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# Content

# Sammendrag

Den zoonotiske parasitten *Echinococcus multilocularis* (*Em*) ble første gang oppdaget i østmarkmus (*Microtus levis*) på Svalbard i 1999. Østmarkmus er en introdusert art og har gjort det mulig for parasitten å etablere seg på Svalbard i en livssyklus med fjellrev (*Vulpes lagopus*) som hovedvert og østmarkmus som mellomvert. I etterkant av den første påvisningen har prevalensen av parasitten vært studert i flere ulike prosjekter. Man antar at parasitten ble introdusert med en fjellrev som har vandret inn fra et område hvor parasitten er endemisk og molekylære analyser har vis nært slektskap mellom *Em* fra Svalbard og Yakutia (Russland).

Denne rapporten oppsummer resultater fra tre ulike prosjekter fra perioden 2009-2023. Prosjektene «Status of *Echinococcus multilocularis* on Svalbard" og "MapEm" var finansiert av Svalbard Miljøvernfond mens et tredje prosjekt «MEmE: Multicentre study on *Echinococcus multilocularis* and *Echinococcus granulosus s.l.* in Europe: development and harmonisation of diagnostic methods in the food chain" ble finansiert av EU gjennom One Health EJP. Prevalens hos fjellrev ble undersøkt i alle prosjektene og to prosjekter undersøkte i tillegg prevalens hos hunder og gnagere i og omkring Longyearbyen og Barentsburg.

Fjellrev på Svalbard blir fangstet på grunn av pelsen på vinteren. Prevalens og infeksjonsbyrde i fjellrev fra fangstsesonger 2009-2023 ble undersøkt med en realtime PCR metode (med magnetiske kuler) i kombinasjon med sedimentasjon av tynntarmsinnhold for telling av antall parasitter. Det ble analysert 700 avføringsprøver med PCR-metoden og *Echinococcus multilocularis* DNA ble påvist i 4,4% [95 % konfidens intervall (KI) 3.1-6.2 %]. Ved sedimentasjon av tarminnhold fra 380 rev ble parasitten påvist i 2,4% [KI = 1,3-4,4]. Infeksjonsintensitet varierte fra 9 til over 80 000 voksne ormer, alle funnet i bakre tredjedel av tynntarmen (jejunum og ileum). Parasitten ble funnet i rev fra de fleste fangstområdene på Svalbard i tillegg til ett individ fra Hopen. Prevalensen avtok med økende avstand fra østmarkmusens kjerneområde i Grumant/Fuglefjella hvor tidligere prosjekter har funnet høy prevalens i musepopulasjonen og i avføring fra fjellrev.

I prosjektene som oppsummeres her ble det ikke undersøkt mus fra Grumant/Fuglefjella, men mus fanget i og omkring Longyearbyen samt en mus fra Barentsburg. Det ble gjennomført makroskopisk undersøkelse av lever og andre indre organer for alveolar ekinokokkose i 157 mus. Det ble ikke gjort positive funn.

Avføringsprøver fra hunder ble samlet inn i to ulike perioder og undersøkt for *Em* samt andre gastrointestinale parasitter. Hundeeiere ble forespurt om å svare på et spørreskjema om risikofaktorer for smitte av *Em*. Prøver av 89 hunder fra perioden 2016-2017 ble undersøkt med den samme metoden benyttet for rev. Prøver av 315 hunder fra 2021 ble undersøkt med en multipleks PCR-metode som i tillegg til Em kunne detektere *E. granulosus* og *Taenia* sp. Ingen prøver fra hunder var positive for *Em*. En prøve fra 2021 var positiv for *Taenia* sp. men ytterligere sekvensering av segmentet kunne ikke skille mellom *T. serialis* og *T. krabbei*. På bakgrunn av kunnskap om disse artenes epidemiologi og tidligere påvisning av *T. krabbei* på Svalbard, ble infeksjon med *T. krabbei* vurdert som mest sannsynlig.

Hovedkonklusjonen er at *Em* fortsatt er utbredt over hele Svalbard. Prevalensen er lav (<5%) men smitterisikoen likevel betydelig da noen rever er infisert med mange tusen voksne ormer

og da skiller ut svært store mengder egg. Det zoonotiske potensialet er klart høyest i området Grumant/Fuglefjella men kan ikke utelukkes i noen områder.

