



The surveillance programme for bovine tuberculosis in Norway 2024

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Authors

Lise Marie Ånestad, Bjørnar Ytrehus, Girum Tadesse Tessema, Øyvør Kolbjørnsen, and Petter Hopp

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Summary

In 2024, the Norwegian Veterinary Institute received organ samples from 11 cattle (including one suspected bovine tuberculosis case), carcasses from 12 alpacas, and lymph node samples from 48 wild red deer collected in 2023 and 42 in 2024, for bovine tuberculosis investigation.

Gross pathology, histopathology, and standard bacteriological examinations of cattle and alpaca samples revealed findings inconsistent with bovine tuberculosis. Therefore, these samples were not tested for mycobacteria. All wild red deer samples were tested, and no *Mycobacterium tuberculosis* complex bacteria (*M. bovis*, *M. caprae*, *M. tuberculosis*) were detected.

Additionally, as part of the investigation following the 2022 outbreak of *M. bovis* in Norway, lymph node samples from nine cattle in an indirect contact herd were examined, after one animal was considered positive (reactor) following two consecutive inconclusive intradermal skin tests. All samples tested negative for *Mycobacterium tuberculosis* complex bacteria.

Introduction

Bovine tuberculosis is a chronic infectious disease caused primarily by *Mycobacterium bovis*, which can infect both animals and humans. When infected, nodular granulomas (tubercles) may develop, most frequently in the lungs and associated lymph nodes, but also in other organs. The infection is often subclinical, but when clinical signs occur, they are non-specific and may include weakness, weight loss, cough, and enlarged lymph nodes. Cattle are the main reservoir of *M. bovis*, though the disease has a broad range of hosts. In some countries, wild animals serve as a reservoir and transmit infection to livestock (1, 2).

In Norway, 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. Within the EU, the infection is categorized under disease category B in cattle, buffalo, and bison; D in other artiodactyls; and E for other mammals. Classification as category B implies that the disease must be controlled in all Member States with the goal of eradicating it throughout the Union. Bovine tuberculosis is also listed by the World Organisation for Animal Health (WOAH).

Norway has been considered free from bovine tuberculosis since 1963, apart from two single-herd outbreaks in Sogn og Fjordane county in 1983 and 1986 (3). Since 1994, the EFTA (European Free Trade Association) Surveillance Authority (ESA) has officially recognised Norway as free from bovine tuberculosis, as described in ESA Decision 032/21/COL.

In 2022, a new case of bovine tuberculosis caused by *M. bovis* was confirmed in a cattle herd in Rogaland County, marking Norway's first case since 1986. The detection followed an official post-mortem inspection at the slaughterhouse, during which a bovine was found with pathological lesions consistent with tuberculosis. Organ samples were subsequently submitted to the Norwegian Veterinary Institute (NVI), where the diagnosis was confirmed. The Norwegian Food Safety Authority (NFSA) initiated outbreak investigations and contact tracing to prevent further spread, eliminate the disease, and identify the source of infection. Additional animals in the primary establishment, and animals in two contact herds tested positive on immunological tests, one in 2022 and another in 2023. Norway's official disease-free status at the national level was not affected by the outbreak. The affected establishments restored their official disease-free status after documentation of fulfilling the disease-specific legislative requirements in Regulation (EU)2020/689.

Since 2000, a national surveillance programme for bovine tuberculosis in cattle has been in place. Today, the programme includes investigation of organ samples from cattle, llamas, alpacas, and both farmed and wild red deer. From 2022, selected abattoirs were asked to submit a minimum number of tissue samples from lungs and lymph nodes with pathological lesions where tuberculosis could not be ruled out (e.g. parasite nodules, enlarged lymph nodes). The broad sampling criterion was introduced in an effort to increase the sample size of the programme. After the 2022 outbreak, the surveillance programme was further expanded in 2023 to include

surveillance of wild red deer in the municipalities surrounding the dairy herd where *M. bovis* was first detected. In red deer, surveillance included an information campaign to raise awareness among hunters, encouraging them to report suspicious lesions to local authorities, and examine lymph nodes collected from clinically healthy felled animals (4).

The NFSA is responsible for implementing the surveillance programme for bovine tuberculosis. The NVI is responsible for planning the programme, coordinating the collection of samples from wild red deer, performing the organ sample investigations, and reporting the results. Organ samples from cattle at abattoirs and from fallen stock (camelids and farmed red deer) in the field, are collected by NFSA inspectors.

Aim

The aim of the surveillance programme is to contribute to documenting the absence of bovine tuberculosis, in accordance with the requirements of Regulation (EU) 2020/689, and to support the maintenance of this favourable situation.

Materials and methods

Collection of material and post-mortem examinations

Organ samples from abattoirs

The NFSA submit representative specimen from lung tissue, lymph nodes, and other organs with pathological lesions where tuberculosis cannot be ruled out (e.g. nodular structures, enlarged lymph nodes), collected during compulsory veterinary inspection of bovine carcasses at slaughter. In addition, the NVI makes a selection of abattoirs where the NFSA is asked to submit lung and lymph node samples from a minimum number of cattle with such lesions. In 2024, this selection included abattoirs slaughtering cattle from herds epidemiologically linked to the herd where bovine tuberculosis caused by *M. bovis* was confirmed in 2022. The selected abattoirs were located in Rogaland and Vestland counties and were asked to submit samples from 70 cattle.

