



The surveillance programme for *Brucella melitensis* in small ruminants in Norway 2023

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Summary

Brucella melitensis was not detected in any sheep flock or goat herd sampled in 2023.

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 females and can cause orchitis and epididymitis in affected males (1, 2). *Brucella melitensis* infection is a zoonosis, and the bacterium causes a serious infection in humans known as Malta fever, characterised by undulant fever, chills, sweat and debilitation (2).

Brucella melitensis is prevalent in sheep and goats in several Mediterranean countries (1), but has never been diagnosed in animals in Norway or any of the other Nordic countries (3, 4). Brucellosis is classified as a list 1 disease in Norway and is notifiable to the World Organisation for Animal health (WOAH). *Brucella* spp. are notifiable (classified as Category B diseases) in the EU, and must be controlled in all member states with the goal of eradicating it throughout the union. Norway has a status as free from *Brucella* in the EU since 2021.

A surveillance programme for *B. melitensis* in sheep was established in 2004, and goats were included in the programme from 2007.

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

Aims

The aims of the programme are to document freedom from *B. melitensis* in sheep and goats according to the demands in Regulation (EU) 2020/689 and to contribute to the maintenance of this favourable situation.

Materials and methods

In sheep, the programme in 2023 was based on serological screening of representative numbers of blood samples from slaughtered animals at 20 larger abattoirs from various parts of Norway. The preferred sampling period was from January to March, but a proportion of the animals were sampled from August to December. A maximum of five animals (>2.5 years old) were to be sampled per herd any given day. Collection of 10,000 blood samples from sheep taken at slaughter was planned.

In addition, two sheep flocks that had submitted samples to NVI from abortions in 2022, and 18 sheep flocks were randomly selected for sampling. In herds of less than 30 animals, all animals were sampled. In herds of 30 to 100, 100 to 200, and more than 200 animals, samples from 30, 35, and 40 animals were sampled, respectively.

In goats, collection of blood sampling was planned in 60 randomly selected goat herds, and in three goat herds that had submitted samples to NVI from abortions in 2022. In herds of less than 30 animals, all animals (>6 months old) were sampled. In herds of 30 to 100, 100 to 200, and more than 200 animals, samples from 30, 35, and 40 animals were sampled, respectively. The preferred sampling period was from January to March, but a proportion of the animals were sampled from September to December. In addition, bulk milk was submitted from a selection of dairy goat farms. The numbers of herds represented in the surveillance programme for *B. melitensis* in small ruminants in 2023 is given in Table 1.

Blood samples were examined for antibodies against *B. melitensis* using the Brucella Rose Bengal Test (RBT) for the initial screening. This test is a simple spot agglutination test, using antigen stained with rose bengal and buffered to a low pH. The antigen and the positive control sera for the RBT was purchased from Bio-Rad Laboratories Inc. (Hercules, CA, USA) and IDvet (Montpellier, France). Positive reactors were re-tested by suitable confirmatory or complementary methods, such as the ID Screen® Brucellosis Serum Indirect Multi-species ELISA (IDvet, Montpellier, France) and/or complement fixation test (APHA , Weybridge, Surrey, UK), to rule out false positive reactions (5).

Bulk milk samples from goat herds were tested with ID Screen® Brucellosis Milk Indirect Multi-ELISA (IDvet, Montpellier, France) and samples with doubtful or positive results were re-tested in duplicates.

Samples with doubtful or positive status in confirmatory or complementary tests were reported, and new blood samples from the suspected animals or herd were requested and tested.

Results

In total, 9,399 blood samples of slaughtered sheep from 3,258 sheep flocks, 373 blood samples sampled in 11 sheep flocks, 1,688 blood samples from 57 goat herds, and 173 bulk milk samples from 169 dairy goat herds, were received in the programme in 2023. From sheep, 136 samples were not suitable for analysis and eight samples had unknown origin, leaving 9,628 samples from 3,239 sheep flocks. From goats, thirteen blood samples were not suitable for analysis, leaving 1,675 samples for analysis. Of the bulk milk samples, two samples had unknown origin, leaving 171 samples for analysis. From 22 of the goat herds both bulk milk and blood samples were submitted. Hence the total number of sampled goat herds were 204. The numbers of tested flocks represent approximately 24.8% of sheep flocks and 13.3% of goat herds in Norway.