Det ble ikke påvist *Em* i hunder eller i smågnagere i Longyearbyen eller omkringliggende områder. Det skyldes antagelig begrenset overlapp av leveområder mellom østmarkmus og fjellrev, samt lav populasjonstetthet av musene. Denne sitasjonen kan endre seg med klimaendringer. En permanent musepopulasjon nærmere Longyearbyen og Barentsburg vil medføre en større risiko for smitte til hunder og økt risiko for human smitte.

Mesteparten av hundeeierne rapporterte at de behandlet hundene sine to ganger i året med antiparasittmiddel. Dette er tilfredsstillende med dagens smittesituasjon. Hunder som har aktivitet omkring Barentsburg/Fuglefjella eller ved økt tilgang på mus, bør vurderes behandlet månedlig med prazikvantel-baserte midler.

Overvåkning av populasjonsdynamikk og utbredelse av både rev og østmarkmus gjøres gjennom COAT prosjektet (COAT – Climate-ecological Observatory for Arctic Tundra). Etablering av et overvåkningsprogram med kontinuerlig lang-tids screening av *Em* i rev, vil gi lokale myndigheter mulighet for en bedre risikovurdering av smittesituasjonen. Analysemetoder som velges for et slikt formål bør være tilstrekkelig sensitive og spesifikke og skal gi estimater for smitteforekomst i revepopulasjonen. Kombinasjonen av realtime PCR (med magnetiske kuler) i kombinasjon med sedimentasjon av tarminnhold fra PCR positive avføringsprøver syntes hensiktsmessig for screening av et stort antall prøver.

# Summary

The zoonotic parasite *Echinococcus multilocularis* (*Em*) was first detected in Eastern European voles (*Microtus levis*) in Svalbard in 1999. This invasive rodent species has enabled a local transmission cycle to establish between Eastern European voles (intermediate hosts) and arctic foxes (*Vulpes lagopus*) definitive hosts, on Svalbard. There has been ad-hoc monitoring of parasite prevalence in a number of different projects subsequent to this initial detection. The parasite is thought to have been introduced with an infected arctic fox migrating from another endemically infected area in the arctic, given that molecular analysis has shown *Em* from Svalbard to be closely related to *Em* from arctic foxes in Yakutia (Russia).

This report summarises the findings from three different projects spanning the period 2009-2023. Two of the projects "Status of *Echinococcus multilocularis* on Svalbard" and "MapEm" were financed through the Svalbard Environmental Fund whilst the third project "MEmE: Multicentre study on *Echinococcus multilocularis* and *Echinococcus granulosus s.l.* in Europe: development and harmonisation of diagnostic methods in the food chain" was part of a larger EU One Health EJP funded project. All three projects investigated infection prevalence in arctic foxes, whilst two also investigated prevalence in rodents around human settlements (Longyearbyen and Barentsburg) and in domestic dogs.

The infection prevalence and abundance in arctic foxes was investigated using a magnetic capture realtime PCR molecular method in combination with sedimentation counting technique to quantify *Em* abundance in the small intestine. The foxes examined had been trapped for their fur during the winter trapping season between 2009-2023. *Echinococcus multilocularis* DNA was detected in 4.4 % [95 % confidence interval (CI) 3.1-6.2 %] of the 700 fox faecal samples screened by PCR. The *Em* abundance was investigated in 380 fox small intestines and the parasite detected in 2.4 % [CI = 1.3-4.4]. The infection intensity ranged from 9 to over 80 000 adults in infected individuals. No adult *Em* were detected in the foremost segment (duodenum) but were found in the remaining three quarters of the small intestine (jejunum and ileum).

Infected foxes were found throughout Svalbard but with decreasing prevalence with increasing distance from the core Eastern European vole area around Grumant/Fuglefjella, including one positive individual in Hopen. Eastern European voles were also investigated for the parasite but not in the core Eastern European vole area where previous investigations had been carried out and high prevalence levels found. Voles were trapped in and around Longyearbyen, and one mouse was submitted from Barentsburg, in 2016-2021. These were macroscopically examined for alveolar echinococcosis of the liver and other internal organs. A total of 157 rodents were submitted and the parasite was not detected in any of them.

