Evaluation of the surveillance programme for *Brucella melitensis* in Norwegian small ruminants

Innhold / Content

Forfattere / Authors
Petter Hopp, Annette H. Kampen,
Siv Klevar, Jorun Jarp

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Summary

The Norwegian Veterinary Institute have evaluated the surveillance programme for *Brucella melitensis* in Norwegian small ruminants from 2004 to 2020 by using a scenario tree modelling approach [1]. The results from this model indicate that in 2020 the probability of freedom for *Brucella melitensis* at the so-called design prevalence of 0.002 at the herd level is more than 99% for the general small ruminant population and approximately 95% for the dairy goat population. The model and assumptions for the model are described in the following.

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 [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.

Brucella melitensis is prevalent in sheep and goats in several Mediterranean countries [2], but has never been diagnosed in animals in Norway or any of the other Nordic countries [3, 4]. Brucellosis is classified as a list A disease in Norway and is notifiable to the Office International des Epizooties.

After the agreement on the European Economic Area in 1994, Norway achieved status as free from *B. melitensis* in small ruminants on a historical basis. However, documentation is required to maintain the status. Hence, 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 aim of the work is to document freedom from *B. melitensis* in sheep and goats according to the demands in EU Directive 91/68/EEC with amendments.

Material and methods

The Norwegian sheep and goat population

As of 2020, the Norwegian sheep population counts approximately 13,600 sheep flocks, with 1 million winter-housed sheep, all for meat production, with mainly indoor housing (Table 2).

Only approximately 300 dairy goat herds and 900 other goat herds, with mainly indoor housing. All goat herds and sheep flocks are on field or rangeland pasture for 3 to 5 months during summer. About 50% of Norwegian goat herds are kept on farms that also have sheep.

The Norwegian goat disease eradication programme, ”Healthier goats” running from 2001 to 2014 aimed at eradicating three common infectious diseases in endemic areas by sanitizing goat herds. The

Goat kids were removed from the dams and environment immediately after birth, fed cow's milk and reared separated from the dams. This is a labour intensive and demanding task to perform, however it has proven an efficient and successful tool to eradicate the infections in the programme from Norwegian goat herds [5].

In 2010, more than half of the Norwegian dairy goat herds had joined the programme, thus the dairy goat population should be considered isolated from the sheep population since 2011. By 2014, all Norwegian dairy goat herds had started the programme, and thus are all mainly kept separated from sheep.

Only about 300 of other goat herds have been included in the eradication programme, thus still more than half of the hobby, wool and meat goat flocks are still in close contact with the sheep population.

The surveillance programme

The main surveillance components identified in the surveillance programme were 1) testing of sheep flocks within ram circles, 2) testing of randomly selected sheep flocks, 3) testing of individual sheep slaughtered at abattoirs, 4) testing of randomly selected goat herds, and 5) testing of goat bulk milk from dairy goat herds. The components that were operating in the different years are summarised in Table 1 and the results of the surveillance programme are summarized in Table 2.

Table 1. The surveillance components in the surveillance programmes for *B. melitensis* from 2003 to 2020 and the years each component was operating.

Species	Surveillance component	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Sheep	Ram circle	x	x	x	x	x	x												
	Randomly selected flocks	x	x	x	x	x	x	x	x	x	x	x							
	Sheep slaughtered at abattoirs												x	x	x	x	x	x	x
Goats	Randomly selected herds					x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Bulk milk from dairy herds																		x

Diagnostic tests

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 (CA, USA) and The Animal and Plant Health Agency (APHA) (Weybridge, Surrey, UK). Positive reactors were re-tested by suitable confirmatory or complementary methods, such as ID Screen® Brucellosis Serum Indirect Multi-species ELISA (ID.Vet, Montpellier, France) and/or complement fixation test (APHA , Weybridge, Surrey, UK), to rule out false positive reactions [6]. 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. In 2020, bulk milk samples from dairy goat herds were examined using the ID Screen® Brucellosis Milk Indirect Multi-species ELISA (ID.Vet, Montpellier, France).

Evaluation of the surveillance programme sensitivity

Principles of the method

The sensitivity of the Norwegian surveillance programme (SSe) was measured as the probability of detecting at least one positive sheep or goat herd at the design prevalence of 0.2% infected herds in the country. The surveillance sensitivity was estimated using stochastic evaluation of scenario trees [7]. In short, the probability of identifying a positive herd was estimated separately for each individual herd using the actual herd sizes and the number of tested animals. Thereafter, the probability of identifying at least one positive sheep or goat herd in the population for each year was estimated. From the annual sensitivity estimates, the annual accumulated posterior probability of freedom was estimated [7]. From 2003 to 2010 the sheep and goat were considered as one population. From 2011, the dairy goats were considered as a separate population, while the sheep and the remaining goat herds was considered as one population.

