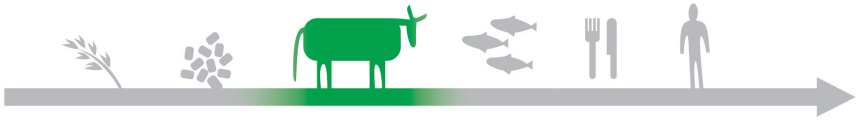
Annual Report

The surveillance programme for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway 2019



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The surveillance program for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway 2019

Content

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Summary

The prevalence of *Echinococcus multilocularis* was based on PCR analysis of faecal samples from 541 red foxes (*Vulpes vulpes*) collected during the licensed fox-hunting season in 2019 and 18 grey wolves (*Canis lupus*) killed in 2019. None of the samples tested positive for *E. multilocularis,* documenting that the prevalence in carnivore hosts (foxes and wolves) were below 1% at a confidence level of at least 95%.

Introduction

Echinococcus multilocularis, the fox tapeworm, is endemic in several regions of the northern hemisphere, including eastern and central parts of Europe (1, 2). During the past decades, the prevalence of *E. multilocularis* in Europe has increased in the known endemic areas (3), and the geographic distribution has expanded to regions where the parasite appeared to be absent previously (4). Similarly, alveolar echinococcosis, the life-threatening zoonotic disease caused by the metacestode stage of this tapeworm, is increasing in prevalence in Europe. A recent European project ranked *E. multilocularis* first amongst the food-borne parasites based on public health concerns (5). The adult tapeworm resides in the small intestine of wild carnivores (definitive hosts) such as red foxes, raccoon dogs and wolves. Domestic dogs and cats can also act as definitive hosts if they prey on infected small mammals, predominantly rodents that serve as intermediate hosts.

In Scandinavia, the first discovery of *E. multilocularis* was on the high-arctic Norwegian islands of Svalbard (6) and in Denmark (7) in 1999. However, there was no evidence of its presence in mainland Fennoscandia (8) until its detection in Sweden in February 2011 (9). Despite analyses of more than 6500 faecal samples from foxes since 2002 (10), *E. multilocularis* has not been reported in mainland Norway.

Anthelmintic treatment of dogs, prior to import from endemic regions, is compulsory in Norway to prevent introduction of the parasite. However, according to the EU Directive 998/2003/EC on pet movement, the maintenance of this national regulation after 2008 requires the documentation of an *E. multilocularis*-free status within the country in question.

Aim

The aim of the surveillance to document the freedom of *E. multilocularis* in mainland Norway.

Materials and methods

In the *E. multilocularis* surveillance of 2019, faecal samples collected from red foxes (*Vulpes vulpes*) hunted during the licensed hunting season (i.e. January to mid-April and mid-July to late December 2019) were included. In addition to faeces from foxes, samples from wolves (*Canis lupus*) killed legally or illegally during 2019 were tested for presence of *E. multilocularis*.

Recruitment of hunters went through the webpages of the Norwegian Veterinary Institute (https://www.vetinst.no/nyheter/registrering-som-provetaker-av-rodrev). In addition, hunters who supplied samples for the surveillance program during previous years were invited by email to participate.

Sampling containers and detailed instructions for sampling were sent to the hunters who volunteered for the program. The samples were submitted to the laboratory with written information on sample locality, date of the sampling, sex (male or female) and estimated age of the animal (juvenile or adult) in pre-paid envelopes. All counties in Norway were included in the sampling regime.

Individual faecal samples (3 g per animal) were subjected to the DNA-fishing (magnetic capture) method combined with real-time PCR detection for the presence of *E. multilocularis* DNA. This procedure involves magnetic capture by specific DNA-hybridisation of capture probes that have a biotin molecule attached to

Echinococcus mtDNA, succeeded by the noncovalent protein-protein binding by streptavidin coated magnetic beads. This allow the physical extraction of this mtDNA/magnetic bead complex by application of a magnetic field, isolating our target from other DNA and from impurities/inhibitors that is present in the solution (11). Detection of the *E. multilocularis* DNA is carried out by real-time PCR (11, 12). If a positive real-time PCR signal is detected, the presence of *E. multilocularis* mtDNA can be verified by an additional independent real-time PCR (12), and a traditional PCR followed by sequencing targeting the *nad1* gene (13). All tests were performed in duplicates with positive control DNA (from adult worms) and negative controls (MilliQ water) included in each run.

The DNA-fishing method is capable of detecting *E. multilocularis* DNA originating from worms as well as eggs. The method is suitable for use during the patent phase of the infection when eggs are shed in the faeces. This period constitutes roughly two-thirds of the entire infection period. The MC-DNA/realtime PCR methods has been shown to be more sensitive than egg isolation by sieving followed by detection of parasite DNA using a multiplex PCR, which was used previously in the Norwegian surveillance program (11, 12). Validation of the current methods in our laboratory has demonstrated a sensitivity of 58% and 91% in 3 g samples spiked with one egg or one worm, respectively (unpublished results).

