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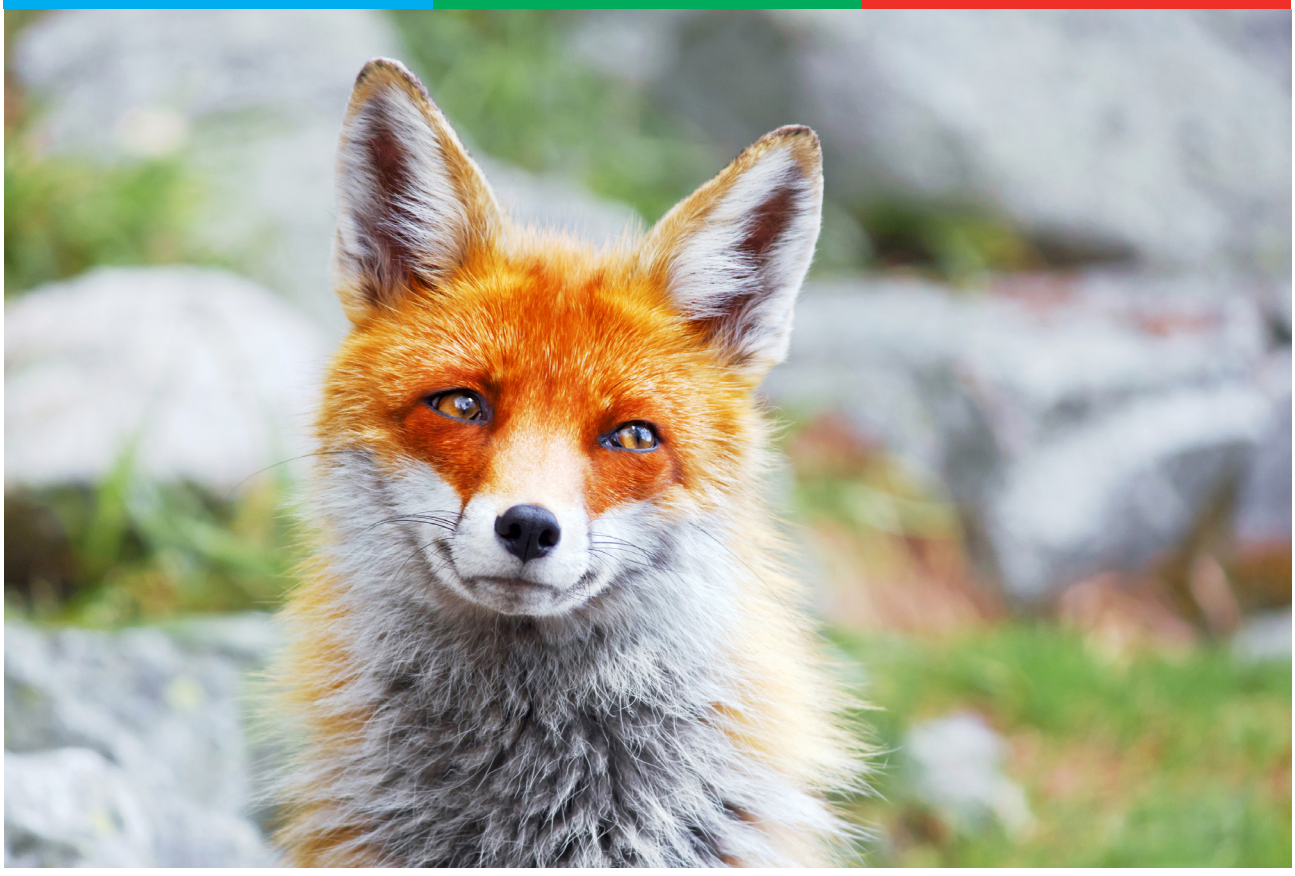
The surveillance programme for *Echinococcus multilocularis* in red foxes (Vulpes vulpes) in Norway 2023



REPORT 35/2024

The surveillance programme for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway 2023

