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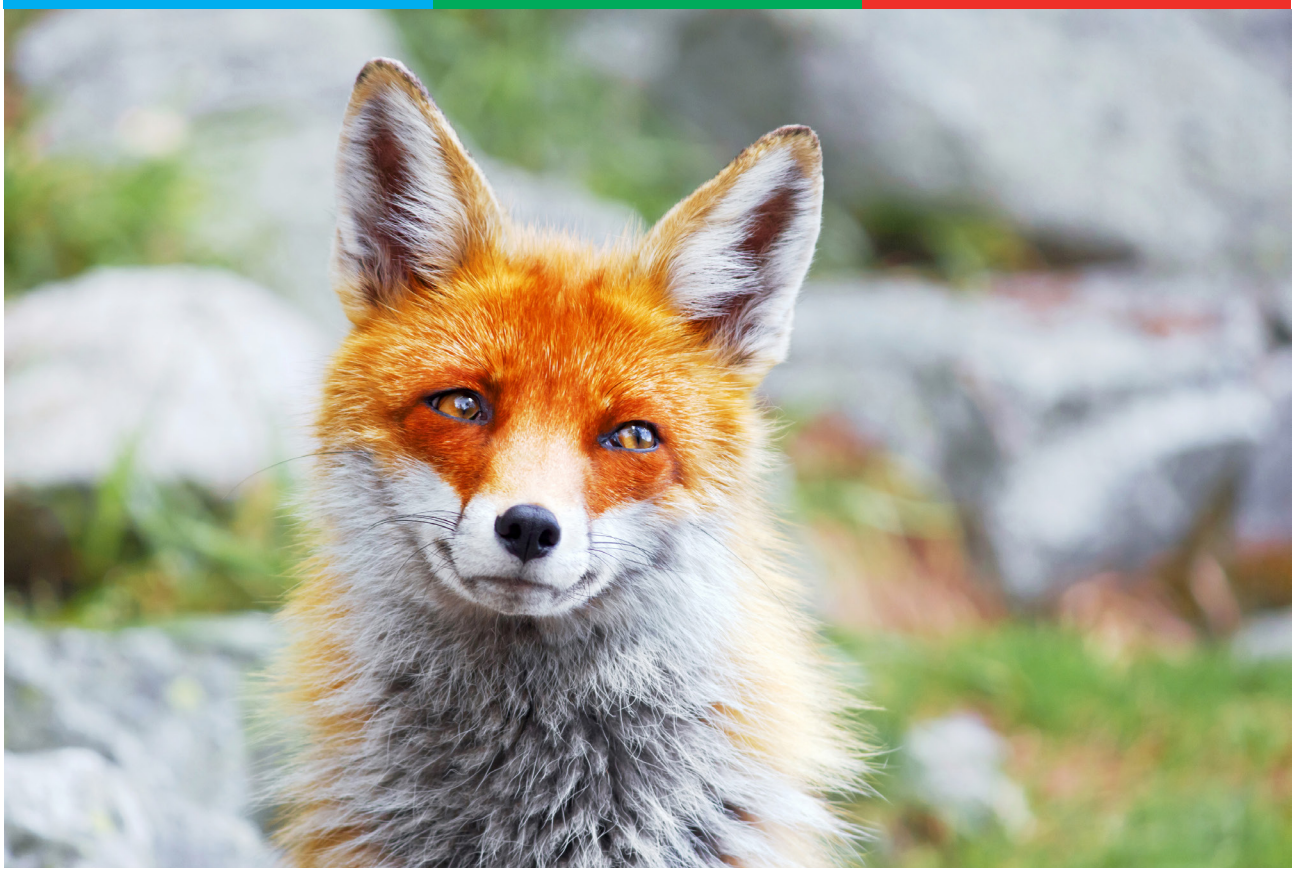
The surveillance programme for Echinococcus multilocularis in red foxes (Vulpes vulpes) in Norway 2020



REPORT 23/2021

The surveillance programme for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway 2020

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Summary

The prevalence of *Echinococcus multilocularis* was based on PCR analysis of faecal samples from 532 red foxes (*Vulpes vulpes*) collected during the licensed fox-hunting season in 2020 and 20 grey wolves (*Canis lupus*) killed in 2020. None of the samples tested positive for *E. multilocularis*, documenting that the prevalence in carnivore hosts (foxes and wolves) were below 1% at a confidence level of at least 95%.

Introduction

Echinococcus multilocularis (Figure 1), the parasite causing alveolar echinococcosis in humans, 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.