Domestic dogs were also screened for the infection with *Em* in addition to other parasites. Canine samples were collected during two different periods and two different methods were used to screen for *Em*. The same magnetic capture realtime PCR method, as for the foxes, was used to screen for *Em* in the dog samples from 2016-2017. In 2021, a different multiplex PCR method was used, which was capable of detecting *E. granulosus s.l.* and *Taenia* sp. in addition to *Em*. Dog owners were also asked to complete questionnaires to investigate potential risk factors for infection. In 2016-2017, samples were received from 89 dogs, whilst 315 dog faecal samples were analysed in 2021. None of the canine samples were positive for *Em*. One of the

samples from 2021 tested positive for *Taenia* sp. Subsequent sequencing of the fragment was unable to differentiate between *T. serialis* and *T. krabbei*. Given the epidemiology of these two species, and the previous detection of *T. krabbei* in Svalbard, it was concluded that this infection was most likely due to *T. krabbei*.

The main results are that *Em* is still present throughout Svalbard. The low prevalence level (<5 %), however, can give a false sense of security if the parasite burden in infected foxes is not taken into consideration. The potential for environmental contamination can be considerable in foxes infected with thousands of adults producing gravid proglottids containing hundreds of eggs each during patency periods of up to a month. Therefore, there is a potential zoonotic risk throughout the archipelago albeit much higher in the areas in and around Grumant/Fuglefjella.

The negative findings in dogs and rodents in and around the human settlements of Longyearbyen and Barentsburg is good news and is thought to probably be a result of limited spatial overlap and low population densities of Eastern European voles and potentially infected foxes. This situation may change in a warming climate as the distribution and density of Eastern European voles increases in and around Longyearbyen. Should permanent Eastern European vole populations manage to establish closer to Longyearbyen or Barentsburg and their respective dog yards, then the risk of transmission to dogs, with a concomitant risk of spill over from dogs into humans, will increase.

Currently most dog owners report deworming their dogs twice yearly which given the current transmission risk seems to be sufficient. However, more frequent deworming can be considered for dogs that have access to Eastern European voles, especially if they have activities in the core Eastern European vole areas around Grumant and Fuglefjella. During periods with potentially high infection risk (high Eastern European vole densities) monthly deworming with praziquantel based anthelmintics may be considered.

It is important to not only continue to monitor the population dynamics and distribution of both Eastern European voles and foxes but also to include screening for *Em*. The longer-term monitoring of changing *Em* prevalence and abundance levels, as well as population densities and distributions in arctic foxes and Eastern European voles would allow local authorities to map infection risk trends using comparable methods. This has not been possible up until now given the different methodological approaches used. The analysis method chosen for longer-term monitoring should be sensitive and specific, concurrently scalable for years with high numbers of trapped foxes and allow for the estimation of parasite abundance. We found the use of the magnetic capture MT-CO1 rtPCR coupled with SSCT of positive samples to be a good compromise for the screening of a high number of samples. Population dynamics and distribution are already monitored for both Eastern European voles and arctic foxes through COAT (COAT – Climate-ecological Observatory for Arctic Tundra). The monitoring of important zoonoses like *Em* are not currently included in any surveillance programs in Svalbard and this needs to be addressed.

# 1 Introduction

Svalbard is a high-Arctic archipelago located between the mainland of Norway and the North-Pole (74–81°N, 15–30°E). Due to its isolated geographic location and low overall productivity of the tundra the archipelago harbours one of the northernmost and simplest tundra ecosystems of the world (Ims et al., 2013). Contrary to what is found in most tundra ecosystems, there are no arctic endemic small rodents, like lemmings, in Svalbard. The only rodent species present is the introduced Eastern European voles that until recently was spatially restricted to the area around the sea bird colony, Fuglefjella (Figure 1). The main predator/scavenger guild is the resident arctic fox (*Vulpes lagopus*) and the migrating glaucous gull (*Larus hyperboreus*; Ims et al., 2013).

