Surveillance programmes for terrestrial and aquatic animals in Norway
Norwegian Veterinary Institute

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Veterinærinstituttet
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The surveillance programme for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway in 2015

Knut Madslien, Charles Albin-Amiot, Malin E Jonsson, Torill Clausen, Kristin Henriksen, Inger Sofie Hamnes, Anne Margrete Urdahl, Berit Tafjord Heier, Øivind Øines, Heidi L. Enemark

Echinococcus multilocularis was not detected in any of the 523 red foxes (*Vulpes vulpes*) examined during the licensed hunting season in 2015. Thus, so far E. multilocularis has not been detected in any of the 4462 foxes that have been tested since the surveillance was initiated in Norway in 2002.

Introduction

The dwarf fox tapeworm *Echinococcus multilocularis* 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). In 1999, *E. multilocularis* was detected for the first time in Denmark (5) and on the high-arctic Norwegian islands of Svalbard (6). Yet, prior to the detection of *E. multilocularis* in Sweden in February 2011 (7) there was no evidence of its presence in mainland Fennoscandia (8). Despite analyses for *E. multilocularis* of more than 3000 foxes/fox scats since 2002 (9), the parasite has not been detected in mainland Norway so far.

Anthelmintic treatment of dogs, prior to import, is compulsory 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 post 2008 requires documentation of an *E. multilocularis* - free status within Norway.

Aim

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

Material and methods

Faecal samples collected from red foxes shot during the licensed hunting season in 2015 (i.e. January to mid-April and mid-July to late December) were included in the surveillance 2015.

All counties in Norway were represented in the sampling regime. Hunters who have provided samples previous years were invited to participate (n=157) and in addition samplers were requested on our homepage. A standard form including information on where and when the fox was hunted, sex (male or female) and estimated age of the animal (juvenile or adult), was completed by each hunter.
In addition, faecal samples from wolves (*Canis lupus*) killed legally or illegally during 2015 were included in the surveillance 2015.

The DNA-fishing method combined with realtime PCR detection, was used for the detection of *E. multilocularis* in the faecal samples. This involves magnetic capture DNA extraction from samples by applying specific DNA-hybridisation, followed by extraction using streptavidin coated magnetic beads and finally detection by realtime PCR (10, 11). The DNA-fishing method is capable of detecting *E. multilocularis* DNA from adult worms as well as eggs. These methods are 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 total infection period. The combination of these methods is more sensitive than the previously used method: egg isolation by sieving followed by detection of parasite DNA using a multiplex PCR (10, 11). Validation of the current methods in our laboratory has demonstrated a sensitivity around 1 egg per 3 g of faeces (Heidi Enemark, personal communication).

A total of 523 samples were analysed individually (3 g faeces per sample). Realtime PCR detection was performed in duplicate. We assumed a test sensitivity of 63 % (10) and a fox population of 151.000 (Olav Hjeljord, Norwegian University of Life Sciences, personal communication). However, the true test sensitivity is probably higher and most likely close to the Swedish method (88% test sensitivity) (10, 12).The apparent prevalence and corresponding confidence interval were estimated using the function epi.prev in package epiR performed in R version 2.6.2 (13). The conservative 63% sensitivity and a specificity of 1 were used for calculating the apparent prevalence.

Results

A total of 524 samples were collected in 2015 of which 523 were suitable for examination (Figure 1). In addition, four samples from wolves (*Canis lupus*) were examined (Table 1). All samples were negative for *E. multilocularis* giving an estimated apparent prevalence of 0% (0 – 0.7%, 95% confidence interval).

In the years 2002 - 2015, a total of 4 462 red fox faecal samples from mainland Norway have been tested for *E. multilocularis* (Table 1).

Table 1. Number and county of the red foxes, and wolwes, sampled and examined for *Echinococcus multilocularis* in Norway during the red fox licensed hunting season in 2015 (January to mid-April and mid-July to late December) and corresponding numbers for the period 2002 - 2015.

County	Number of red foxes tested		Other species tested
	2015	Total 2002-2015	
Østfold	91	528	
Akershus	76	531	
Oslo	12	94	
Hedmark	110	743	1 wolf
Oppland	39	305	2 wolves
Buskerud	27	169	
Vestfold	2	59	
Telemark	18	179	
Aust-Agder	3	89	
Vest-Agder	3	65	
Rogaland	7	87	
Hordaland	6	149	
Sogn og Fjordane	7	218	
Møre og Romsdal	4	119	
Sør-Trøndelag	22	349	1 wolf
Nord-Trøndelag	20	319	
Nordland	5	122	
Troms	42	207	
Finnmark	29	130	
Total	**523**	**4 462**	4 wolves

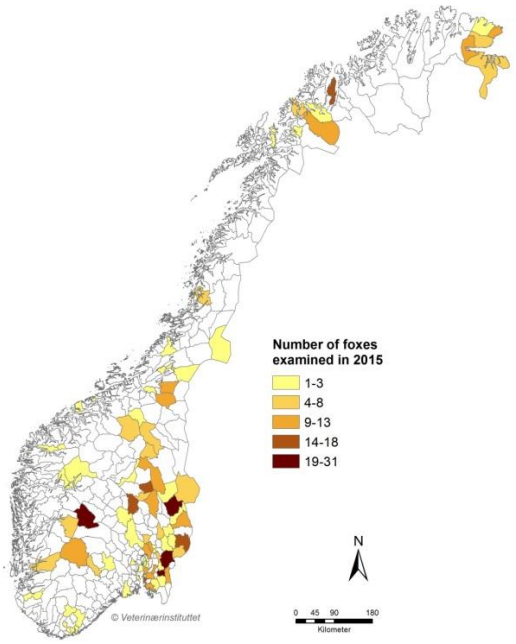

Figure 1. Map of Norway showing numbers and hunting municipality of red foxes sampled and examined for *Echinococcus multilocularis* during the red fox licensed hunting period in 2015

Discussion

No faecal samples collected from Norwegian foxes during the surveillance programme in 2015 were positive by PCR for *E. multilocularis*, which is in agreement with results from previous years. According to requirements of Regulation (EU) No 1152/2011, Annex II, the pathogen-specific surveillance programme must be designed to detect a prevalence of ≤ 1% at confidence level of at least 95%.

The number of samples collected in Norway in 2015 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 (9), and surveillance in Denmark has recently demonstrated its prevalence in a new region in Denmark (14). 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 (15).

This is worrisome since Norwegian studies have exposed lack of compliance with the anthelmintic treatment requirements for pets entering the country from after having visited endemic areas (16, 17). As a consequence, an annual surveillance programme 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.

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