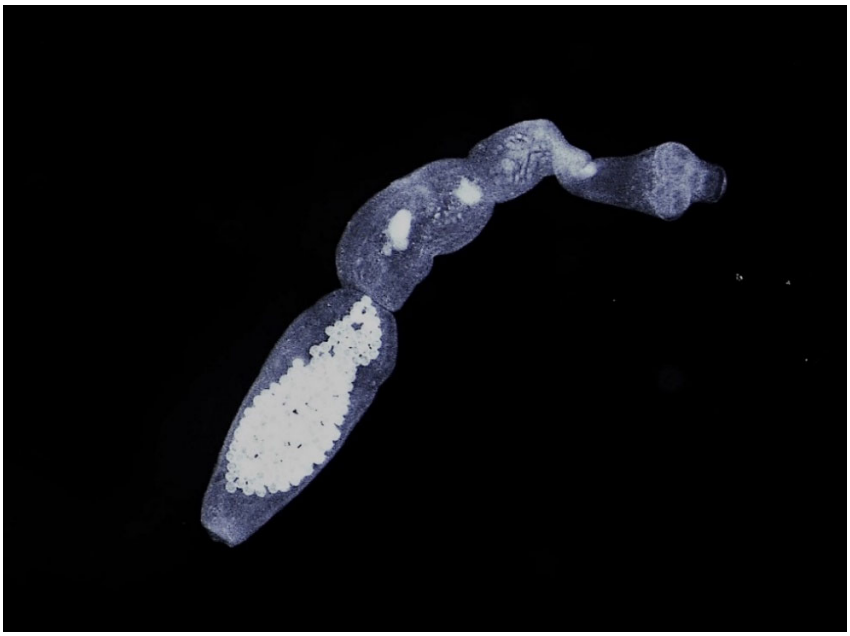

Figure 1: Echinococcus multilocularis*, adult worm used for spiking of positive controls included in the PCR analyses. The sack-like uterus containing hundreds of eggs is clearly visible. Worms used as controls were inactivated by kept frozen for <75 C for several days, and subsequently stored in 70% ethanol. Professor Peter Deplazes, University of Zurich, kindly donated the depicted worm. Photo: Øivind Øines, Norwegian Veterinary Institute*

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 was no evidence of its presence in mainland Fennoscandia (8) until its detection in Sweden in February 2011 (9). Despite analyses of more than 7000 faecal samples from foxes 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. According to the EU Directive 576/2013 on pet movement, the maintenance of this national regulation requires the documentation of an *E. multilocularis*-free status within the country in question.

Aim

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

Materials and methods

In the *E. multilocularis* surveillance of 2020, 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 2020) were included. In addition to faeces from foxes, samples from wolves (*Canis lupus*) killed legally, or illegally during 2020, were tested for the presence of *E. multilocularis*.

Recruitment of hunters was done through the webpages of the Norwegian Veterinary Institute (https://www.vetinst.no/nyheter/registrering-som-provetaker-av-rodrev). In addition, hunters who had supplied samples for the surveillance program during previous years were invited to participate by email.

Sampling containers and detailed instructions for sampling were sent to the hunters who volunteered for the program. The samples were submitted to the laboratory with written information on sample locality, date of the sampling, sex (male or female) and estimated age of the animal (juvenile or adult) in pre-paid envelopes. All counties in Norway were included in the sampling regime.

Individual faecal samples (3 g per animal) were analysed using the sensitive DNA-fishing (magnetic capture) method combined with real-time PCR detection of *E. multilocularis* mtDNA. This procedure involves magnetic capture of biotin tagged DNA-hybridisation probes targeting a locus on the *E. multilocularis* mtDNA. The biotin attached to the hybridisation probe/target DNA-complex is coupled by a noncovalent protein-protein binding interaction to streptavidin molecules which are coated onto magnetic beads. This allow extraction of parasite mtDNA from inhibitors and other DNA in the sample, by using a magnet (11).

Detection of the *E. multilocularis* DNA was carried out by real-time PCR (11, 12). If a positive real-time PCR signal is detected, the presence of *E. multilocularis* mtDNA can be verified by an additional independent real-time PCR (12), and /or using a standard PCR targeting the nad1 gene followed by Sanger-sequencing (13). All tests are performed in duplicates with each run including two positive control DNA samples (from adult worms) and negative controls (MilliQ water) included in each run.