Initially, a test sensitivity of 63% and a specificity of 100% were assumed (11). However, our internal validation has demonstrated an overall sensitivity close to the Swedish method (88% test sensitivity) (11, 14). The apparent prevalence and corresponding confidence interval were estimated using Epitools (15), with a test sensitivity of 63% and a specificity of 100%.

Results and Discussion

In 2019, 559 faecal samples from wild carnivores were analysed for *E. multilocularis*: 541 samples from red foxes (Table 1, Figure 2) and 18 samples from wolves (*Canis lupus*) (Table 1, Figure 3). All samples tested negative for *E. multilocularis* giving an estimated apparent prevalence of 0% (0.0 – 0.7%, 95%CI).

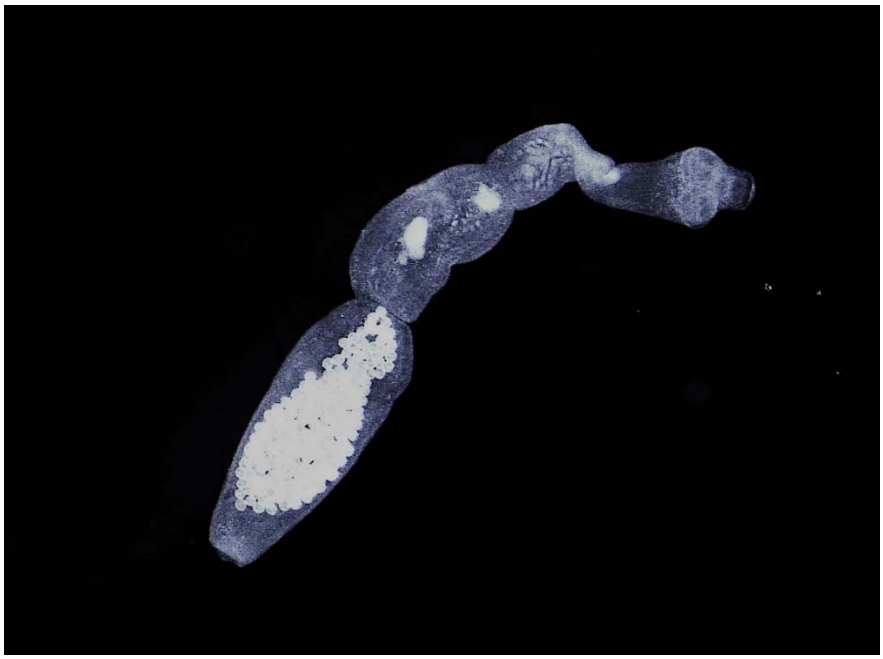

Figure 1. *Echinococcus multilocularis*, adult worm used for spiking of positive controls included in the PCR analyses. The sack-like uterus containing hundreds of eggs is clearly visible. Worms used as controls were inactivated by kept frozen for <75 C for several days, and subsequently stored in 70% ethanol. Professor Peter Deplazes, University of Zurich, kindly donated the depicted worm. Photo: Øivind Øines, Norwegian Veterinary Institute.

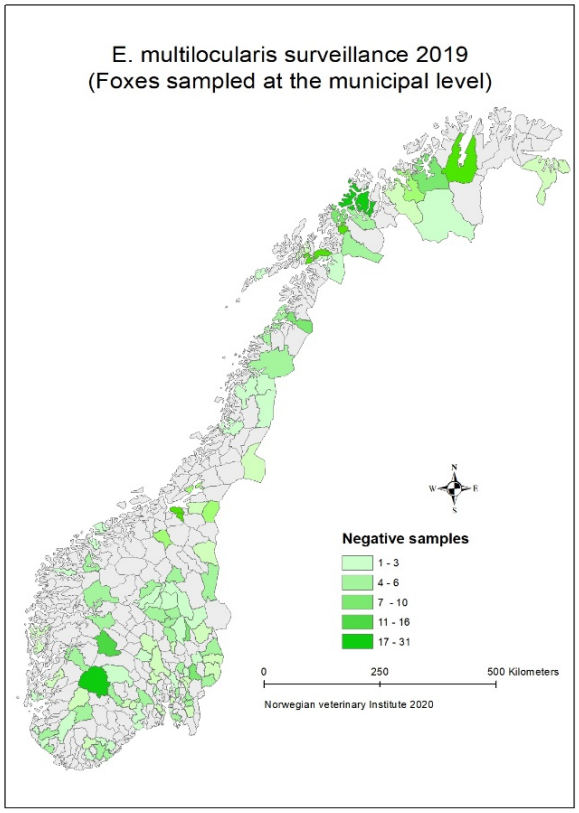

Figure 2. Map of Norway showing the origin of red foxes by municipality, tested for *Echinococcus multilocularis* during the red fox licensed hunting season in 2019 (January to mid-April and mid-July to late December).

