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

<table>
<thead>
<tr>
<th>County</th>
<th>Number of red foxes tested</th>
<th>Other species tested 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2017</td>
<td>Total 2002-2017</td>
</tr>
<tr>
<td>Østfold</td>
<td>76</td>
<td>705</td>
</tr>
<tr>
<td>Akershus</td>
<td>49</td>
<td>628</td>
</tr>
<tr>
<td>Oslo</td>
<td>10</td>
<td>140</td>
</tr>
<tr>
<td>Hedmark</td>
<td>53</td>
<td>883</td>
</tr>
<tr>
<td>Oppland</td>
<td>34</td>
<td>377</td>
</tr>
<tr>
<td>Buskerud</td>
<td>28</td>
<td>235</td>
</tr>
<tr>
<td>Vestfold</td>
<td>5</td>
<td>75</td>
</tr>
<tr>
<td>Telemark</td>
<td>28</td>
<td>230</td>
</tr>
<tr>
<td>Aust-Agder</td>
<td>7</td>
<td>101</td>
</tr>
<tr>
<td>Vest-Agder</td>
<td>20</td>
<td>86</td>
</tr>
<tr>
<td>Rogaland</td>
<td>2</td>
<td>92</td>
</tr>
<tr>
<td>Hordaland</td>
<td>18</td>
<td>201</td>
</tr>
<tr>
<td>Sogn og Fjordane</td>
<td>14</td>
<td>244</td>
</tr>
<tr>
<td>Møre og Romsdal</td>
<td>11</td>
<td>136</td>
</tr>
<tr>
<td>Sør-Trøndelag</td>
<td>26</td>
<td>392</td>
</tr>
<tr>
<td>Nord-Trøndelag</td>
<td>41</td>
<td>399</td>
</tr>
<tr>
<td>Nordland</td>
<td>23</td>
<td>166</td>
</tr>
<tr>
<td>Trons</td>
<td>45</td>
<td>306</td>
</tr>
<tr>
<td>Finnmark</td>
<td>5</td>
<td>136</td>
</tr>
<tr>
<td>Total</td>
<td>495</td>
<td>5 532</td>
</tr>
</tbody>
</table>

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


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