The DNA-fishing method is capable of detecting *E. multilocularis* DNA originating from any developmental stage of the parasite, including worms, and eggs in high volume samples. 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 MC-DNA/realtime PCR methods has been shown to be more sensitive than egg isolation by sieving followed by detection of parasite DNA using a multiplex PCR, used previously in the Norwegian surveillance program (11, 12). Validation of the current methods in our laboratory has demonstrated a sensitivity of 58% and 88% in 3 g samples spiked with 5 eggs or one worm, respectively (unpublished results).

Initially, a test sensitivity of 63% and a specificity of 100% were assumed (11). However, our internal validation has demonstrated an overall sensitivity of 70%. For samples spiked with ≥ 10 eggs the sensitivity is 86% which is close to the method of Isaksson et al. (12) (88% test sensitivity) (11, 14). The apparent prevalence and corresponding confidence interval were estimated using Epitools (15), with a test sensitivity of 63% and a specificity of 100%.

Results and Discussion

In 2020, 552 faecal samples from wild carnivores were analysed for *E. multilocularis*: 532 samples from red foxes (Table 1, Figure 2) and 20 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 – 0.7%, 95%CI).

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 2020, were negative by PCR for *E. multilocularis*.

According to requirements of Regulation (EU) No 2018/772, Annex I, the disease freedom status must have a pathogen-specific surveillance program designed to detect a prevalence of ≤ 1% at minimum confidence level of 95%. The number of samples collected and analysed in Norway in 2020 was sufficient to document a current prevalence of *E. multilocularis* below 1%.

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 2020 (January to mid-April and mid-July to late December) and corresponding numbers for the period 2002 - 2019.*

County 2020	County 2019	Number of red foxes tested		Other species tested 2020
		2020	Total 2002-2019	
Viken	Østfold	51	871	
	Akershus	22	762	1
	Buskerud	26	362	1
Oslo	Oslo	6	162	
Innlandet	Hedmark	58	1 031	14
	Oppland	35	471	3
Vestfold og Telemark	Vestfold	12	103	
	Telemark	27	326	
Agder	Aust-Agder	23	142	
	Vest-Agder	14	144	
Rogaland	Rogaland	17	110	
Vestland	Hordaland	34	311	
	Sogn og Fjordane	24	271	
Møre og Romsdal	Møre og Romsdal	18	174	
Trøndelag	Trøndelag	47	963	1
Nordland	Nordland	44	280	
Troms og Finnmark	Troms	55	469	
	Finnmark	19	153	
Total	**Total**	**532**	**7 105**	**20 wolves**

However, it is worrying that the rising prevalence in countries close to Norway has increased the risk of introduction of the parasite to Norway. In Sweden, there are already detections of *E. multilocularis* in four different regions (10), and surveillance in Denmark has demonstrated its presence in two regions (16). Studies in Sweden have discovered *E. multilocularis* in the intermediate hosts of field vole (*Microtus agrestis*) and water voles (*Arvicola amphibious*) in two study areas (20). 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 with the surveillance 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 continuing national regulation for compulsory anthelmintic treatment of imported dogs to minimize the risk of an introduction of *E. multilocularis* to Norway.

