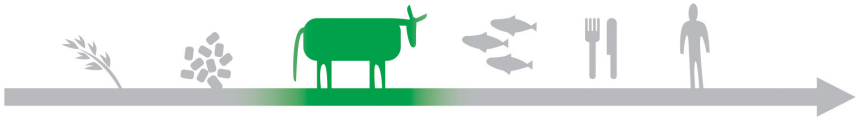
Annual Report

The surveillance programme for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway 2018



Veterinærinstituttet
Norwegian Veterinary Institute

Mattilsynet

The surveillance program for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway 2018

Content

Authors
Heidi L. Enemark, Kristin Henriksen, Chiek Er, Berit Tafjord Heier, Elliott Lambert, Knut Madslien, Øivind Øines

Commissioned by

Mattilsynet

ISSN 1894-5678

Design Cover: Reine Linjer
Photo front page: Colourbox

Summary

The prevalence of *Echinococcus multilocularis* was based on PCR analysis of faecal samples from 536 red foxes (*Vulpes vulpes*) and 34 grey wolves (*Canis lupus*) collected during the licensed fox-hunting season in 2018. None of the samples tested positive for *E. multilocularis* by which we deduce that the prevalence in carnivore hosts (foxes and wolves) were below 1% with 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 is no evidence of its presence in mainland Fennoscandia (8) until its detection in Sweden in February 2011 (9). Despite analyses of more than 5 500 foxes/fox scats 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 2018, 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 2018) were included. In addition to faeces from foxes, samples from wolves (*Canis lupus*) killed legally or illegally during 2018 were tested for presence of *E. multilocularis*.

By email invitations, hunters who supplied samples for the surveillance program during previous years were invited to submit samples for the 2018 sampling season. Further recruitment of hunters was via the webpages of the Norwegian Veterinary Institute (https://www.vetinst.no/nyheter/registrering-som-provetaker-av-rodrev). Sampling containers and detailed instructions for sampling were send to the hunters who volunteered for the program. The samples an forms with information on the origin of the fox, date of the hunt, sex (male or female) and estimated age of the animal (juvenile or adult) were submitted to the laboratory in pre-paid envelopes. With the exception of Rogaland, all counties in Norway were part of the sampling regime.

The DNA-fishing method combined with real-time PCR detection was used for detection of *E. multilocularis*. This procedure involves magnetic capture DNA extraction from samples by applying specific DNA-hybridisation, succeeded by extraction using streptavidin coated magnetic beads and

detection by real-time PCR (11, 12). Following a positive real-time PCR signal the presence of *E. multilocularis* DNA is verified by PCR and sequencing targeting the *nad1* gene (13) and an additional independent real-time PCR (12). Individual faecal samples (3 g per animal) were tested and the real-time PCR reactions were performed in duplicate with positive (DNA from adult worms) and negative (MilliQ water) controls included in each reaction.

The DNA-fishing method is capable of detecting *E. multilocularis* DNA from adult 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 combination of these 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% was 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 the function epi.prev in package epiR performed in R version 2.6.3 (15).

Results and Discussion

In 2018, 570 faecal samples from wild carnivores were analysed for *E. multilocularis*: 536 samples from red foxes (Table 1, Figure 2) and 34 samples from wolves (*Canis lupus*) (Table 1, Figure 3). All samples tested negative for *E. multilocularis* giving an estimated apparent prevalence of 0% (0 – 0.8%, 95% confidence interval

