

The surveillance programme for bovine tuberculosis in Norway 2023



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Authors

Marie Myklatun Krosness, Petter Hopp, Øyvor Kolbjørnsen, Girum Tadesse Tessema, Bjørnar Ytrehus

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Summary

In 2023, organ samples from 67 cattle, one wild red deer and four alpacas were submitted to the Norwegian Veterinary Institute for the surveillance of bovine tuberculosis. Of these, samples from five cattle and one alpaca were submitted due to suspicion of bovine tuberculosis. Mycobacterium tuberculosis complex (M. bovis, M. caprae, M. tuberculosis) was not detected in any of the samples. Organ samples from animals with positive immunological tests in the outbreak investigation following the detection of Mycobacterium bovis in a herd in 2022, are not included in this report.

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 2022 bovine tuberculosis was detected in organ samples from several cattle in one herd in Norway. The herd was humanely slaughtered. Norway still has official free status for bovine tuberculosis from the ESA.

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 red deer. From 2022 specific abattoirs were selected to submit a minimum number of tissue samples from lungs and lymph nodes with pathological lesions. In 2023, lymph nodes from wild red deer in Suldal municipality submitted through the surveillance programme for Chronic Wasting Disease were kept for analysis for bovine tuberculosis. These will be analysed in 2024 and are not further discussed in this report.

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 2023, 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. In 2023 these were samples from abattoirs that slaughter cattle from herds with an epidemiological link to the herd where bovine tuberculosis was confirmed in 2022. The abattoirs are located in Rogaland and Vestland counties.

Farmed red deer, llamas and alpacas are selected when the NFSA receive reports of dead or euthanized sick animals. Organ samples from wild red deer are submitted either by NFSA when they find suspicious pathological changes during wildlife meat inspection or when they are called by hunters that have observed such lesions, or hunters submit samples themselves. The NVI 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 2023, samples from 62 bovine, one wild red deer and three alpacas were submitted. Infection due to *Mycobacterium* spp. was not detected in any of these samples. The pathological lesions were not highly suspicious for tuberculosis and the samples from cattle consisted mostly of pulmonary processes of various types, including regional lymph nodes. Histopathological examination was performed on material from all submitted cases. None of the samples were further investigated by bacteriological culturing for the detection of *Mycobacteria*.

Organ samples from one wild deer was submitted from the NFSA. Histopathological and Ziehl-Neelsen staining of tissue samples demonstrated the presence of acid fast bacteria. The samples were further analysed with real-time PCR and *Mycobacterium avium subsp. avium* was detected. Infection due to the *Mycobacterium tuberculosis* complex was not detected.

In addition, organ samples from five bovines were submitted from the NFSA post mortem inspection due to suspicion of bovine tuberculosis. One alpaca was also submitted due to suspicion of tuberculosis. Tissue samples from two bovines were further investigated by bacteriological culturing for the detection of *Mycobacteria*. Infection due to *Mycobacterium* spp. was not detected in any of these samples.

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. Since 2022, an effort has been 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; resulting in an increased number of organ samples being submitted in 2022 and 2023.

Table 1: Samples submitted for testing of bovine tuberculosis from 2000 to 2023, 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	70¹	69	1	6	5	0	1 ²	1 ²	0
2023	67 ¹	56	0	4 ³	2	0	1 ²	1 ²	0

 $^{1\ \}text{Includes samples submitted following suspicion of bovine tuberculosis, 1 and 5 samples in 2022 and 2023\ respectively.}$

Acknowledgement

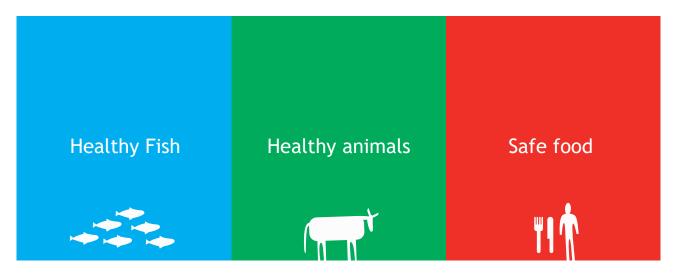
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² Wild animal from one municipality.

³ Includes samples submitted following suspicion of bovine tuberculosis, 1 sample in 2023.

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