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Content

Summary

The prevalence of *Echinococcus multilocularis* was investigate by analyzing PCR results from faecal samples of 512 red foxes (*Vulpes vulpes*) collected during the licensed fox-hunting season in 2023 and 12 grey wolves (*Canis lupus*) that were killed in 2023. The absence of positive results for *E. multilocularis*, indicate that the prevalence in carnivore hosts (foxes and wolves) was 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, particularly in eastern and central parts of Europe (1, 2). Over the past decades, the endemic areas (3) of *E. multilocularis* in Europe has risen and its geographic distribution has expanded to regions where the parasite appeared to be absent previously (4). This expansion has led to an increased incidence of alveolar echinococcosis, the life-threatening zoonotic disease caused by the infections of the metacestode stage of this tapeworm. 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 (*Nyctereutes procyonoides*) and wolves. Domestic dogs and cats can also act as definitive hosts if they prey on infected small mammals that serves as intermediate hosts, predominantly rodents. Intermediate hosts become infected after ingestion of eggs that has been excreted by the carnivore hosts into the environment. The *E. multilocularis* eggs are resilient to disinfectants and can survive for long periods in the environment with experiments indicating survival up to 240 days (22).

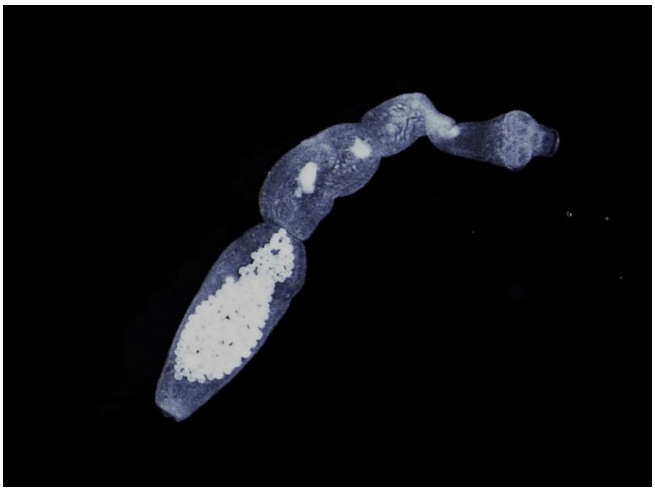

Figure 1: Echinococcus multilocularis, *adult worm used for spiking of positive controls included in the PCR analyses. The sack-like uterus containing hundreds of eggs is clearly visible. Worms used as controls were inactivated by kept frozen for <75 C for several days, and subsequently stored in 70% 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 8,600 faecal samples from foxes since 2002 (10, present report), *E. multilocularis* has not been reported in mainland Norway.

Anthelmintic treatment of dogs, prior to import from endemic regions, is compulsory in Norway and has been implemented to prevent introduction of the parasite. According to the EU Directive 576/2013 and Commission Delegated Regulation (EU) 2018/772 on pet movement, the maintenance of this national regulation requires the documentation of an *E. multilocularis*-free status within the country in question. The results of the investigations in the surveillance programme to EFSA every year to document freedom of *Echinococcus multilocularis* in mainland Norway. Every year the datasets from participating countries is subject to assessment and the resulting report; "Annual assessment of *Echinococcus multilocularis* surveillance reports submitted in 20xx in the context of Commission Delegated Regulation (EU) 2018/772" is published in the EFSA journal.

The Norwegian Food Safety Authority (NFSA) is responsible for implementing the surveillance programme. The Norwegian Veterinary Institute (NVI) is responsible for sampling plans, laboratory investigations and the reporting components of the programme to EFSA and the annual national report.

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 2023, 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 2023) were included. In addition to faeces from foxes, samples from wolves (*Canis lupus*) killed legally, or illegally during 2023, were tested for the presence of *E. multilocularis* (Figure 6.). One sample from a wild arctic fox (from mainland Norway population, necropsied at Norwegian Veterinary Institute) was also tested.

The RiBESS tool (https://shiny-efsa.openanalytics.eu/app/ribess) was used to estimate the sample size required to substantiate the absence of the parasite from the target population with a confidence level of 95%. For the calculation we used sensitivity value 0.63, specificity value 1.00 (11), together with an estimated population size of 151,000 red foxes.