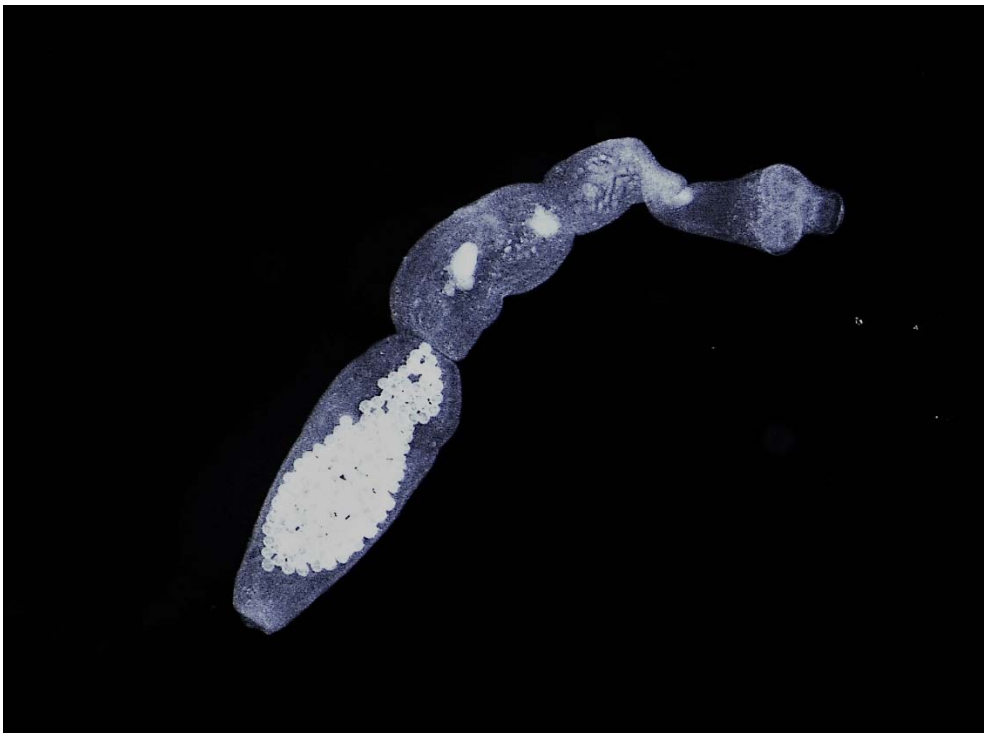


Figure 1. *Echinococcus multilocularis*, adult worm used for spiking of positive controls included in the PCR analyses. The sack-like uterus containing hundreds of eggs is clearly visible. Worms used as controls were stored in 70% ethanol and not infective to humans. Professor Peter Deplazes, University of Zurich, kindly donated the depicted worm. Photo: Øivind Øines, Norwegian Veterinary Institute.

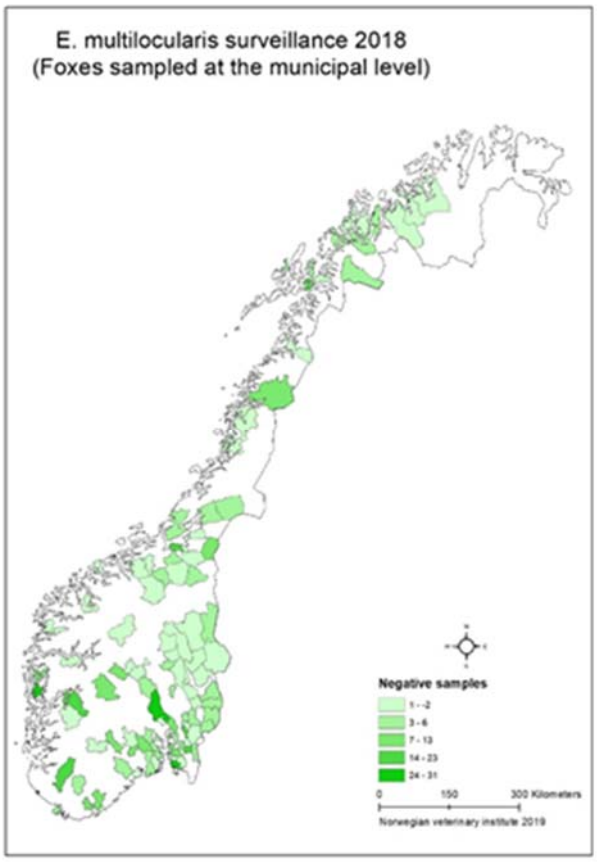


Figure 2. Map of Norway showing the origin (municipality) of red foxes tested for *Echinococcus multilocularis* during the red fox licensed hunting season in 2018 (January to mid-April and mid-July to late December).