Table 2. The Norwegian sheep population and the number of samples and sheep flocks examined in the Norwegian surveillance programme for *Brucella melitensis* from 2003 to 15th November 2020.

Year	Total number of									
	Flocks [1]		Animals		Flocks tested		Animals tested		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	13 734	1 209	936 203	71 159	3 259	58	8 951	1 751	0	0
2020	13 634	1 304	950 782	72 882	2 763	38 + 188[3]	8 227	1 034	0	0

[1] Based on data from the register of production subsidies as of 31th July the respective year until 2016, for 2017 per 1st May and thereafter, as of 1st March.
[2] Probably unspecific reaction.
[3] Blood samples collected in 38 herds and bulk milk samples collected in 188 herds.

Model input

Inputs to the simulation model were: demographic information on the Norwegian sheep and goat population (Table 2), the number of tested animals, flocks or herds, and ram circles (Table 2), the diagnostic test sensitivities, the assumed brucellosis prevalence at flock or herd level, ram circle level and population level if infection should be present, and the probability of introduction (Table 3). Probability distributions were used to account for uncertainty in the input parameters.

Brucella melitensis has never been detected in Norwegian animals. Introduction of live animals carrying the infection is considered the main route for spread of the infection to small ruminants in new regions and countries [8]. From 2003 to 2019 a total of 500 sheep and goats have been introduced into Norway, varying from 9 to 92 animals annually. All sheep and goats originated from Denmark, Sweden, Finland, Austria and Germany; which all are countries considered free from *B. melitensis* [9]. All the imported animals were tested for Brucella before entering a Norwegian small ruminant farm. Therefore, the probability of introduction of the infection (*PIntro*) was considered to be very low and not more than 1% annually. The annual *PIntro* was set to 1% (Table 3).

Table 3. Input parameters in a stochastic simulation model for evaluation of the Norwegian surveillance programme for *Brucella melitensis* from 2003 to 2015.

	Input parameter		
Input variable	**Expected**	**Distribution**	**Source**
Infected flock design prevalence	0.002	fixed	[10]
Within-flock prevalence	0.05[1]	lnorm(-2.95,1.37), truncated [0,1]	Portugeese data
Sensitivity of RBT	0.925	Beta(3037.5,246.5)	[11]
Sensitivity of C-ELISA	0.931	Beta(43.4,3.2)	[11]
Sensitivity of CFT	0.926	Beta(1249.3,100.2)	[11]
Combined sensitivity 2003-2005	0.870	Beta(584.4,86.7)	Estimated based on *SeRBT* and *SeELISA*
Combined sensitivity 2006-2008	0.843	Beta(764.1,142.0)	Estimated based on *SeRBT* and *SeELISA*
Combined sensitivity 2009-2013	0.826	Beta(1016.8,214.7)	Estimated based on *SeRBT* and *SeCFT*
Combined sensitivity 2014-2020	0.832	Beta(977.0,196.7)	Estimated based on *SeRBT* and *SeCFT*
Sensitivity of bulk milk samples	0.889	fixed	[12]
Annual probability of introduction	0.01	fixed	This publication
Prior probability of infection 2003	0.5	fixed	Uninformed prior

[1] Geometric mean.

Simulation model

The simulation model was run using R v4.0.2 [13]. For each simulation of a scenario with a unique combination of input parameters, 1000 iterations were run. The results were presented with an annual estimate for the surveillance sensitivity and an estimate of the accumulated probability of freedom for sheep and goat population. The uncertainty in the estimates was given by the 2.5 % - 97.5% credibility interval around the median; i.e. that 95% of the estimates from 1000 iterations were within the credibility interval.

