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The surveillance programme for *Echinococcus multilocularis* in red foxes (Vulpes vulpes) in Norway 2025

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Content

Summary

The prevalence of *Echinococcus multilocularis* (*E. multilocularis*) in definitive hosts (red fox, wolf) was investigated by analysing PCR results from faecal samples of 505 red foxes (*Vulpes vulpes*) collected during the licensed fox-hunting season in 2025, and one grey wolf (*Canis lupus*) that also was sampled in 2025. All samples tested negative. 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% as required to maintain the *Echinococcus multilocularis*-free status for mainland Norway.

Introduction

E. 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 initiative ranked *E. multilocularis* the most important food-borne parasite in Europe based on the high severity associated with human infections and the predicted increase in spread of this parasite within Europe (5).

Human cases of alveolar echinococcosis have been reported in Norway (25); however, these have all been classified as import cases, based on thorough case-by-case analysis. The disease has been classified as a list-2-zoonosis and is notifiable through the Notifiable System for Communicable Diseases (MSIS) managed by the Norwegian Institute of Public Health (FHI) (https://www.fhi.no/en/).

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 (21).

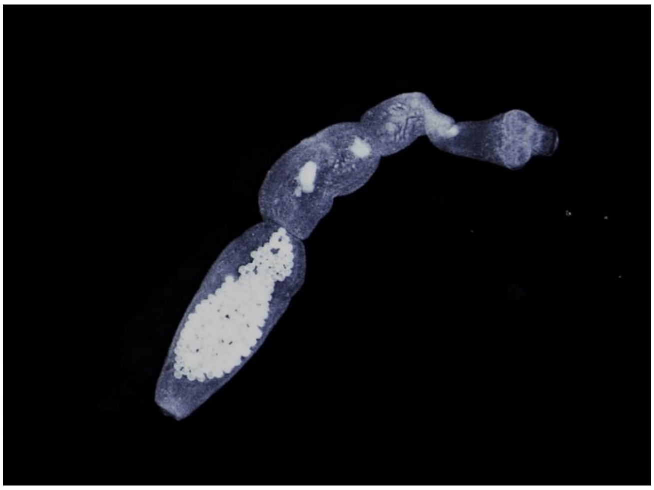

Figure 1. *Echinococcus multilocularis*, adult worms and eggs are used for spiking of positive controls included in the PCR analyses. The sack-like uterus containing hundreds of eggs is clearly visible. Worms used as controls were inactivated by kept frozen for <75 C for several days and subsequently 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 discoveries of *E. multilocularis* were 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 9,600 faecal samples from foxes since 2002 (Table 1), *E. multilocularis* has not been found 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 are reported to EFSA every year to document freedom of *E. multilocularis* in mainland Norway. Every year, the datasets from countries with *E. multilocularis*-free status (i.e. Finland, Ireland and Norway), is subject to assessment by the EFSA Scientific Network for Risk Assessment in Animal Health and Welfare, subgroup *Echinococcus multilocularis* 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 for this parasite. The Norwegian Veterinary Institute (NVI) is responsible for sampling plans, laboratory investigations and the reporting components of the programme to EFSA and the production of the annual national report.

Aim

The aim of the surveillance-programme is to document the freedom of *E. multilocularis* in mainland Norway by analyses of samples from definite hosts.

Materials and methods

The RiBESS tool (https://r4eu.efsa.europa.eu) was used to estimate the sample size required to substantiate the absence (i.e. prevalence < 1%) of the parasite from the target population with a confidence level of 95%. For the calculation we used the published (11) sensitivity value 0.63, specificity value 1.00 (11), together with an estimated population size of 151,000 red foxes.

Using these parameters, results from a minimum of 474 examined samples must be examined and reported to EFSA to meet the criteria given in EU Directive 576/2013 and Commission Delegated Regulation (EU) 2018/772.