Svalbard is influenced by the West Spitsbergen Current branch of the North Atlantic Current derived from the Gulf Stream, which makes the climate mild compared to other locations at the same latitude. In recent decades Svalbard has experienced dramatic changes in climate which makes Svalbard one of the fastest warming regions in the Arctic and on earth (Isaksen et al., 2016; Nordli et al., 2020; Zdanowicz et al., 2023).

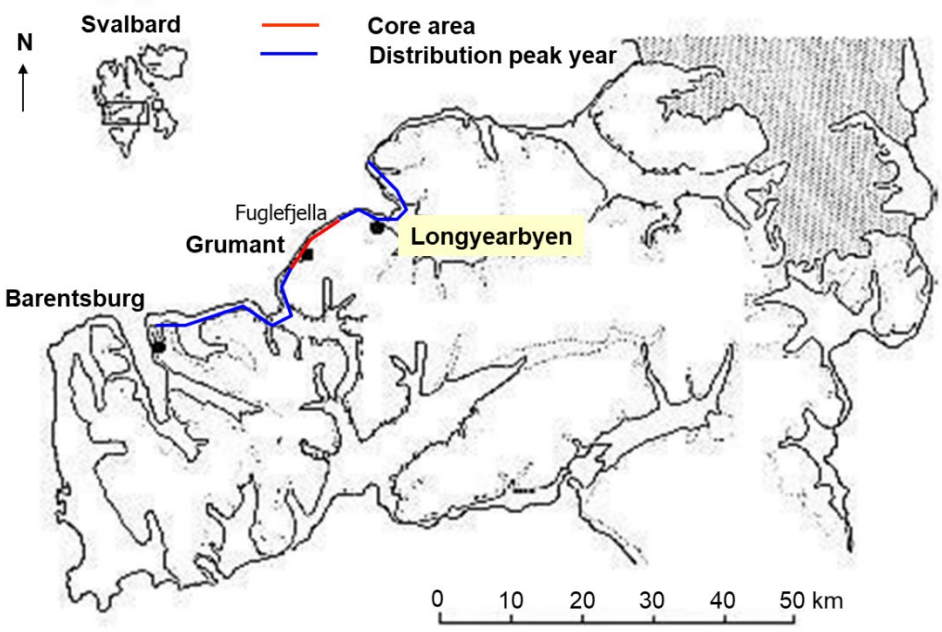


*Figure 1. The distribution of Eastern European voles showing the core area (red line) in Grumant and Fuglefjella (copied with permission from Fuglei et al. (2008)). The blue lines show the wider distribution area in peak vole population years.*

*Echinococcus multilocularis* (*Em*) is a zoonotic cestode with an indirect lifecycle (Davidson et al., 2016; Oksanen et al., 2016; Woolsey and Miller, 2021). Canines, both wild (wolves, foxes, racoon dogs, coyotes) and domestic dogs, act as the definitive hosts whilst rodents like voles, are intermediate hosts (Davidson et al., 2016; Oksanen et al., 2016; Woolsey and Miller, 2021). Accidental spillover of eggs from canines to humans can cause alveolar echinococcosis

(Hillenbrand et al., 2017; Kern et al., 2017); a fatal, unless treated, debilitating disease. This parasite was first detected in the population of introduced Eastern European voles in Svalbard in 1999 and further examination of arctic fox faeces in the surrounding area revealed patent infections in the definitive hosts, with higher *Em* prevalence around the geographic area in which voles have colonised (Fuglei et al., 2008; Henttonen et al., 2001; Stien et al., 2009).

These findings confirmed that this parasite was now endemic to Svalbard. It has been hypothesised that the introduction of the Eastern European vole has allowed a local *Em* lifecycle to establish, whilst the initial infection is thought to have been brought with arctic foxes migrating from other endemic areas, like Russia (Henttonen et al., 2001). Genetic analysis of the parasite in Svalbard has confirmed a close relationship with *Em* found in arctic foxes from Yakutia (Russia) and St Lawrence Island (Alaska) (Knapp et al., 2012; Lallemand et al., 2024; Santoro et al., 2024).