Results

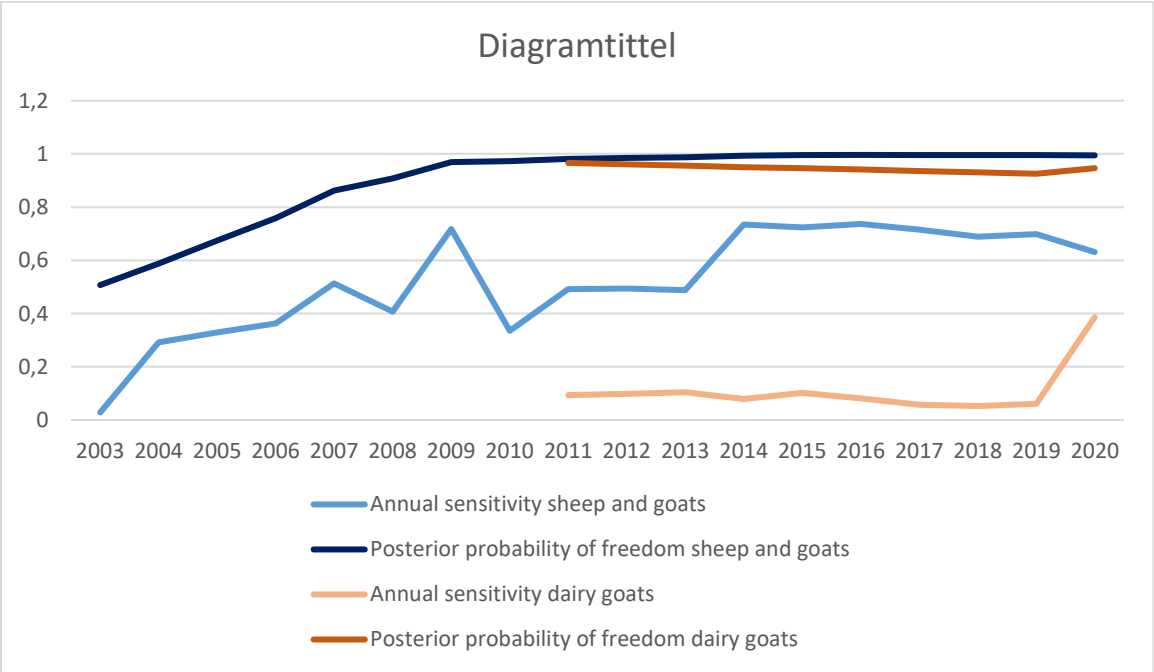
Sampling in sheep and goat population

From 2004 to 2013 the annual sensitivity for detecting at least one infected flock if the prevalence was 0.2% or more varied from 29.2% (28.0 – 30.4) (95% credibility interval) in 2004 to 71.8% (70.8 – 72.7) in 2009. From 2014 to 2020 the annual sensitivity varied from 63.1% (61.6 – 64.7) in 2020 to 74.1% (72.4 – 75.0) in 2016.

When aggregating the results of the surveillance over several years, the probability that less than 0.2% flocks were infected with *B. melitensis* in the sheep and goat population increased from 50.7% (50.6 – 50.8) in 2003 to 99.4% (99.3 – 99.4) in 2014. Thereafter, the posterior probability of freedom varied between 99.5% and 99.6% (Figure 3).

Sampling in dairy goat herds

From 2011 to 2019, the estimated annual sensitivity for detecting at least one infected flock if the prevalence was 0.2% or more varied between 5.3 % (4.5 – 5.3) in 2018 to 10.4% (9.4 – 11.4) in 2013. When aggregating the results of the surveillance over several years, the probability that less than 0.2% flocks were infected with *B. melitensis* in the sheep population decreased from 96.6% (96.5 – 96.7) in 2011 to 94.7% (94.6 – 94.8) in 2020.



Discussion

During the years 2004-2008, ram circles and their member flocks registered by The Norwegian Sheep and Goat Breeders Association 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 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 Sheep and Goat Breeders Association were selected for sampling in 2009.The low sensitivity of the programme during this period is due explained by the fact that the ram circle members only constitute 10% to 15% of the sheep herds giving a low coverage of the total population.

During 2010-2013 sheep and goat herds were randomly selected for sampling with a number of animals per herd. From 2014, individual sheep were sampled at slaughterhouses. Thereby, the results show that the posterior probability that less than 0.2% flocks were infected with *B. melitensis* in the sheep and goat population increased to above 99%. So, the posterior surveillance sensitivity is according to the required level for demonstration of freedom.

However, the corresponding posterior probability for the dairy goat population at the design prevalence was estimated to 94.7%. After finalizing the eradication programme “Healthier goats”, the dairy goat population should be considered a separate population that doesn’t mix with the

remaining small ruminant population. During the years from 2000 to 2008 approximately 300 live goats were imported, mainly of breeds kept for meat and wool production (Animalia.no), and during the years from 2009 to 2020 no live goats were imported to Norway (Animalia.no) indicating that there are minor risk that *B. melitensis* should have been introduced to Norway during that period.

Conclusions

The Norwegian Veterinary Institute have evaluated the surveillance programme for *Brucella melitensis* in Norwegian small ruminants from 2004 to 2020 by using a scenario tree modelling approach. The results from this model indicate that in 2020 the probability of freedom for *Brucella melitensis* at the so-called design prevalence of 0.002 at the herd level is more than 99% for the general small ruminant population and approximately 95% for the dairy goat population.

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