The red fox is the main target species for this surveillance programme. There are no scientific studies describing the Norwegian red fox population size. In 2020 report by Hansen et al., 2020 (27) on red fox as cause of loss of lambs on pasture, it was estimated that the Norwegian red fox winter population could be up to close to 100,000 adults, and a summer population (including pups) of minimum twice that size, about 200,000 individuals. Data from Statistics Norway (www.ssb.no) shows that, for the hunting season 2024-2025, Norwegian hunters reported 18,180 red foxes hunted in mainland Norway (23). There has been an annual decline in number of reported hunted red foxes in mainland Norway since 2019 (23), but to our knowledge, there are no scientific data that indicates a decline in the red fox population. In the absence of more accurate alternatives, we used an estimate for the population of Norwegian red foxes of 151,000 for calculations of desired sampling size. This population estimate was provided by professor emeritus Olav Hjeljord at the Norwegian University of Life Sciences and was partly based on the spatial distribution of preferred fox habitat and hunting statistics. The red fox is geographically distributed all over mainland Norway (Figure 2). The population density during spring is (roughly estimated) varying from 1 red fox/10 km^2 in mountain areas, to 3 red foxes/10 km^2 in forest/marsh lands and to 10 red foxes/10 km^2 in urban/agricultural areas such as parts of eastern Norway (personal communication Prof. emeritus Olav Hjeljord, 2020). As for many other predator species in Scandinavia the reproduction and survival rate of red fox pups fluctuates by following the fluctuations in the small rodent populations. Both the number of litters and the litter size vary significantly with the prevalence and thus accessibility of small rodents. The latter fluctuates greatly in 3-5 years cycles, usually with high populations of rodents every fourth year often designated as a “rodent year”. In such rodent years, rodents dominate the red fox diet, thus more and bigger litters are born. However, the peaks in rodent populations does not necessarily occur in the same year in different parts of Norway, making it even more of a challenge to estimate the red fox population accurately any given year. In years with shortage of food, the mortality among the pups is presumably high (22).

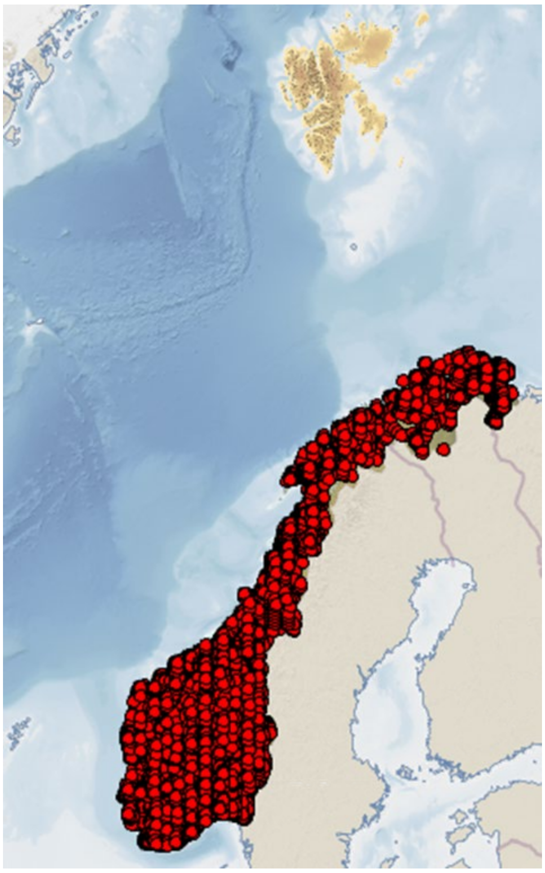

Figure 2. Map showing observations of red fox in Norway. Online service where citizens can logon and register their observations of fauna and flora in Norway. Source: Norwegian Biodiversity Information Centre. https://artsdatabanken.no/arter/takson/31176

Norway also harbours much smaller populations of other potential definitive hosts for *E. multilocularis*. Notably, there are wolves (*Canis lupus*) and Arctic foxes (*Vulpes lagopus*), with occasional reports also mentioning observation of raccoon dogs (*Nyctereutes procyonoides*). No raccoon dogs were registered in Statistics Norway’s hunting bag database for 2024-2025. The arctic fox is a critically endangered species in Mainland Norway and is closely monitored. A re-establishing programme to increase the number of Arctic foxes in mainland Norway is currently ongoing. For 2025 the mainland population was estimated to be 309 adult foxes (26). A small and tightly regulated population of wild wolves inhabits Norway. (*Canis lupus lupus*). During the winter of 2024–2025, there were 40-47 wolves recorded in Norwegian territories and an additional 19 wolves residing in territories spanning both Norway and Sweden. This is a decline from previous years (24). Both the arctic fox and wolf are endangered species in Norway. For the arctic fox in mainland Norway no hunting is allowed. As for the wolves a limited number are taken out every year by licensed hunting. Occasionally NVI receives carcasses of arctic fox or wolves for postmortem examination, and in that occasion intestinal content samples are collected and examined for *Echinococcus multilocularis* by the method used in the surveillance programme. A limited number of wolf samples are usually submitted annually from Norwegian Institute for Nature Research. On top of the 505 red foxes tested between in 2025 as part of our official surveillance program, one sample from a wolf was submitted and it was also included in the surveillance examinations for *E. multilocularis.*

Recruitment of hunters was done through the webpage of the Norwegian Veterinary Institute. The hunters enter their name and municipality via the webpage of the Norwegian Veterinary Institute; Registrering som prøvetaker av rødrev 2025 - Veterinærinstituttet. 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 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 allows 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 in proficiency tests for detection and identification of *Echinococcus* spp. in mucosa and molecular identification of *Echinococcus* spp., organised by the European Reference Laboratory for Parasites (EURL-P).