Mainland Norway is considered to be free from *Em* and has a national annual surveillance program in red foxes for this parasite (Davidson et al., 2016). Surveillance of *Em* in Svalbard is not included in this program and as such more ad-hoc screening approaches have occurred. Since the detection of *Em* in Svalbard in 1999 there have been a number of studies investigating the prevalence of this zoonotic parasite in the archipelago.

| Project period | Project name | RiS no. | Species sampled | Funding body |
|---|---|---|---|---|
| 2002-2006 | The role of climatic variation in the dynamics and persistence of an Arctic predator-prey/host-parasite system in Svalbard | 2330 | Voles, fox faecal scats | Norwegian Research Council (NRC) |
| 2001-2014 | Monitoring rabies, parasites and infectious diseases or agents in the arctic fox population in Svalbard | 2300 | Arctic fox carcasses | Svalbard Environmental Fund (SEF) |
| 2016-2018 | Status of *Echinococcus multilocularis* on Svalbard 2016 | 10496 | Faeces arctic foxes and dogs, voles (Longyearbyen) | SEF |
| 2017 | Parasitic fauna of East European voles (*Microtus levis*) in Svalbard (ParaVole) | 10852 | Fox faecal scats, voles (Longyearbyen) | The CzechPolar2 project (LM2015078) Ministry of Education Youth and Sports of the Czech Republic |
| 2018-2020 | Spredning av østmarkmus og parasitt på Svalbard (Mus og parasitt på Svalbard) | 11010 | Voles | SEF |
| 2020-2022 | MEmE: Multicentre study on *Echinococcus multilocularis* and *Echinococcus granulosus* s.l. in Europe: development and harmonisation of diagnostic methods in the food chain, 2020-2022, | 2091 | Voles (Longyearbyen and Barentsburg), arctic fox carcasses and faeces, dog faecal samples | EU One Health EJP project - grant agreement no. 773830 |
| 2022-2023 | MapEm | 2091 | Arctic fox carcasses and faeces | SEF |

*Table 1. An overview of the different research projects that have investigated the prevalence of* Echinococcus multilocularis *in Svalbard since the parasite was first detected in 1999. The Research in Svalbard (RiS) number is shown.*

Previous studies have found a declining gradient of infection in fox faecal scats from the core Eastern European vole area in Grumant, around the bird cliffs, Fuglefjella, in which the voles are most abundant, radiating out across Nordenskiöld Land (Figure 1) (Fuglei et al., 2008; Stien et al., 2009). There are large annual fluctuations in the vole population, partly driven by weather stochasticity (Fauteux et al., 2021; Hansen et al., 2013; Stien et al., 2009), and in good years the voles can spread as far as Longyearbyen and Barentsburg (Fuglei et al., 2016) (Figure 1).

A new vole monitoring system was established in 2020, which has documented that they are spreading to new areas. In winter 2020-2021, the monitoring system documented that voles had established at a new location at Hatten in Tempelfjorden. However, the following winter (2021-2022) they were extinct again at this location (unpublished data). The spread of the voles close to human settlements increases the risk of domestic dogs becoming infected with *Em*. The infection of domestic dogs would consequently increase the risk for spillover infection and alveolar echinococcosis in humans. It is therefore important to monitor not only the status of the vole population but also the level of infection in arctic foxes and domestic dogs in order to provide up to date health and safety recommendations to the inhabitants and visitors of Svalbard.

This report summarises the results from three different projects spanning the period of 2009-2023 (Table 1). Two of the projects were financed through the Svalbard Environmental Fund (Status of *Echinococcus multilocularis* on Svalbard and MapEm) whilst the third was part of a larger EU One Health EJP project MEmE. All three projects investigated infection prevalence in arctic foxes, whilst MEmE and Status of *Echinococcus multilocularis* on Svalbard also investigated prevalence in rodents around human settlements (Longyearbyen and Barentsburg) and in domestic dogs.

The project “Status of *Echinococcus multilocularis* on Svalbard” was the first to screen domestic dogs as well as foxes using molecular methods to detect *Em* and asked dog owners to complete a questionnaire to identify potential risk factors for *Em* infection. Voles trapped in Longyearbyen were also submitted to the project.

The EU One Health EJP project “MEmE” was a large international collaboration that developed harmonised methods for evaluating and diagnosing *Echinococcus* in endemic regions. Svalbard was one of the regions included and investigated arctic foxes as well as samples from domestic dogs and rodents from Longyearbyen and Barentsburg.

