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The surveillance and control programme for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway. Hunting season 2012-2013

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The surveillance programme for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway. Hunting season 2012-2013

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Echinococcus multilocularis *was not detected in any of the 625 red foxes* (Vulpes vulpes) *examined during the 2012-2013 licensed hunting season.*

Introduction

Echinococcus multilocularis is endemic in large parts of the northern hemisphere, including eastern and central parts of Europe (1, 2). In 1999, *E. multilocularis* was detected in Denmark (3) and on the high-arctic Norwegian islands of Svalbard (4).

There was no evidence that this parasite had established in mainland Fennoscandia (5) prior to its detection in Sweden in February 2011 (6).

E. multilocularis has yet to be detected in mainland Norway, and 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 2012-2013 licensed hunting season (from mid-July 2012 to April 2013) were included in this year's program. All regions of Norway were represented in the sampling regime. Hunters were invited to participate based on a list of registered fox hunters. A standard form that included information on where and when the fox had been hunted, as well as the sex (male or female) and presumed age of the animal (juvenile or adult), was completed by each hunter.

The method used for the detection of *E. multilocularis* in the faecal samples was the newly developed DNA-fishing technique. This involves targeted DNA extraction from samples by applying specific DNA-hybridisation, followed by isolation using streptavidin coated magnetic beads (Mats Isaksson, National Veterinary Institute, personal communication) and finally detection using a realtime PCR (7). The new method is also capable of detecting DNA from adult worms, in addition to eggs. These methods are targeted for use during the patent phase of the intestinal infection, more precisely when DNA from the eggs will be shed in the faeces. This period constitutes roughly two-thirds of the total infection period. The combination of the new methods were shown to be more sensitive than the previously used method; egg isolation using physical sieving followed by detection of parasite DNA using a multiplex PCR (8).

A total of 625 samples were run individually (3 g faeces examined per sample). We assumed a fox population of 70,000 (Olav Hjeljord, Norwegian University of Life Sciences, personal communication), a test sensitivity of 63% (7) for the last two hunting seasons, and a sensitivity of 30% for the preceding hunting seasons to account for the lower sensitivity of the test used until 2011. The apparent prevalence and corresponding confidence interval were estimated using the function epi.prev in package epiR performed in R version 2.6.2 (9). We used the average sensitivity for the tests used during 2009-2013 (30% sensitivity for 2009-2011 and 63% sensitivity for 2012-2013, i.e. 46% sensitivity for the whole period) when calculating the apparent prevalence for the last five years.

Results

A total of 656 fox samples were collected during the 2012-2013 hunting season, of which 625 were suitable for examination (Figure 1). All samples were negative for *E. multilocularis* giving an estimated apparent prevalence of 0% (0 - 0.6%, 95% confidence interval). During the last five hunting seasons (from 2008-2009 to 2012-2013) a total of 2168 foxes have been examined. All foxes have tested negative giving an estimated prevalence of 0% (0 - 0.2%). In total, 3405 red fox faecal samples, from mainland Norway, have been tested for *E. multilocularis* between 2002 and 2013 (Table 1).

Table 1. Number and county of the red foxes sampled and examined for *Echinococcus multilocularis* in Norway during the red fox licensed hunting season from July to April, 2002-2013.

	No. red foxes sampled		
County	2002-2012	2012-2013	Total 2002-2013
Østfold	208	123	331
Akershus	309	85	394
Oslo	64	4	68
Hedmark	397	78	475
Oppland	216	24	240
Buskerud	102	22	124
Vestfold	49	7	56
Telemark	101	28	129
Aust-Agder	76	9	85
Vest-Agder	60	2	62
Rogaland	70	10	80
Hordaland	130	7	137
Sogn og Fjordane	193	6	199
Møre og Romsdal	98	5	103
Sør-Trøndelag	274	34	308
Nord-Trøndelag	129	126	255
Nordland	115	1	116
Troms	116	25	141
Finnmark	73	17	90
Total	2780	625	3405

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 periods from July to April, 2012-2013.



Discussion

The 2012/2013 result is in agreement with the results from previous years with no positive samples detected. The cumulative sample size during the last five years is sufficient to confirm that the prevalence is less than 1%. This means that Norway fulfils the criteria, as given by EFSA (10), to document that *E. multilocularis* infection is absent from the national fox population. However, the criteria set by EFSA allow for samples to be collected over a five year period without taking into account the probability of introduction during the same period. Wahlström et al (5) showed that, even when taking into consideration the probability of introduction of infection, the number of samples collected until 2009 in Norway was sufficient to document that the prevalence was lower than 1%.

The detection of *E. multilocularis* in Sweden in 2011 and recently also in a new region in Denmark (11) have increased the risk of introduction of the parasite to Norway. 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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