***Figure 2**: Map showing observations of red fox in Norway. Online service where citizens can logon and register their observations of fauna and flora in Norway. Source: Norwegian Biodiversity Information Centre. https://artsdatabanken.no/Pages/180936*

Recruitment of hunters was done through the webpages of the Norwegian Veterinary Institute. The hunters enter their name and municipality via the webpages of the Norwegian Veterinary Institute (https://www.vetinst.no/nyheter/registrering-som-provetaker-av-rodrev-2023). This registration is announced on NVI's web page and at the NVI's Facebook page. Those that have contributed to the program previous years are invited by e-mail to register, but the registration is also open for new hunters. The selection of hunters is the based on residence and previous quality of their submitted samples. In addition, the selection also includes some hunters that are new to the programme and therefore covers some new regions.

Sampling containers and detailed instructions for sampling were sent to the selected hunters. 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 through 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 followed by washing-steps (11).

Detection of the *E. multilocularis* DNA is 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). The NVI-staff participate annually on molecular proficiency tests for detection and identification of *Echinococcus* spp. organised by the EURL.

All tests are performed in duplicates with each run including two positive control DNA samples (from adult worms) and negative controls (MilliQ water) and EBK 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).

Initially, a test sensitivity of 63% and a specificity of 100% were assumed (11). However, our internal validation has demonstrated an overall sensitivity of 0.82.For samples spiked with ≥ 10 eggs the sensitivity is 0.91 which is close to the method of Isaksson *et al.* (12) (Se 0.88) (11, 14). The apparent prevalence and corresponding confidence interval were estimated using Epitools (15), with a test sensitivity of 0.63 and a specificity of 1.00.

Results and Discussion

In 2023, 529 faecal samples from wild carnivores were analysed for *E. multilocularis*: 512 samples from red foxes (Table 1, Figure 3, 4 and 5), 12 samples from wolves (*Canis lupus*) (Table 1 and Figure 6) and one from artic fox (*Vulpes lagopus*). 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 2023, 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 spatial distribution of the fox samples is somewhat uneven (Figure 3, 5 and Table 1), but all counties were represented. The topography of Norway (large areas with mountains) entails scattered settlements, and hunters do the fox sampling voluntarily in the proximity of their homes. When compared with the fox hunting statistics for 2022-2023 (Statistics Norway), the counties of Viken and Innlandet reported the highest numbers of hunted foxes.