Lastly the project “MapEm” built upon the methods used in MEmE to continue to screen fox faecal samples for *Em* and to document the intestinal abundance of the parasite in PCR positive foxes.

Photo: An arctic fox with summer coat in Svalbard (Colourbox).

# 2 *Echinococcus multilocularis* in arctic foxes (*Vulpes lagopus*)

## 2.1 Arctic fox population on Svalbard

Arctic foxes are endemic to the Arctic and an apex predator and scavenger in Svalbard. Its function in the ecosystem is through its effects on terrestrial prey species – through ground nesting birds such as rock ptarmigan and geese, and reindeer carcasses, and through marine resources such as sea birds, seal cubs and seal carcasses (Eide et al., 2012; Nater et al., 2021). Rapid climate change is impacting the population dynamics of the arctic fox through multiple drivers, such as access to carcasses, marine subsidies and zoonoses. Harvesting has long traditions in Svalbard and arctic foxes have been trapped for hundreds of years. Arctic foxes are also the main host for dangerous zoonoses like rabies and *Em* (Ims et al., 2013).

Trapping continues to be an important recreational activity for residents in Svalbard today, in restricted areas only, as well as for some commercially operated harvest stations (https://www.sysselmesteren.no/nb/jakt-fangst-og-fiske/revefangst/). Trapping is regulated by the Svalbard Environmental Protection Act (Svalbardmiljøloven; LOVDATA, 2001) and states that "All harvesting of species in Svalbard shall be done such that the natural productivity and diversity of species are preserved and that the composition and development of populations are not significantly altered" (Fuglei et al., 2013; LOVDATA, 2002). Trapping is allowed between 1st November and 15th March in 25 restricted trapping areas (23 at Nordenskiöld Land and two south of Ny-Ålesund; Figure 2). Legislation allows for the use of two traps which are the "Svalbard trap" and the traditional dead-fall trap (https://www.sysselmesteren.no/en/hunting-trapping-and-fishing/fox-hunting/).

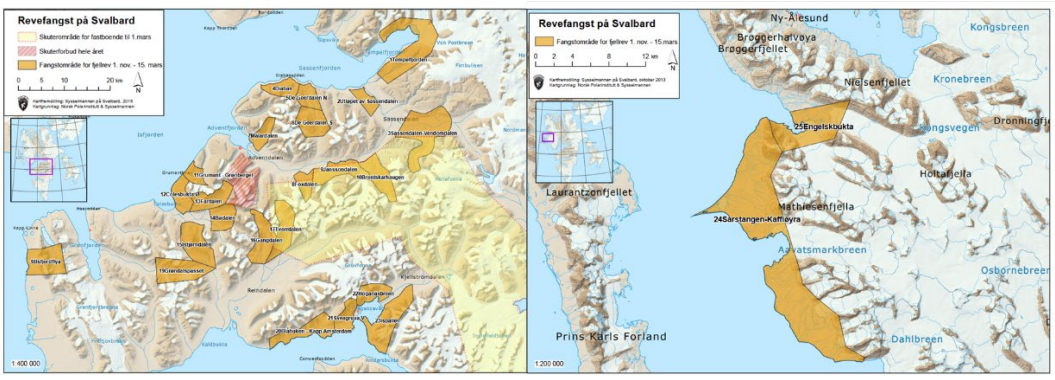


*Figure 2. Overview maps showing the 23 restricted arctic fox trapping areas on Nordenskiöld Land and the two areas around Ny Ålesund, Svalbard. Maps copied with permission from Sysselmesteren.no.*

Arctic foxes breeding population are annually monitored in two areas of West Spitsbergen using den monitoring (Environmental Monitoring of Svalbard and Jan Mayen (MOSJ): https://mosj.no/en/indikator/fauna/terrestrial-fauna/arctic-fox/; Climate-ecological Observatory for Arctic Tundra (COAT): https://coat.no/en/Arctic-fox/Arctic-fox-Svalbard). Six to nine breeding dens have been monitored every summer in Brøggerhalvøya/Adventfjorden since 1993 and 32 breeding dens have been monitored in Adventdalen/Sassendalen, first from

1982 to 1989, and annually since 1997. The population dynamics over time have been relatively stable (Nater et al., 2021).