All samples are submitted to the NVI for further examination. When the NFSA strongly suspect bovine tuberculosis, samples are submitted as suspicious cases, with specific protocols for notification and precautions prior to examination.

At the NVI, all abattoir samples undergo gross and histopathological examinations, with many also analysed by routine bacteriology. Samples showing lesions consistent with tuberculosis are further tested by PCR and/or bacteriological culture for mycobacteria.

Organ samples from fallen stock

Farmed red deer, llamas, and alpacas are included in the programme when the NFSA receive reports of dead or euthanized sick animals. Organ samples from wild red deer are submitted either by the NFSA when they find suspicious pathological changes during wildlife meat inspection, or when they are called by hunters that have observed such lesions. In some cases, hunters, rangers, and wildlife managers submit samples directly to the NVI. The NVI performs most of the post-mortem examinations of the farmed deer, llamas, and alpacas. Some necropsies are performed in the field. If no pathological lesions consistent with tuberculosis are identified, no further examination is performed.

Organ samples from wild red deer in specific municipalities

In 2023 and 2024, retropharyngeal lymph nodes from wild red deer older than 1.5 years, felled in Suldal municipality and collected by hunters through the chronic wasting disease surveillance programme, were subjected to PCR analysis for bovine tuberculosis. Additionally, in 2024, hunters in the neighbouring municipalities, Vindafjord and Tysvær, were asked to submit lymph node samples from all wild red deer older than 1.5 years, for the same purpose.

Results from both years are included in this report, as the analyses were conducted after the publication of the 2023 report.

Gross and histopathological examinations and microbiological analyses

Gross examination

At the 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 standard routine protocol, embedded in paraffin, sectioned at 3 µm, and stained with haematoxylin and eosin (HE). Ziehl-Neelsen (ZN) 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 direct PCR test is based on the amplification of the insertion sequence segment IS6110 target, present within the members of the *Mycobacterium tuberculosis* complex (MTC) from tissue samples (5).

Bacteriological examination

Samples are examined as described in the WOAHP manual (1). 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

Organ samples from the surveillance program

In 2024, organ samples from 10 cattle and carcasses of 12 alpacas were submitted to the surveillance programme for bovine tuberculosis. Gross and histopathological examinations were performed on tissues from all submitted cases. None of the samples had findings indicative of infection due to *Mycobacterium* spp. In cattle, the most common finding was bronchopneumonia in various forms. Reactive lymphadenopathy was frequently observed in the regional lymph nodes. As no pathological lesions were consistent with tuberculosis, no PCR or bacteriological culture for mycobacteria were performed.

Lymph node samples from 48 wild red deer collected in 2023 and 42 collected in 2024 were examined by real-time PCR. *Mycobacterium tuberculosis* complex was not detected in any of the samples. The number of submitted samples from wild red deer in 2024 was relatively low compared to the total harvest of 1,166 deer across the three municipalities that year, including 366 deer older than 1.5 years. Therefore, the limited sample size may be insufficient to draw conclusions about the presence or absence of bovine tuberculosis in the deer population.

Suspicious organ samples submitted

Organ samples from one additional bovine were submitted by the NFSA after post-mortem inspection due to suspicion of bovine tuberculosis, according to the requirements of Regulation (EU) 2020/689. Gross-, histopathological-, and standard bacteriological examinations of the lungs and lymph nodes were consistent with pyogranulomatous pneumonia caused by *Actinomyces bovis*. Since tuberculosis was not suspected, no further analyses for *Mycobacterium* spp. were conducted.

The number of samples submitted and the number of positive samples since the programme started in 2000, are presented in Table 1.

Table 1. Samples submitted for testing of bovine tuberculosis from 2000 to 2024, 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/ municipalities	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
2022	70 ¹	69	1	6	5	0	1 ²	1 ²	0
2023	67 ¹	56	0	4 ³	2	0	48 ^{2,4}	1 ²	0
2024	11 ¹	11	0	12	7	0	42 ²	3 ²	0

1 Includes samples submitted following suspicion of bovine tuberculosis: one sample in 2022, five in 2023, and one in 2024.

2 Wild animals from one municipality in 2022, one in 2023, and three in 2024.

3 Includes samples submitted following suspicion of bovine tuberculosis, one sample in 2023.

4 The samples from wild red deer were not included in the 2023 report, as their analyses were conducted after its publication.

Prior to 2022, relatively few samples were submitted from cases with suspicious lesions, or from lesions where tuberculosis could not be ruled out during post-mortem inspection, suggesting a low prevalence of such findings. Since 2022, after selected abattoirs were asked to submit samples from a minimum number of cattle, submissions increased in 2022 and 2023. In 2024, however, the number of submitted samples declined again, likely due to a limited number of organs with lesions fulfilling the submission criteria.

Organ samples from the 2022 outbreak investigation

In 2024, as part of follow-up to the 2022 outbreak investigation, an indirect contact herd was screened using the comparative intradermal skin test. One bovine animal returned two consecutive inconclusive results and was therefore considered positive (reactor). This animal, along with eight cohort cattle, was subsequently slaughtered. No suspicious lesions were observed during post-mortem examinations. Samples from lymph nodes associated with the head, lungs, and intestines of all nine animals were submitted to the NVI. Gross and histopathological examinations did not indicate bovine tuberculosis. All samples were analysed by direct PCR and all samples from seven animals were cultured for mycobacteria. No *Mycobacterium tuberculosis* complex bacteria were detected in any of the samples. The herd continues to be monitored by the NFSA.

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postmottak@vetinst.no

vetinst.no