b>70<sup>1</sup></b>	<b>69</b>	<b>1</b>	<b>6</b>	<b>5</b>	<b>0</b>	<b>1<sup>2</sup></b>	<b>1<sup>2</sup></b>	<b>0</b>

1 One of the samples was submitted following suspicion of bovine tuberculosis

2 Wild animal from one municipality

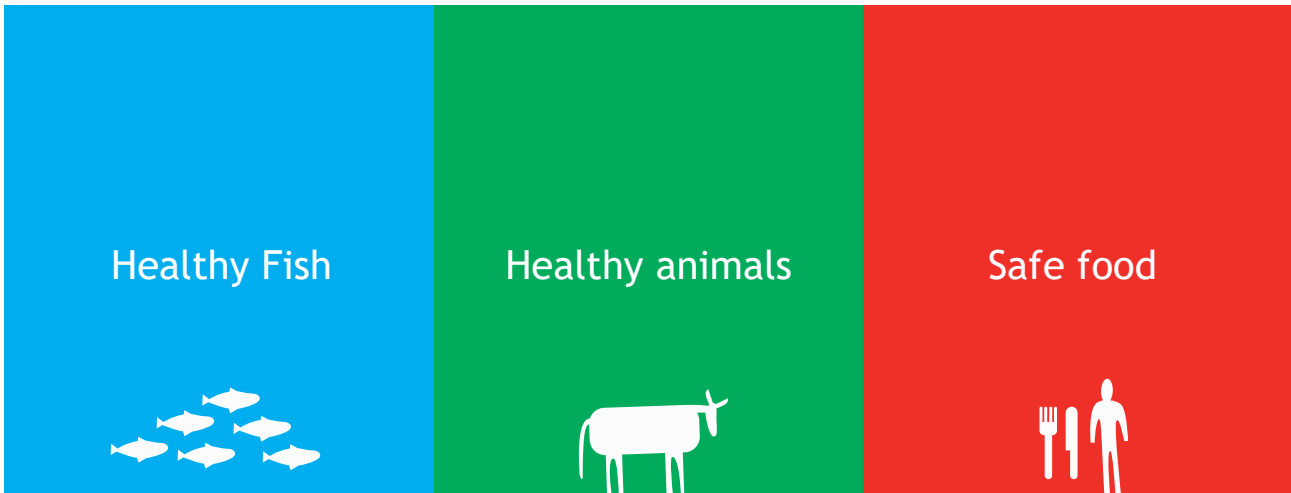
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