## 2.2 *Echinococcus multilocularis* in arctic foxes

*Echinococcus multilocularis* prevalence has been investigated in arctic foxes in a number of different studies over the years. Different methods and sampling techniques have been used during different years and seasons.

| Year | Locality | Sample type | Diagnostic method | Season | N | *% Em* prevalence [95% CI] | Ref. |
|---|---|---|---|---|---|---|---|
| 1996-2004 | Spitsbergen (trapping) | Intestines | Intestinal scraping technique (Tackmann et al., 2006) | Winter | 353 | 8.5 [6.0-11.9][a] | (Stien et al., 2009) |
| 2000 | Grumant | Faecal scats | Copro-ELISA (Raoul et al., 2001) | Summer | 35 | 20 [10-36] | (Fuglei et al., 2008) |
| | Bjørndalen | | | | 13 | 0 [0-23] | |
| | Nordenskiöld Land | | | | 91 | 0 [0-4] | |
| | Distant[b] | | | | 0 | | |
| 2004 | Grumant | | | | 224 | 60 [54-66] | |
| | Bjørndalen | | | | 9 | 0 [0-30] | |
| | Nordenskiöld Land | | | | 74 | 0 [0-5] | |
| | Distant[b] | | | | 27 | 7 [2-23] | |
| 2012 | Longyearbyen, Sassendalen and Billefjorden | Faecal scats | PCR (Trachsel et al., 2007) | Summer | 10 | 0 | (Mysková et al., 2019) |
| 2013 | | | | | 12 | 0 | |
| 2015 | | | | | 40 | 0 | |
| 2009-2023 | Spitsbergen (trapping) | Faeces | PCR (Øines et al., 2014) | Winter | 700 | 4.4 [3.1-6.2] | This study |
| 2016-2023 | | Intestines | Segmented sedimentation counting technique (Umhang et al., 2011) | | 380 | 2.4 [1.3-4.4] | |

*Table 2. A summary of* Echinococcus multilocularis *prevalence in arctic foxes in Svalbard using different diagnostic methods and seasons of sampling.* [a] *Highest Em prevalence seen in arctic foxes trapped within 10 km from vole core area in Grumant (35% [95% CI = 21-50%]) although infected foxes are also seen at 70 km distance from vole core site.* [b] *Distant to Grumant: from Ny Ålesund or Hornsund*

## 2.3 Study animals

A total of 791 arctic foxes carcasses were collected during 2009-2017, whilst 437 carcasses were collected between 2019 and 2023 as part of the Norwegian Polar Institute's project "Annual arctic fox (*Vulpes lagopus*) den monitoring and population dynamic in Svalbard project" (RiS 2091). A subsample of these in 2009-2017 (N=292) and in 2019-2023 (N=415) were subsequently analysed at the Norwegian Veterinary Institute for *Em*. The arctic foxes had either been found dead (a few individuals) or been trapped for their fur (the vast majority). It should be noted that the hunting bag size was restricted in 2022-2023 given uncertainty around the impact of the newly discovered biting lice (*Linognathus* sp.) (Buhler et al., 2023) on the arctic fox population numbers and sustainability. This resulted in just 36 arctic foxes being sampled as part of the MapEm project.

The carcasses were frozen to -80°C for a minimum of 7 days prior to shipment to Tromsø. A total of 707 arctic fox faecal samples were taken from these foxes for *Em* analysis. In addition to analysing faecal samples from these foxes, the intestines were also collected, when possible. During 2016-2017 and 2022-2023 only those that tested PCR positive for *Em* in their faeces were investigated for parasite abundance in their intestine, whilst in the MEmE project in 2019-2022, all intestines, regardless of PCR status were investigated. Each trapper reported the date and geographic location of where each fox had been trapped.

The age, sex and body condition (fat index) of the fox was evaluated and recorded during the skinning process in Svalbard after which the carcasses were refrozen and shipped to Tromsø for further sampling and *post-mortem* analysis. Faecal samples were taken per rectum during *post-mortem* analysis in Tromsø and the small intestine divided into four equal length segments. These samples were then refrozen until later molecular and parasitological analysis.