All real time tests from samples are performed in duplicates with each run including two positive control DNA samples and negative controls (MilliQ water) and Extraction Blank Controls (EBC) 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).

For the method used, a test sensitivity of 63% and a specificity of 100% were assumed (11) although our internal validation has demonstrated the overall sensitivity to be higher. Based on results of spiked samples over time (2015-2024), the overall sensitivity of the method appears to be 0.82, 95% CI [0,77-0.86] (source EFSA). The apparent prevalence with a corresponding confidence interval of 95% were estimated using Epitools (14), using a test sensitivity of 0.63 and a specificity of 1.00.

Results and Discussion

In the *E. multilocularis* surveillance of 2025, 511 faecal samples were collected from red foxes hunted during the licensed hunting season (i.e. January to mid-April and mid-July to late December 2025). Six samples were unfit, leaving 505 faecal samples from red foxes for analysis. In addition, one sample from a hunted wolf, provided by Norwegian Institute for Nature Research, was tested for the presence of *E. multilocularis* (Table 1, Figure 5). All samples tested negative for *E. multilocularis* giving an estimated apparent prevalence of 0%, 95% CI [0.0 – 0.8%]. Surveillance results were no different from earlier years.

Table 1. Number and origin (county) of red foxes and number of other animals examined for *Echinococcus multilocularis* in Norway in 2025 and corresponding numbers for the period 2002-2025.

County	Number of examined red fox samples in 2025	Total number of examined samples from red foxes 2002-2025	Number of samples from other species examined in 2025
Østfold	33	1 085	
Akershus	30	979	
Oslo	15	222	
Innlandet	81	1 829	1
Buskerud	48	557	
Telemark	26	175	
Vestfold	26	490	
Agder	24	410	
Rogaland	9	189	
Vestland	74	925	
Møre og Romsdal	26	324	
Trøndelag	49	1 155	
Nordland	26	366	
Troms	23	674	
Finnmark	15	238	
Overall	**505**	**9 618**	**1 wolf**

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 Norwegian *E. multilocularis* Surveillance and Control Programme fulfils these criteria.

The geographical distribution of the fox samples is somewhat uneven (Table 1, Figure 3, 4), 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 2024-2025 (23), the counties of Innlandet, Trøndelag and Vestland reported the highest numbers of hunted red foxes.

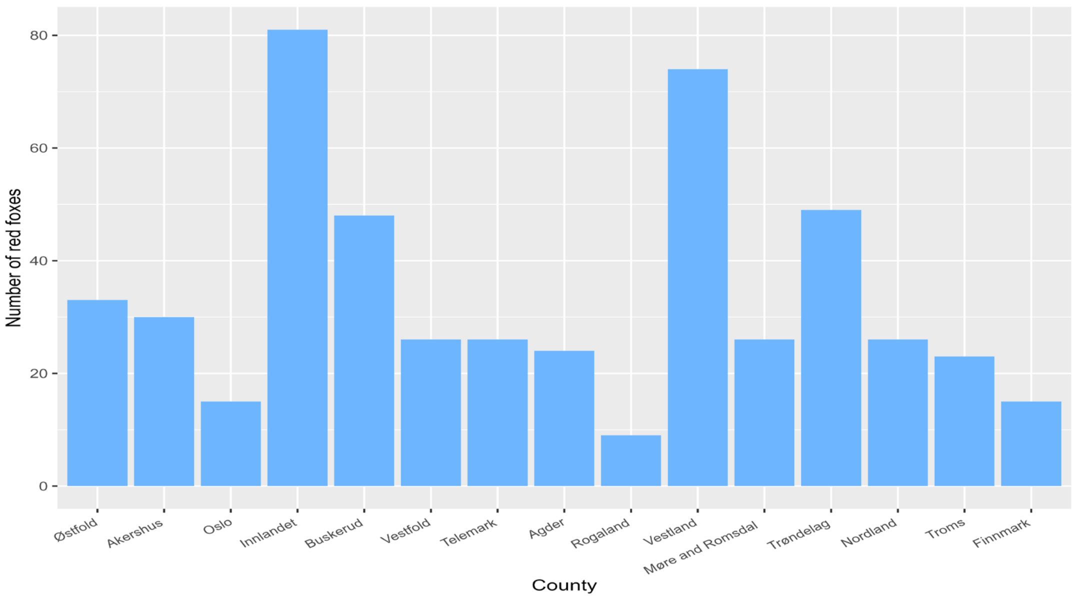

Figure 3. Geographical distribution of examined red foxes (n = 505) in the Norwegian *E. multilocularis* surveillance programme 2025.