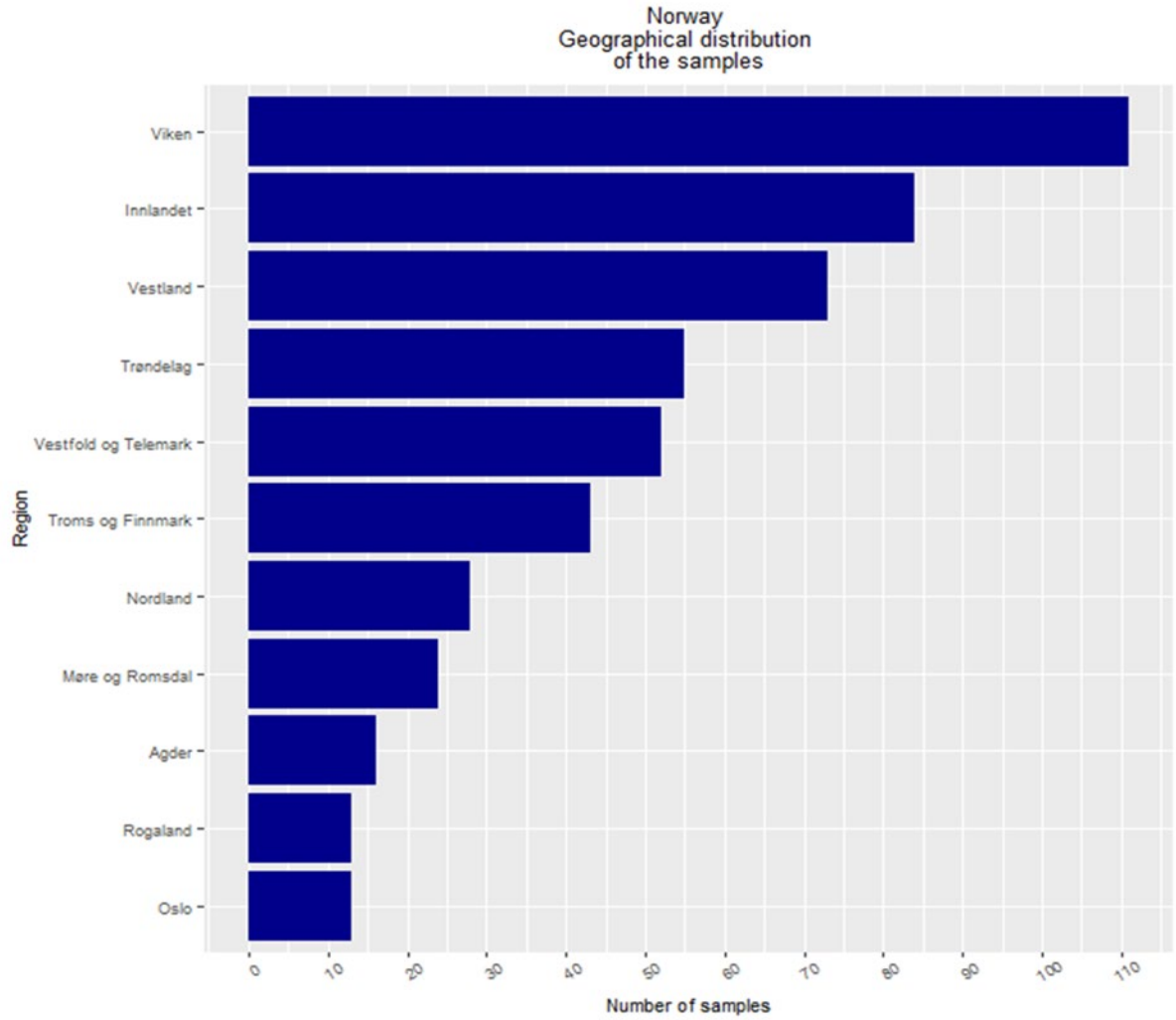

Figure 3: Norway - Geographical distribution of red fox samples in 2023. (Source: EFSA)

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 2023 (January to mid-April and mid-July to late December) and corresponding numbers for the period 2002 - 2022.*

County 2023	County 2019	Number of red foxes tested		Other species tested 2023
		2023	Total 2002-2022	
Viken	Østfold	51	1 079	
	Akershus	19	873	1
	Buskerud	41	471	
Oslo	Oslo	13	197	
Innlandet	Hedmark	64	1 171	11
	Oppland	20	559	
Vestfold og Telemark	Vestfold	12	129	
	Telemark	40	407	
Agder	Aust-Agder	10	214	
	Vest-Agder	6	170	
Rogaland	Rogaland	13	157	
Vestland	Hordaland	42	421	
	Sogn og Fjordane	28	337	
Møre og Romsdal	Møre og Romsdal	27	241	
Trøndelag	Trøndelag	55	1 076	
Nordland	Nordland	28	383	
Troms og Finnmark	Troms	23	585	
	Finnmark	20	181	
Total	**Total**	**512**	**8 651**	**12 wolves**

The temporal distribution of samples is also somewhat uneven (Figure 4). This is most likely due to preferred hunting conditions during winter (January–March) and banned hunting between 15 April and 15 July (and between 24th and 31st December). In September and October, it is also hunting season for wild cervids such as moose (*Alces alces*) and red deer (*Cervus elaphus*) (and in which many Norwegian hunters participate), which might be an explanation for the low numbers of red fox samples from these months.

