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

![Image of Echinococcus multilocularis](image.png)

**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 wolves 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.

<table>
<thead>
<tr>
<th>County</th>
<th>Number of red foxes tested 2018</th>
<th>Other species tested 2018</th>
<th>Number of red foxes tested Total 2002-2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Østfold</td>
<td>68</td>
<td>3</td>
<td>781</td>
</tr>
<tr>
<td>Akershus</td>
<td>50</td>
<td></td>
<td>677</td>
</tr>
<tr>
<td>Oslo</td>
<td>11</td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>Hedmark</td>
<td>30</td>
<td></td>
<td>936</td>
</tr>
<tr>
<td>Oppland</td>
<td>20</td>
<td></td>
<td>411</td>
</tr>
<tr>
<td>Buskerud</td>
<td>68</td>
<td></td>
<td>263</td>
</tr>
<tr>
<td>Vestfold</td>
<td>17</td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>Telemark</td>
<td>22</td>
<td></td>
<td>258</td>
</tr>
<tr>
<td>Aust-Agder</td>
<td>13</td>
<td></td>
<td>108</td>
</tr>
<tr>
<td>Vest-Agder</td>
<td>34</td>
<td></td>
<td>106</td>
</tr>
<tr>
<td>Rogaland</td>
<td></td>
<td></td>
<td>94</td>
</tr>
<tr>
<td>Hordaland</td>
<td>57</td>
<td></td>
<td>219</td>
</tr>
<tr>
<td>Sogn og Fjordane</td>
<td>3</td>
<td></td>
<td>258</td>
</tr>
<tr>
<td>Møre og Romsdal</td>
<td>11</td>
<td></td>
<td>147</td>
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<tr>
<td>Tøndelag</td>
<td>64</td>
<td></td>
<td>858</td>
</tr>
<tr>
<td>Nordland</td>
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<td>Troms</td>
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<td></td>
<td>351</td>
</tr>
<tr>
<td>Finnmark</td>
<td>2</td>
<td></td>
<td>141</td>
</tr>
<tr>
<td>Total</td>
<td>536</td>
<td>34 wolves</td>
<td>6 027</td>
</tr>
</tbody>
</table>

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 $\leq 1\%$ 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


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