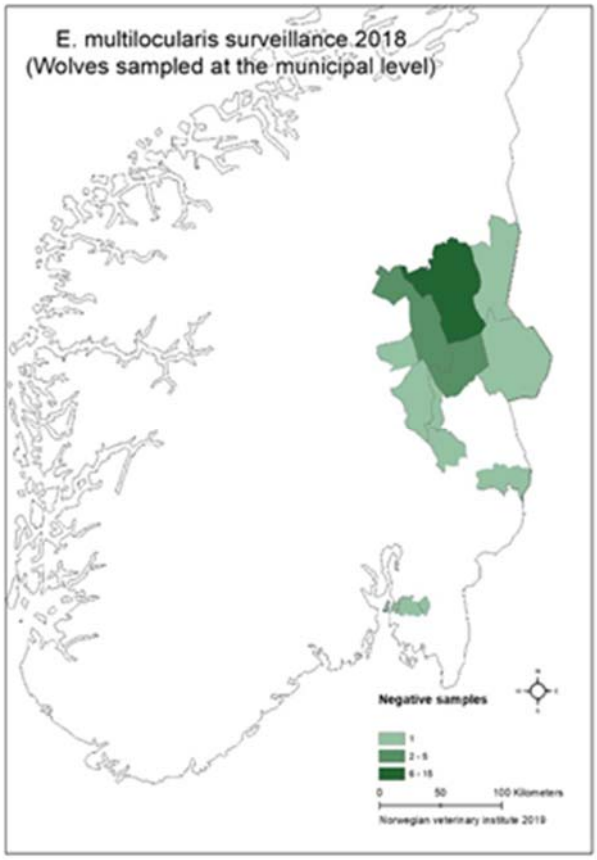


Figure 2. Map of Norway showing the origin (municipality) of wolfs tested for *Echinococcus multilocularis* in 2018.

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 2018 (January to mid-April and mid-July to late December) and corresponding numbers for the period 2002 - 2017.

County	Number of red foxes tested		Other species tested 2018
	2018	Total 2002-2017	
Østfold	68	781	3
Akershus	50	677	
Oslo	11	150	
Hedmark	30	936	30
Oppland	20	411	1
Buskerud	68	263	
Vestfold	17	80	
Telemark	22	258	
Aust-Agder	13	108	
Vest-Agder	34	106	
Rogaland		94	
Hordaland	57	219	
Sogn og Fjordane	3	258	
Møre og Romsdal	11	147	
Trøndelag	64	858	
Nordland	41	189	
Troms	26	351	
Finnmark	2	141	
Total	536	6 027	34 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 2018, 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 confidence level of at least 95%. The number of samples collected and analysed in Norway in 2018 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. In Sweden, there are already detections of *E. multilocularis* in four different regions (10), and surveillance in Denmark has demonstrated its presence in a new region in Denmark (16). 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 to monitor 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 *E. multilocularis* introduction to Norway.

References

1. Schweiger A, Ammann RW, Candinas D, Clavien PA, Eckert J, Gottstein B, Halkic N, Meullhaupt B, Prinz BM, Reichen J, Tarr PE, Torgerson PR, Deplazes P. Human alveolar echinococcosis after fox population increase, Switzerland. Emerg Infect Dis 2007; 13: 878-882.

2. Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev 2004; 17: 107-135.

3. Romig T, Dinkel A, Mackenstedt U. The present situation of echinococcosis in Europe. Parasitol Int. 2006;55(Suppl):S187–91 10.1016/j.parint.2005.11.028

4. Combes B, Comte S, Raton V, Raoul F, Boue F, Umhang G, Favier S, Dunoyer C, Woronoff N, Giraudoux P. Westward spread of *Echinococcus multilocularis* in foxes, France, 2005-2010. Emerg Infect Dis 2012, 18:2059-2062.

5. Bouwknegt M, Devleesschauwer B, Graham H, Robertson LJ, van der Giessen JWB, The Euro-FBP workshop participants Prioritisation of food-borne parasites in Europe, 2016. Euro Surveill. 2018; 23(9):pii=17-00161. https://doi.org/10.2807/1560-7917.ES.2018.23.9.17-00161

6. Dahlberg T, Evans R, Slettbakk T, Ottesen P, Blystad H. *Echinococcus multilocularis* påvist på Svalbard. MSIS-rapport 2000; 28: 23.

7. Kapel CMO, Saeed I. *Echinococcus multilocularis* - en ny zoonotisk parasit i Danmark. DVT 2000; 83: 14-16.

8.Wahlström H, Isomursu M, Hallgren G, Christensson D, Cedersmyg M, Wallensten A, Hjertqvist M, Davidson RK, Uhlhorn H, Hopp P. Combining information from surveys of several species to estimate the probability of freedom from *Echinococcus multilocularis* in Sweden, Finland and mainland Norway. Acta Vet Scand 2011, Feb 11;53:9.

9. Osterman LE, Juremalm M, Christensson D, Widgren S, Hallgren G, Ågren EO, Uhlhorn H, Lindberg A, Cedersmyg M, Wahlström H. First detection of *Echinococcus multilocularis* in Sweden, February to March 2011. Euro Surveill. 2011;16(14):pii=19836.