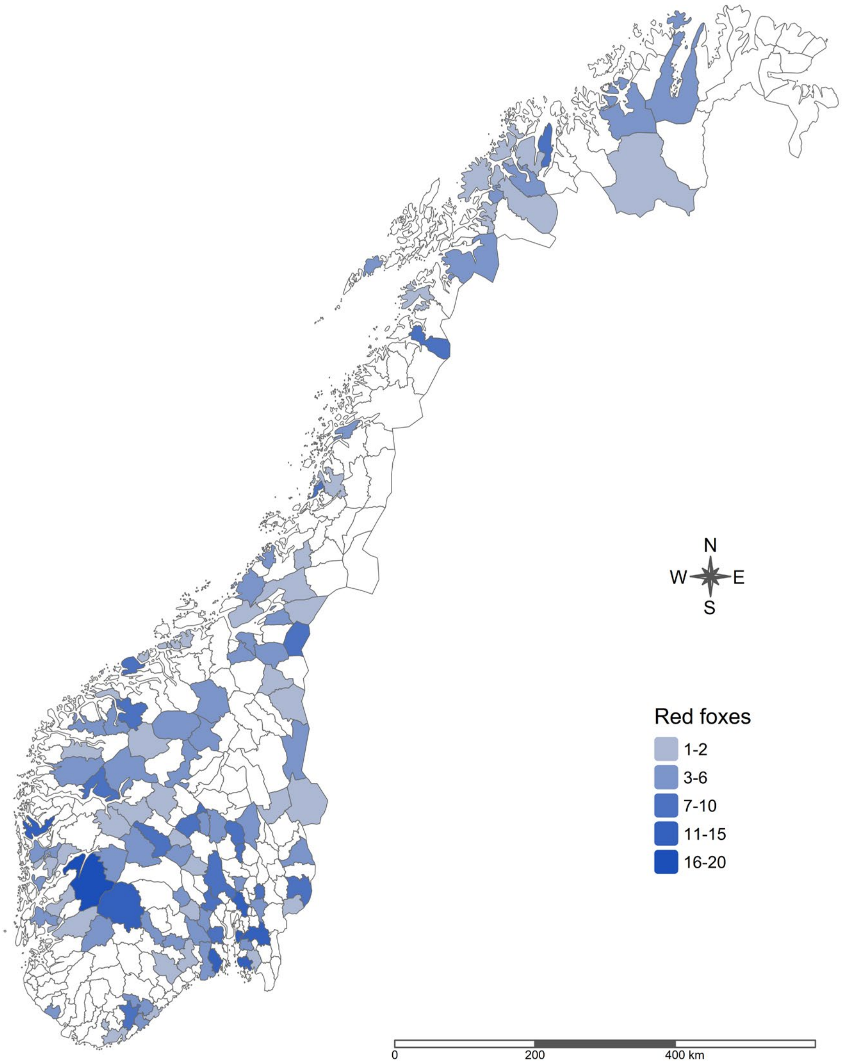

Figure 4. Map of Norway showing the origin of red foxes by municipality, tested for *Echinococcus multilocularis* during the red fox licensed hunting season for red fox in 2025.