Of the screened blood samples, all except for six sheep samples and one goat sample were negative in RBT (0.06%). The RBT positive sample was re-tested in the Indirect Multi-species ELISA with negative result for *B. melitensis*.

Two bulk milk samples tested positive (1.2%) and three had doubtful results, whereas the other bulk milk samples were negative. At retesting in duplicates the samples with doubtful and one of the samples with positive results by screening, were negative. For one bulk milk sample both screening and retesting in duplicates were positive. The flock of origin of the positive bulk milk sample was followed up by blood sampling of 35 goats that were all seronegative for *B. melitensis* in the RBT test.

In conclusion, all samples tested for antibodies against *B. melitensis* in 2023 were negative. The results from the surveillance programme are shown in Table 1.

Table 1: Results and total number of flocks within the frame of the Norwegian surveillance programme for *Brucella melitensis* in small ruminants in the years 2004-2023

Year	Total number of									
	Flocks in Norway ¹		Animals in Norway		Flocks tested		Animals tested (blood samples)		Positive samples	
	Sheep	Goats	Sheep >1 year	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats
2004	17 439		918 500		1 655		50 501		0	
2005	16 500		927 400		935		28 406		1 ²	
2006	15 800		894 100		911		27 812		0	
2007	15 400	1 300	854 000	71 500	1 004	183	29 633	5 734	0	0
2008	15 059	1 308	891 427	69 637	783	80	23 235	2 399	0	0
2009	14 800	1 300	877 400	67 800	816	104	24 011	3 124	0	0
2010	14 800	1 300	887 600	67 600	269	25	8 160	779	0	0
2011	14 500	1 300	882 000	66 900	467	93	13 629	2 698	0	0
2012	14 300	1 300	868 500	65 400	479	86	13 989	2 562	0	0
2013	14 242	1 276	871 976	64 112	468	95	13 550	2 827	0	0
2014	14 218	1 150	755 987	55 894	3 489	89	9 703	2 528	0	0
2015	14 425	1 177	784 558	58 048	3 353	97	9 418	3 048	0	0
2016	14 500	1 300	951 000	68 500	3 492	86	9 821	2 313	0	0
2017	14 463	1 227	984 832	72 658	3 444	61	9 017	1 712	0	0
2018	14 337	1 246	1 005 793	69 636	3 267	61	8 636	1 691	0	0
2019	13 693	1 209	936 203	71 159	3 259	58	8 951	1 751	0	0
2020	13 506	1 270	947 283	72 526	2 927	215 ³	8 701	1 498 ³	0	0
2021	13 389	1 359	943 912	74 512	3 118	52	9 028	1 540	0	0
2022	13 219	1 414	926 700	75 230	3 136	198 ³	9 609	1 798	0	0
2023	13 048	1 529	912 371	75 644	3 239	204 ³	9 628	1 675	0	0

¹ Based on data from the register of production subsidies as of 31st July the respective year until 2017. Thereafter, as of 1st March.

² Probably an unspecific reaction.

³ Includes bulk milk samples from dairy goat herds.

Discussion

During the years 2004-2008, ram circles and their member flocks registered by The Norwegian Association of Sheep and Goat Farmers constituted the target population for the programme. Approximately 90% of the Norwegian sheep flocks participating in ram circles were screened for antibodies against *B. melitensis* during 2004 and 2005. Most flocks participating in the ram circles were retested in the programme during 2006 to 2008. Breeding flocks of other sheep breeds than those regulated by The Norwegian Association of Sheep and Goat Farmers were selected for sampling in 2009.

During 2010-2013 sheep and goat herds were randomly selected for sampling. From 2014, sheep have been sampled at slaughterhouses. This resulted in better surveillance of the total population with the use of less resources than needed when sampled on farm. However, less animals were tested per herd, giving less accurate results on the herd level. In goats, the surveillance is based on sampling live animals in the herds. However, in 2020, 2022 and in 2023, bulk milk samples were added to the surveillance programme. In 2022 and in 2023, sheep flocks and goat herds with registered abortions were included in the programme for a more risk-based and targeted surveillance.

The surveillance programme for *B. melitensis* in sheep has been evaluated using scenario tree modelling (6). When taking into account results accumulated from 2004 to 2018, it was estimated that there is a 99% probability that the prevalence of sheep flocks being positive for *B. melitensis* is lower than 0.2% (7). The results of the programme from 2019 to 2023 have further confirmed this conclusion.

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