10. Wahlström H, Enemark Hl. Davidson RK, Oksanen A. Present status, actions taken and future considerations due to the findings of *Echinococcus multilocularis* in two Scandinavian countries. Vet Parasitol 2015, 213:178-181.

11. Øines Ø, Isaksson M, Hagstöm Å, Tavornpanich S and Davidson RK. Laboratory assessment of sensitive molecular tools for detection of low levels of *Echinococcus multilocularis*-eggs in fox (*Vulpes vulpes*) faeces. Parasites & Vectors 2014, 7:246.

12. Isaksson M, Hagström Å, Armua-Fernandez MT, Wahlström H, Ågren EO, Miller A, Holmberg A, Lukacs M, Casulli A, Deplazes P and Juremalm M. A semi-automated magnetic capture probe based DNA extraction and real-time PCR method applied in the Swedish surveillance of *Echinococcus multilocularis* in red fox (*Vulpes vulpes*) faecal samples. Parasites & Vectors 2014, 7:583.

13. Trachsel D, Deplazes P, Mathis A, 2007. Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. Parasitology 134, 911-920.

14. Davidson RK, Øines Ø, Madslien K, Mathis A. *Echinococcus multilocularis* – adaptation of a worm egg isolation procedure coupled with a multiplex PCR assay to carry out large scale screening of red foxes (*Vulpes vulpes*) in Norway. Parasitol Res 2009; 104 (3): 509-514.

15. R development Core Team. R: A language and environment for statistical computing 2008

http://www.R-project.org

16. Petersen HH, Al-Sabi MNS, Enemark HL, Kapel CMO, Jørgensen JA, Chriel M. *Echinococcus multilocularis* in Denmark 2012-2015: high local prevalence in red foxes. Parasitol Res 2018 https://doi.org/10.1007/s00436-018-5947-y

17.Marcinkuté A, Šarkunas M, Moks E, Saarma U, Jokelainen P, Bagrade G, Laivacuma S, Strupas K, Sokolovas V, Deplazes P. *Echinococcus* infections in the Baltic region. Vet Parasitol 2015, 213: 121-131.

18.Davidson RK, Robertson LJ. European pet travel: misleading information from veterinarians and government agencies. Zoonoses Public Health 2012, 59: 575-583.

19.Hamnes IS, Klevar S, Davidson RK, Høgåsen HR, Lund A. Parasitological and serological investigation of samples from stray dogs imported into Norway from Eastern European countries [in Norwegian]. In Norwegian Veterinary Institute's Report Series: report 15 (Norwegian Veterinary Institute), p 20.

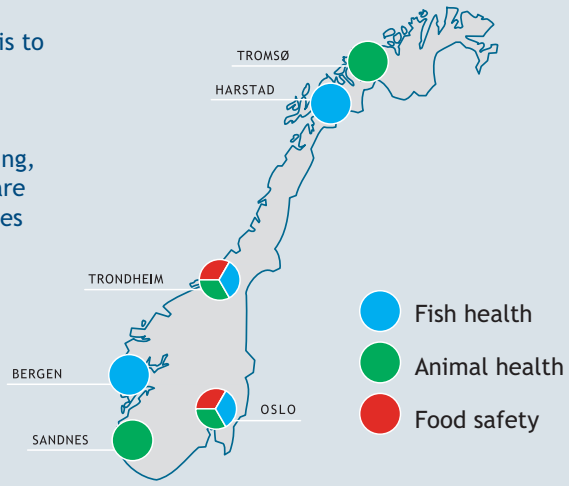
Scientifically ambitious, forward-looking and cooperatively oriented – for integrated health

The Norwegian Veterinary Institute is a national research institute that operates in the fields of animal and fish health, food safety and feed hygiene; its primary task is to provide the authorities with independently generated knowledge.

Emergency preparedness, diagnostic services, monitoring, reference functions, consulting, and risk assessments are all important areas of activity. Our products and services include research results and reports, analyses and diagnoses, studies and advice.

The Norwegian Veterinary Institute's central laboratory and administration lie in Oslo, and we operate regional laboratories in Sandnes, Bergen, Trondheim, Harstad and Tromsø.

The Norwegian Veterinary Institute collaborates with a large number of national and international institutions.



Fish health

Animal health

Food safety

Oslo	Trondheim	Sandnes	Bergen	Harstad	Tromsø
postmottak@vetinst.no	vit@vetinst.no	vis@vetinst.no	post.vib@vetinst.no	vih@vetinst.no	vitr@vetinst.no

www.vetinst.no

Veterinærinstituttet
Norwegian Veterinary Institute