Annual Report

The surveillance programme for *Echinococcus multilocularis* in red foxes (*Vulpes Vulpes*) in Norway 2017

Veterinærinstituttet
Norwegian Veterinary Institute

Mattilsynet

The surveillance program for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway 2017

Content

Authors
Heidi L. Enemark, Kristin Henriksen, Malin E Jonsson, Elliot Lambert, Ian D. Woolsey, Inger Sofie Hamnes, Berit Tafjord Heier, Knut Madslien, Charles Albin-Amiot, Øivind Øines

Commissioned by
Mattilsynet

ISSN 1894-5678

Design Cover: Reine Linjer
Photo front page: Colourbox

Summary

The prevalence of *Echinococcus multilocularis* was analysed by PCR of faecal samples from 495 red foxes (*Vulpes vulpes*) and 11 gray wolfs (*Canis lupus*) collected during the licensed hunting season in 2017. *E. multilocularis* was not detected in any of the samples corresponding to a prevalence in carnivore hosts below 1% with at a confidence level of at least 95%.

Introduction

Echinococcus multilocularis, the fox tapeworm, is endemic in large parts of the northern hemisphere, including eastern and central parts of Europe (1, 2). During the past decades, 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 was not previously detected (4). Likewise, alveolar echinococcosis, the life-threatening zoonotic infection caused by the metacestode stage of this tapeworm, is increasing in prevalence in Europe. Thus, based on public health concerns *E. multilocularis* was recently ranked first amongst the food-borne parasites in Europe (5). The adult tapeworm resides in the small intestine of wild carnivores (definitive hosts) such as e.g. red foxes, raccoon dogs and wolfs. Domestic dogs and cats may be involved in the lifecycle as definitive hosts if they prey on infected small mammals, predominantly rodents, serving as intermediate hosts.

In Scandinavia, *E. multilocularis* was detected for the first time on the high-arctic Norwegian islands of Svalbard (6) as well as 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 for *E. multilocularis* of more than 5 500 foxes/fox scats since 2002 (10), the parasite has not been detected in mainland Norway so far.

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

Aims

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

Materials and methods

Faecal samples collected from red foxes (*Vulpes vulpes*) shot during the licensed hunting season in 2017 (i.e. January to mid-April and mid-July to late December) were included in the surveillance 2017.

Hunters that contributed to the surveillance program previous years were invited by e-mail to submit samples for the 2017- sampling season. Additional hunters were recruited via webpages of the Norwegian Veterinary Institute (https://www.vetinst.no/nyheter/registrering-som-provetaker-av-rodrev). Sample containers and detailed instructions for sampling were forwarded to hunters who volunteered for the program. The samples together with information concerning 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. In addition to faeces from foxes, samples from wolves (*Canis lupus*) killed legally or illegally during 2017 were included in the surveillance. All counties in Norway were represented in the sampling regime.

We used the DNA-fishing method combined with real-time PCR detection 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 presence of *E. multilocularis* DNA is verified by PCR/sequencing targeting the *nad1* gene (13) and an additional independent real-time PCR (12). Faecal samples (3 g per animal) were analysed individually, and 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 targeted for use during the patent phase of the infection when DNA from the eggs is shed in the faeces. This period constitutes roughly two-thirds of the entire infection period. The combination of these methods is 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 around 1 egg per 3 g of faeces (unpublished results).

We assumed a test sensitivity of 63% and a specificity of 100% (11). However, the true test sensitivity is probably higher and most likely 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

From foxes, a total of approximately 530 faecal samples were collected in 2017 of which 495 were suitable for examination (Figure 1, Table 1). In addition, 11 samples from wolves (*Canis lupus*) were analysed. All samples were negative for *E. multilocularis* giving an estimated apparent prevalence of 0% (0 – 0.8%, 95% confidence interval).