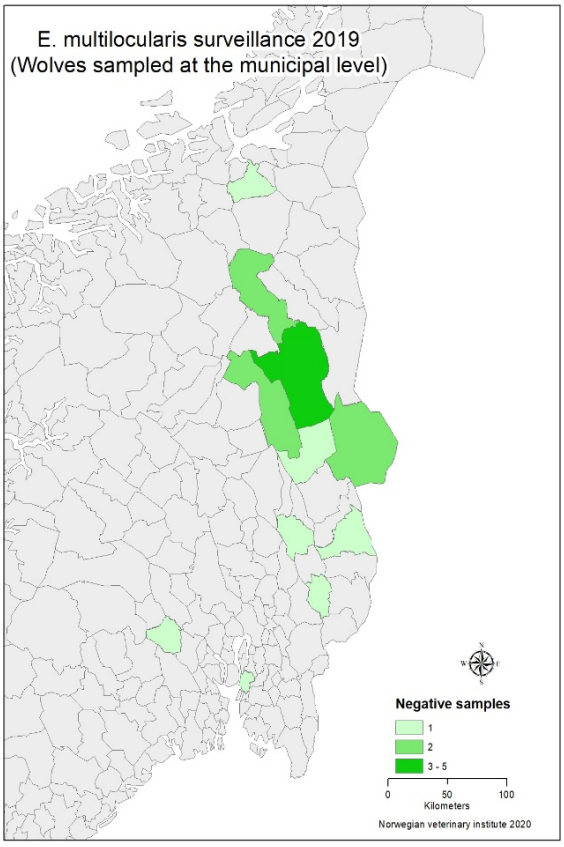

Figure 2. Map of Norway showing the origin of wolfs by municipality, tested for *Echinococcus multilocularis* in 2019. The wolf from Finnmark County not shown.

Table 1. Number and origin (county) of red foxes and wolves examined for *Echinococcus multilocularis* in Norway during the red fox licensed hunting season in 2019 (January to mid-April and mid-July to late December) and corresponding numbers for the period 2002 - 2018.

County	Number of red foxes tested		Other species tested 2019
	2019	Total 2002-2018	
Østfold	22	849	
Akershus	35	727	
Oslo	1	161	
Hedmark	65	966	15
Oppland	40	431	
Buskerud	31	331	1
Vestfold	6	97	
Telemark	46	280	
Aust-Agder	21	121	
Vest-Agder	4	140	
Rogaland	16	94	
Hordaland	35	276	
Sogn og Fjordane	10	261	
Møre og Romsdal	16	158	
Trøndelag	41	922	1
Nordland	50	230	
Troms	92	377	
Finnmark	10	143	1
Total	541	6564	18 wolves

Surveillance results were no different from earlier years. All faecal samples collected from wild carnivores in mainland Norway as part of the surveillance program in 2019, were negative by PCR for *E. multilocularis.*

According to requirements of Regulation (EU) No 1152/2011, Annex II, the disease freedom status must have a pathogen-specific surveillance program designed to detect a prevalence of ≤ 1% at minimum confidence level of 95%. The number of samples collected and analysed in Norway in 2019 was sufficient to document a current prevalence of *E. multilocularis* of below 1%.

However, it is worrying that the rising prevalence in countries close to Norway, has increased the risk of introduction of the parasite to Norway. In Sweden, there are already detections of *E. multilocularis* in four different regions (10), and surveillance in Denmark has demonstrated its presence in two regions (16). Studies in Sweden have discovered *E. multilocularis* in the intermediate hosts of field vole (*Microtus agrestis*) and water voles (*Arvicola amphibious*) in two study areas (20). Moreover, studies in the Baltics have shown a wider distribution of the tapeworm than previously anticipated, which has caused an increasing number of alveolar echinococcosis cases in humans (17). This is worrying, as a lack of compliance with the anthelmintic treatment requirements for pets entering the Norway after having visited endemic areas has been demonstrated (18, 19). The above-mentioned points illustrate why it is imperative to continue with the surveillance for *E. multilocularis* in Norway to document and ensure Norway has a continuous disease free status via the annual surveillance program.

Our results support the maintenance of the national regulation for compulsory anthelmintic treatment of imported dogs to minimize the risk of an introduction of *E. multilocularis* to Norway.

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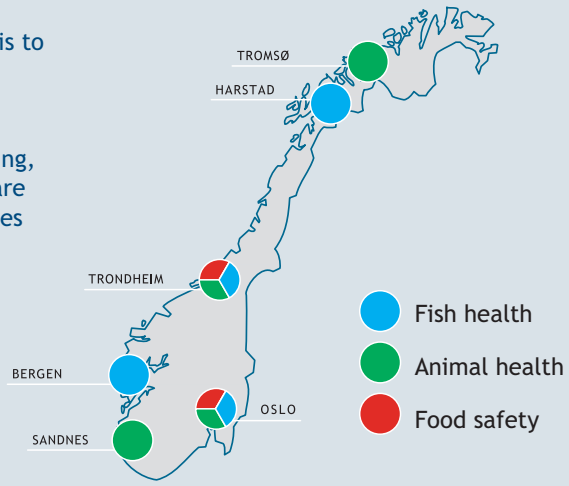

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