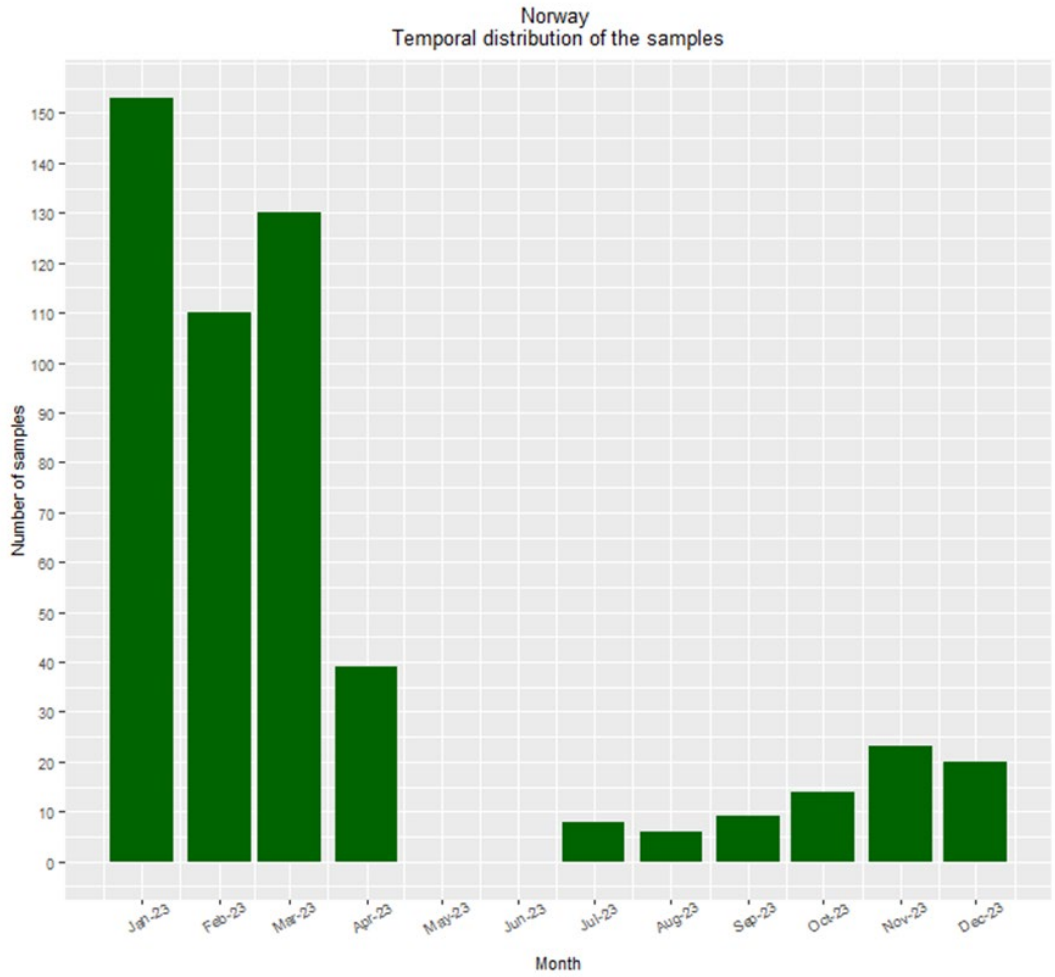

Figure 4: Temporal distribution of red fox samples in 2023 (Source: EFSA). In Norway red fox hunting is allowed all year except between 15th April – 15th July.

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, 21), 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.