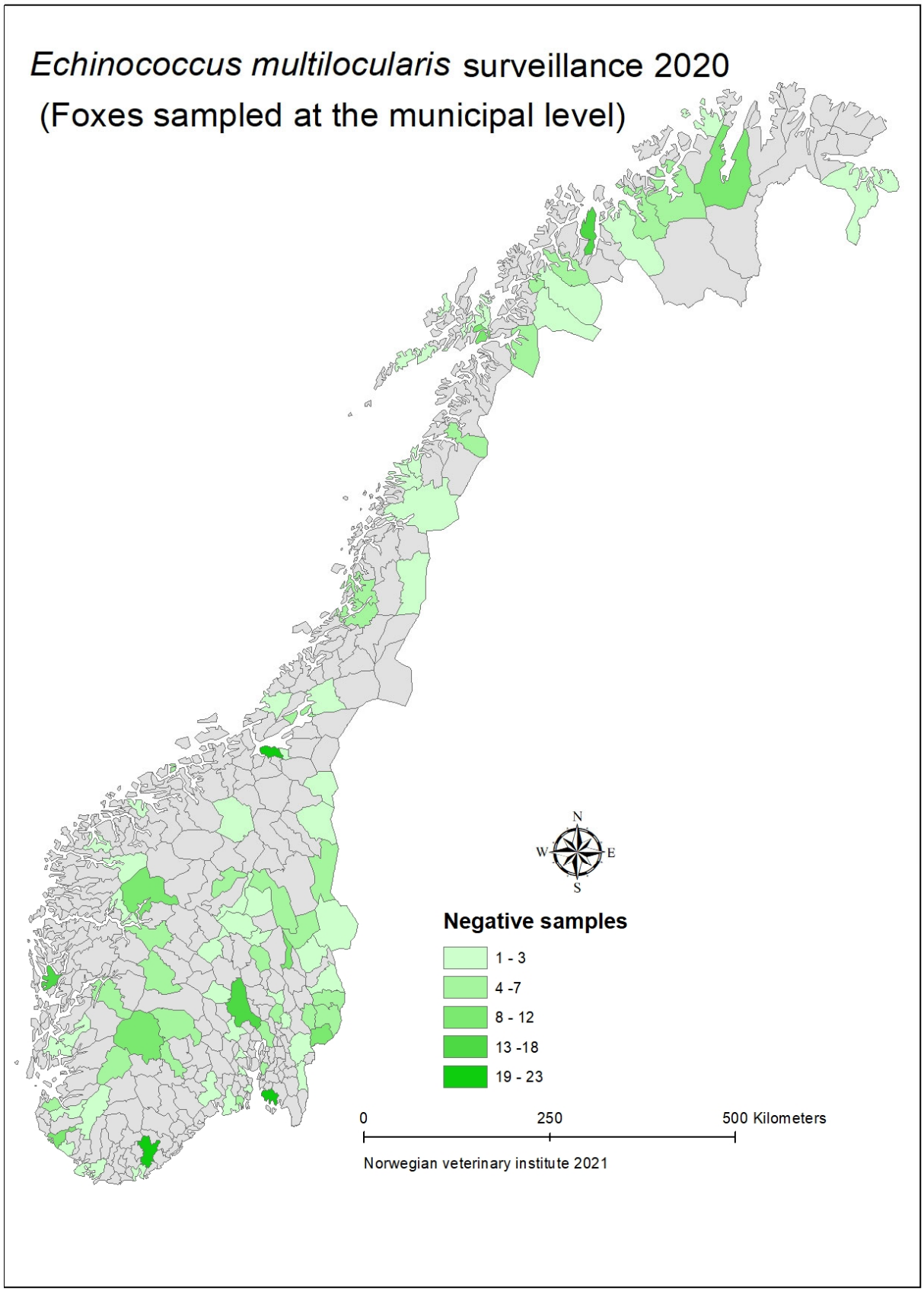

***Figure 2**: Map of Norway showing the origin of red foxes by municipality, tested for* Echinococcus multilocularis *during the red fox licensed hunting season in 2020 (January to mid-April and mid-July to late December)*