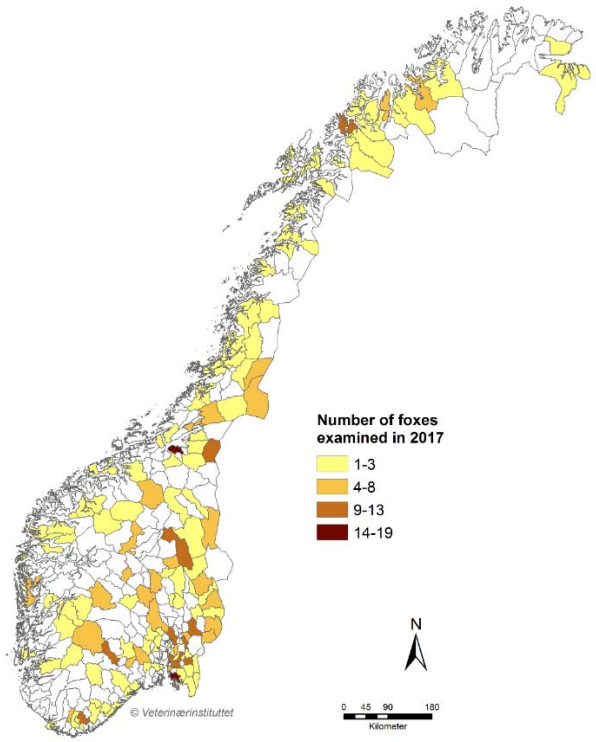


Figure 1. Map of Norway showing numbers and origin (municipality) of red foxes sampled and examined for *Echinococcus multilocularis* during the red fox licensed hunting season in 2017 (January to mid-April and mid-July to late December).

Table 1. Number and origin (county) of red foxes and wolves sampled and examined for *Echinococcus multilocularis* in Norway during the red fox licensed hunting season in 2017 (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 2017
	2017	Total 2002-2017	
Østfold	76	705	
Akershus	49	628	
Oslo	10	140	
Hedmark	53	883	9 wolves
Oppland	34	377	1 wolf
Buskerud	28	235	
Vestfold	5	75	
Telemark	28	230	
Aust-Agder	7	101	
Vest-Agder	20	86	
Rogaland	2	92	
Hordaland	18	201	
Sogn og Fjordane	14	244	
Møre og Romsdal	11	136	
Sør-Trøndelag	26	392	
Nord-Trøndelag	41	399	1 wolf
Nordland	23	166	
Troms	45	306	
Finnmark	5	136	
Total	495	5 532	11 wolves

In agreement with results from previous years, no faecal samples collected from carnivores, as part of the Norwegian surveillance program in 2017, were positive by PCR for *E. multilocularis.*

According to requirements of Regulation (EU) No 1152/2011, Annex II, the pathogen-specific surveillance program must be designed to detect a prevalence of ≤ 1% at confidence level of at least 95%. The number of samples collected and analysed in Norway in 2017 was sufficient to document a current prevalence of *E. multilocularis below* 1%. However, increasing prevalence in nearby regions has increased the risk of introduction of the parasite to Norway. In Sweden, *E. multilocularis* has now been found in four different regions (10), and surveillance in Denmark has recently demonstrated its prevalence in a new region in Denmark (16). Furthermore, studies have shown the parasite to be wider distributed in the Baltics than previously anticipated leading to increasing numbers of alveolar echinococcosis in humans (17). This is a cause for concern since Norwegian studies have revealed lack of compliance with the anthelmintic treatment requirements for pets entering the country after having visited endemic areas (18, 19). Consequently, the annual surveillance program is necessary to document a continuous disease free status.

Our findings support the maintenance of the national regulation for compulsory anthelmintic treatment of imported dogs to minimise 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. Enemark HL, Al-Sabi MN, Knapp J, Staahl M, Chríel M. Detection of a high-endemic focus of *Echinococcus multilocularis* in red foxes in southern Denmark, January 2013. Euro Surveill. 2013;18(10):pii=20420.

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.

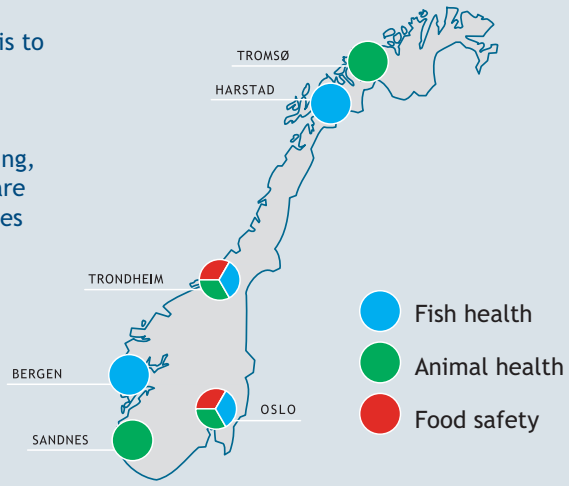
Scientifically ambitious, forward-looking and cooperatively oriented – for integrated health

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