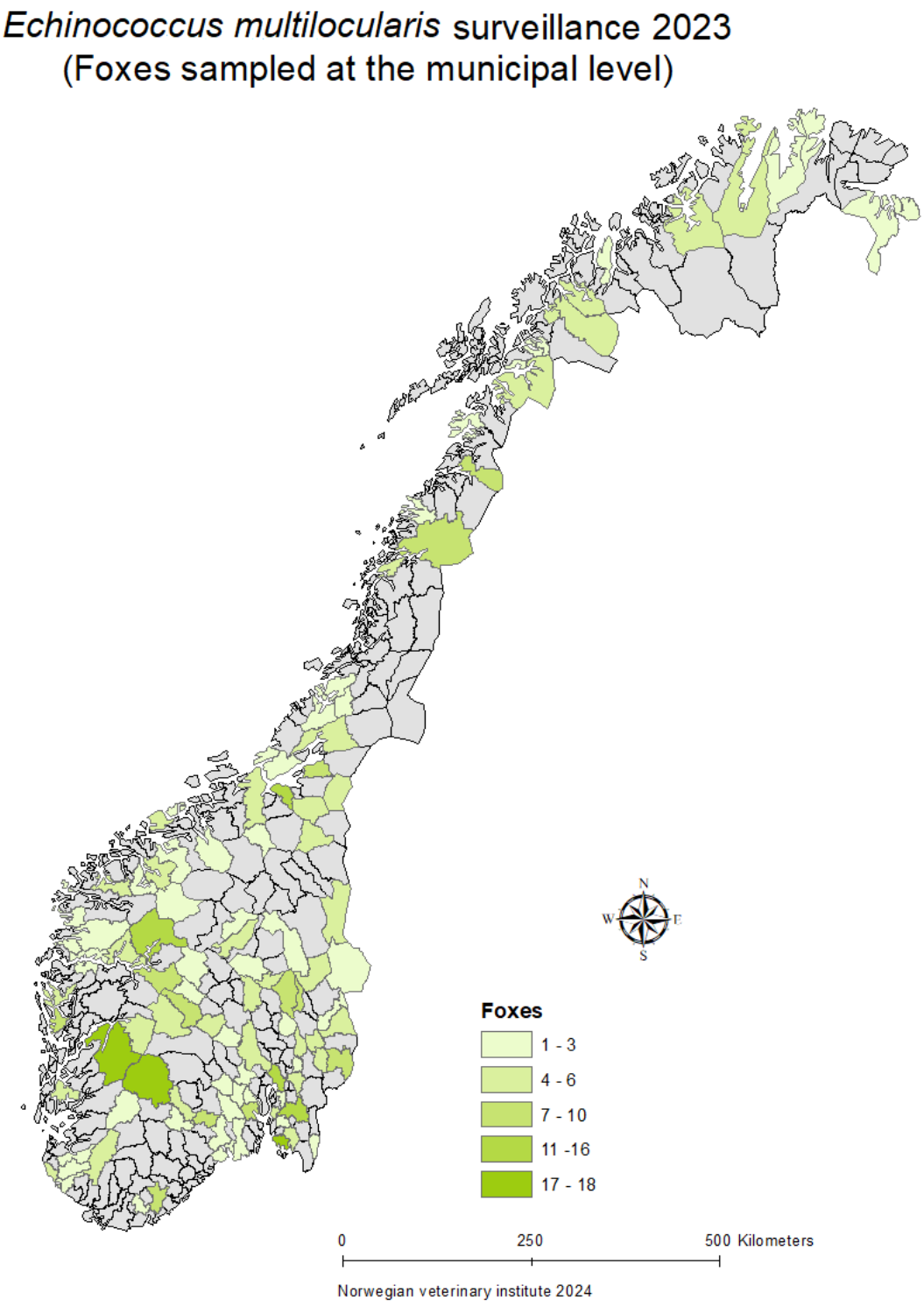

Figure 5: Map of Norway showing the origin of red foxes by municipality, tested for Echinococcus multilocularis *during the red fox licensed hunting season for red fox in 2022.*