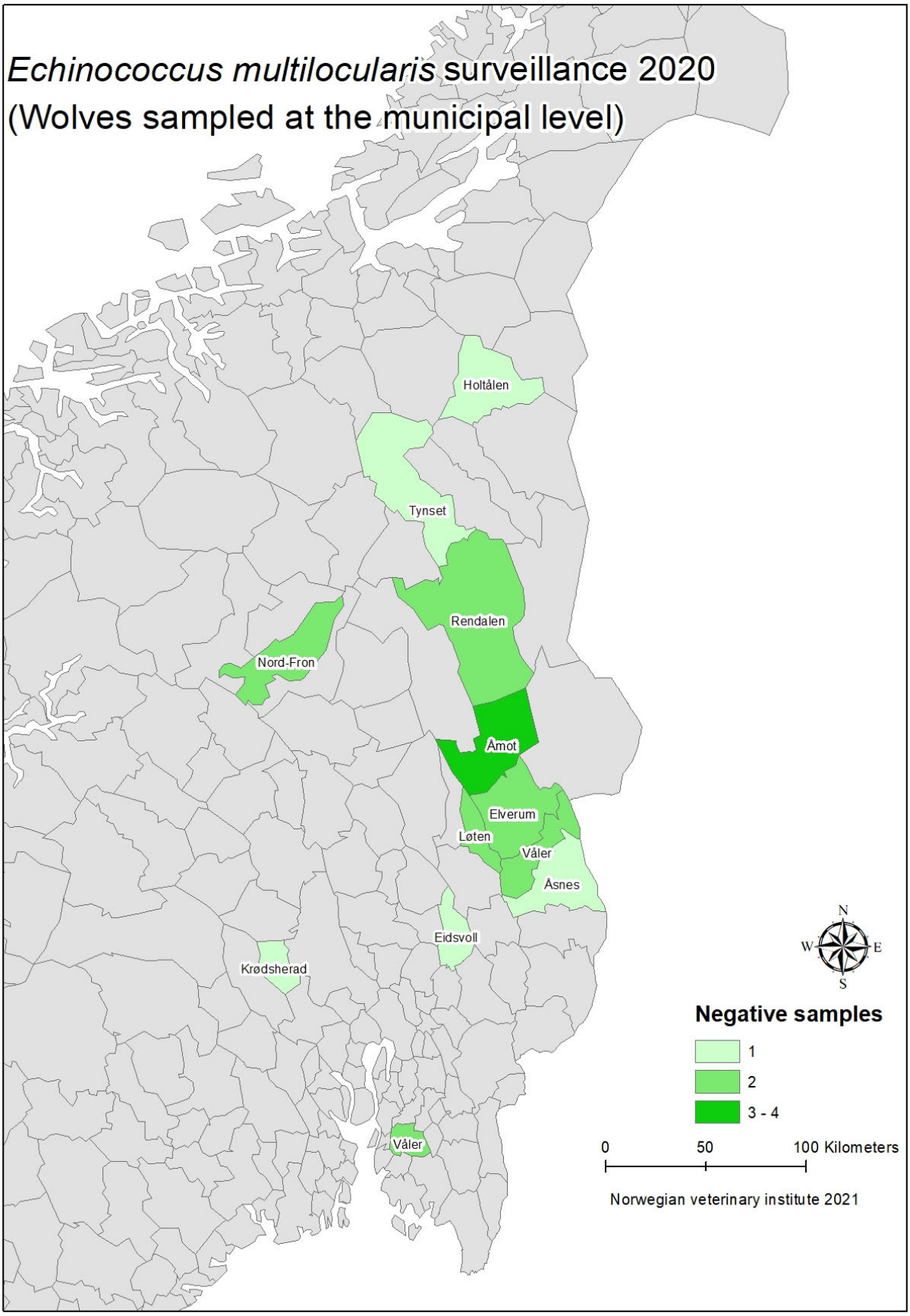

Figure 3: Map of Norway showing the origin of wolfs by municipality, tested for Echinococcus multilocularis *in 2020.*

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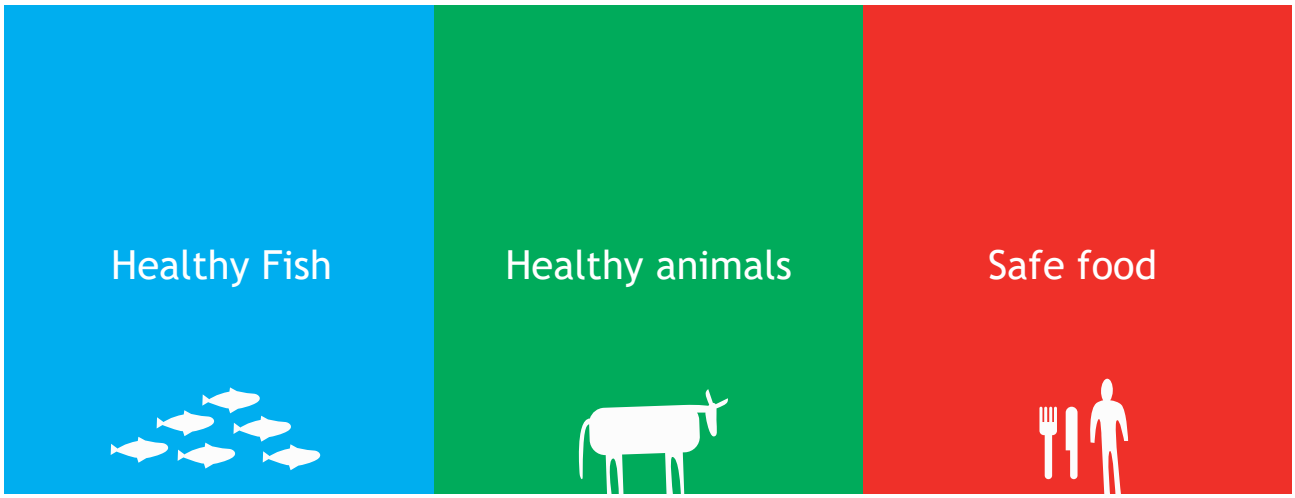

Scientifically ambitious, forward-looking and collaborative- for one health!

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