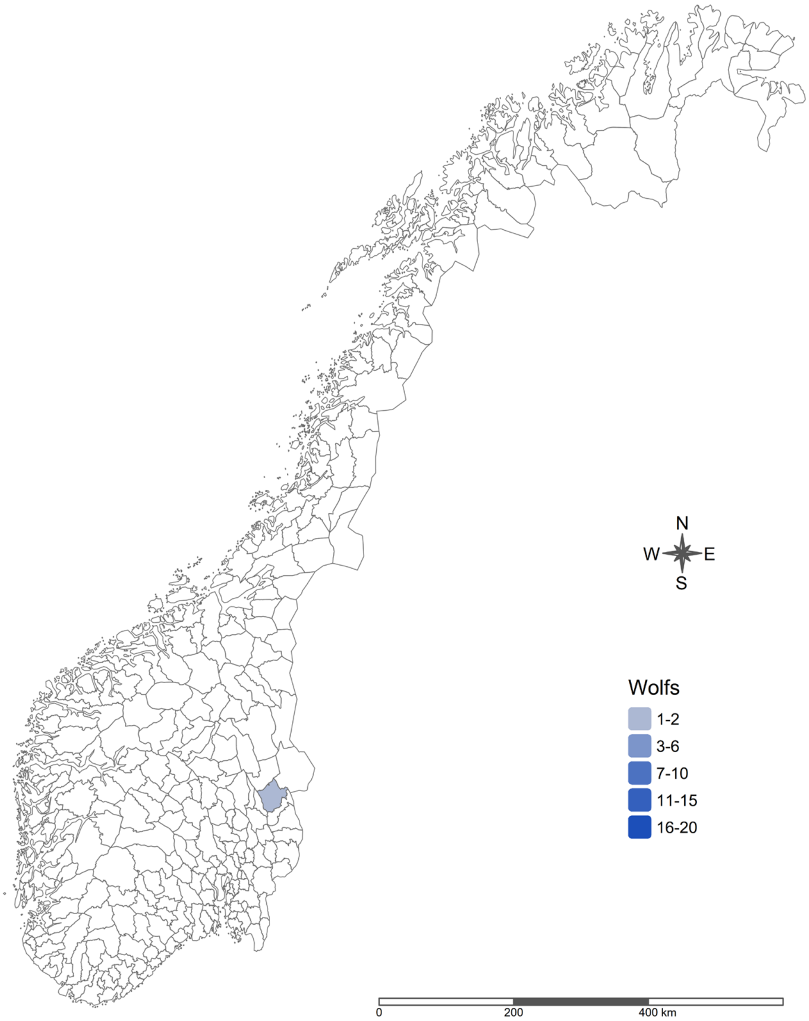

Figure 5. Map of Norway showing the origin of wolfs by municipality, tested for *Echinococcus multilocularis* in 2025

The temporal distribution of samples is also somewhat uneven (Figure 6). This is most likely due to preferred hunting conditions during winter (January–March) and banned hunting between 15 April and 15 July (and between 24 and 31 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. Two samples from red foxes were submitted in May and June; these were animals found dead/in very poor condition in urban environments and submitted for autopsy and sampling by the local wildlife management authority.

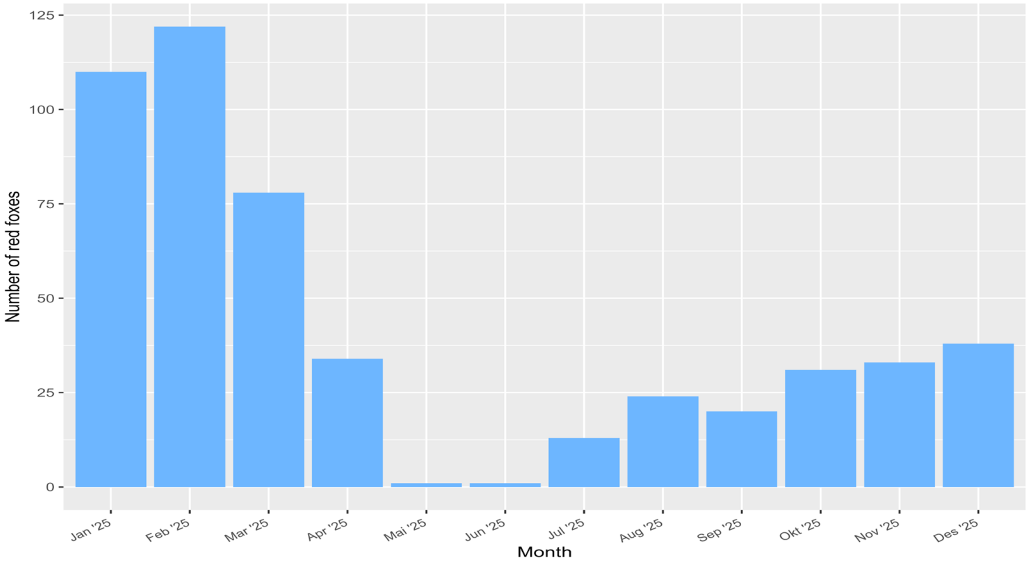

Figure 6. Sampling months of red foxes (n = 505) examined in the Norwegian *E. multilocularis* surveillance programme 2025.

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, *E. multilocularis* has been detected in four different regions (10, 20), and surveillance in Denmark has demonstrated its presence in two regions (15). 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 (19). 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 (16). This is worrying, as a lack of compliance with the anthelmintic treatment requirements for pets entering Norway after having visited endemic areas has been demonstrated (17, 18). The above-mentioned points illustrate why it is imperative to continue with the surveillance for *E. multilocularis* in Norway to document and enable up-to-date data to support the continuation of the disease-free status of Norway, via this 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.

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