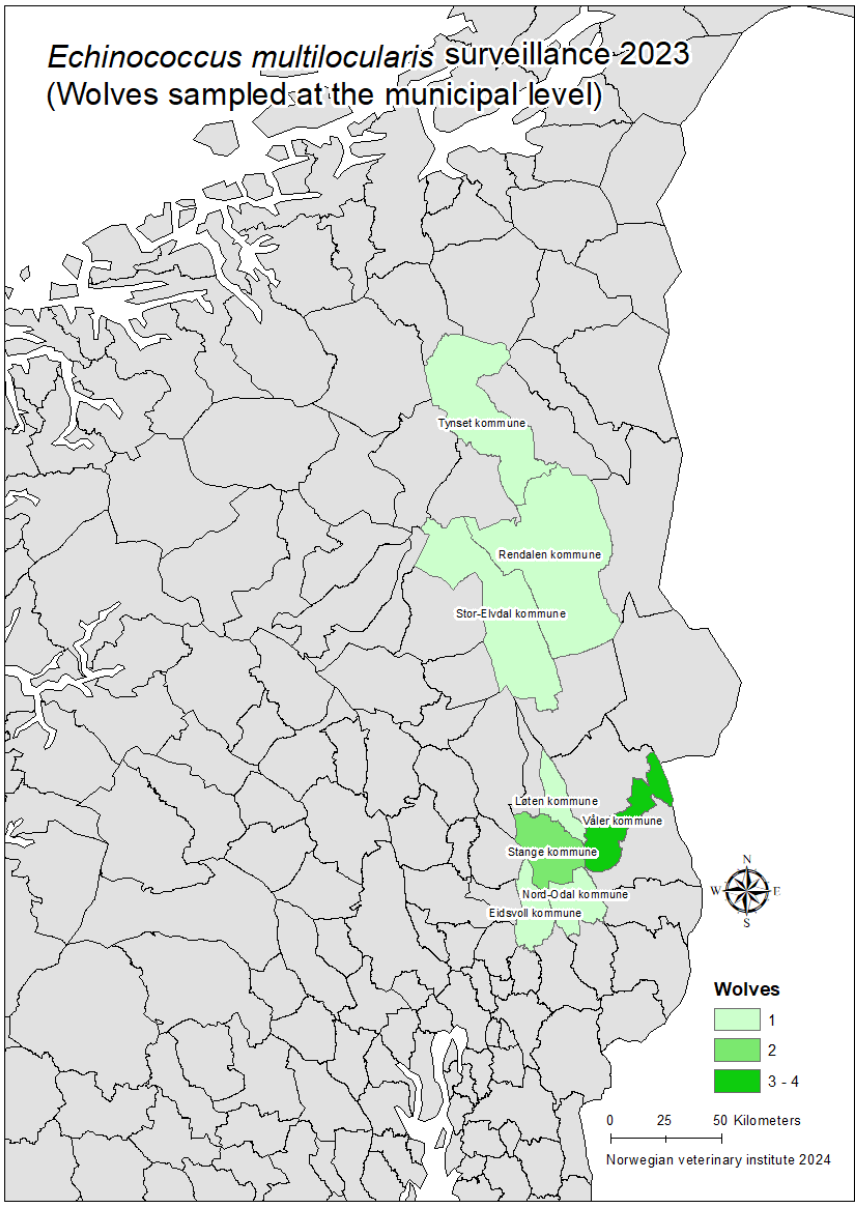

Figure 6: Map of Norway showing the origin of wolfs by municipality, tested for Echinococcus multilocularis *in 2022*

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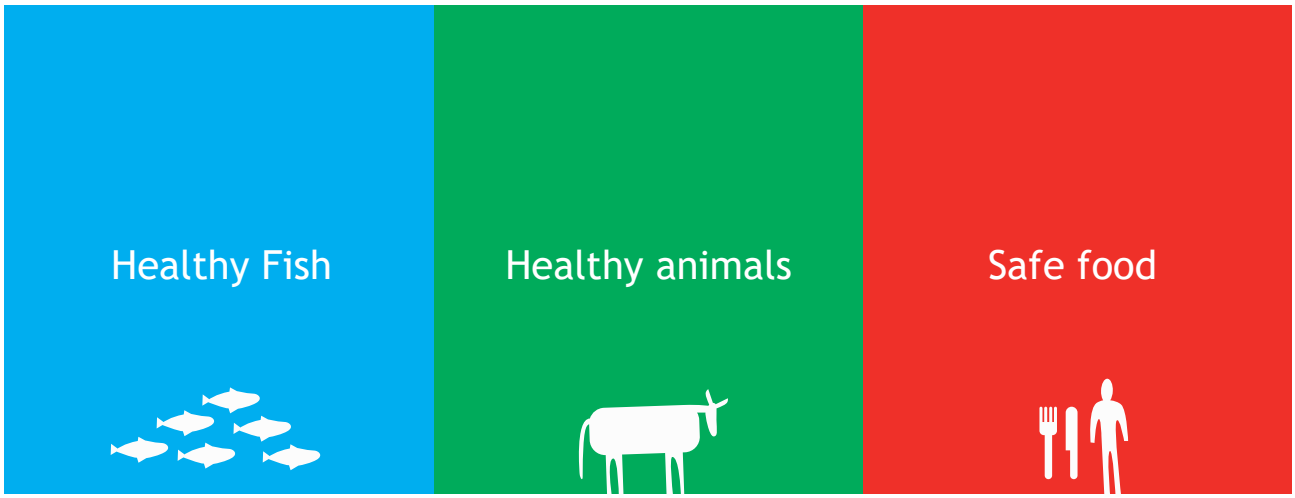

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