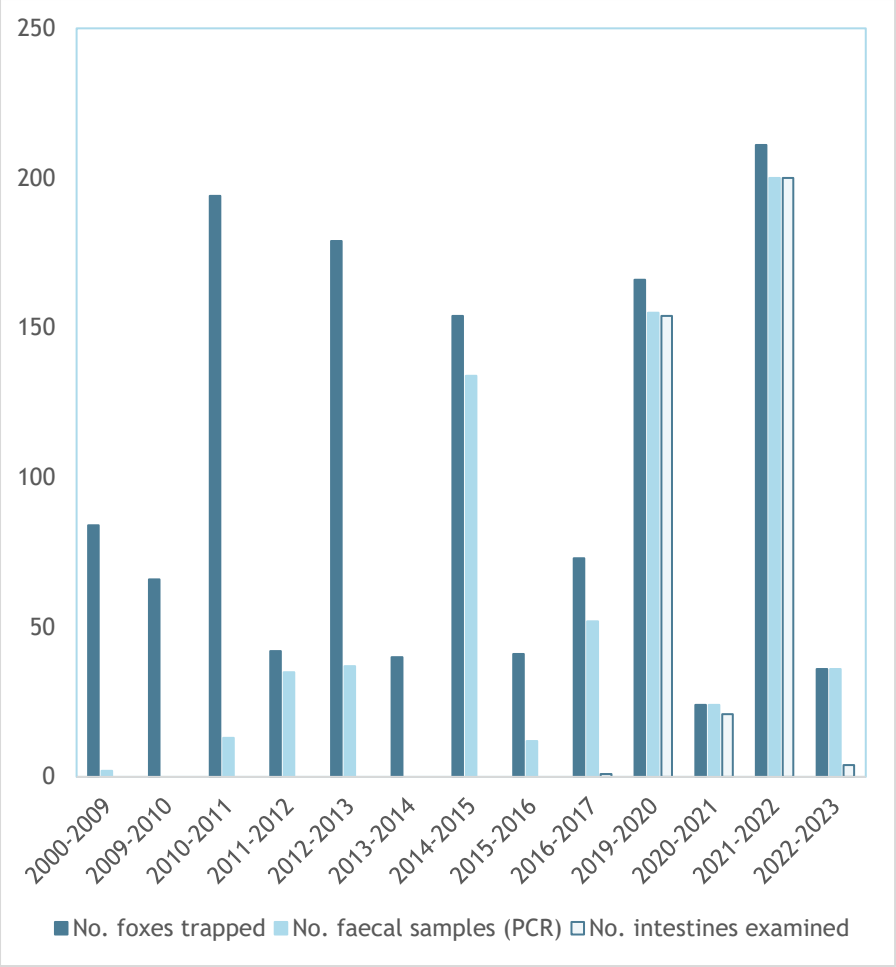


*Figure 3. The number of arctic foxes trapped or submitted to the Norwegian Polar Institute between 2000 and 2023. Also shown are the number of samples tested for* Echinococcus multilocularis *by rt-PCR (faeces) with validated PCR results and the sedimentation counting method (no. small intestines examined).*

## 2.4 Materials and methods

### 2.4.1 Detection of *Em* eggs in faeces

All the faecal samples from arctic foxes were analysed using magnetic capture method targeting *Em* mitochondrial DNA (mtDNA) followed by realtime PCR (rt-PCR) detection targeting the

mitochondrial CO1 gene (Isaksson et al., 2014; Øines et al., 2014). This method is hereafter referred to as the MC CO1 rt-PCR method, or just the rt-PCR method. A total of 707 arctic fox faecal samples were screened for the presence of *Em* mtDNA. However, seven samples had to be excluded from the results as faecal material for these samples was not available for re-testing after a quality control issue was discovered in one of the PCR runs. All other positive samples on this plate had DNA extraction carried out a second time and were retested successfully.

### 2.4.2 Evaluation of abundance of parasites in small intestine

The segmented sedimentation counting technique (SSCT) method was used to evaluate the abundance of *Em* in the small intestines (Umhang et al., 2011). A total of 380 arctic fox intestines were investigated for *Em* abundance using the SSCT method. All the foxes collected during 2020-2022 as well as PCR positive foxes from 2022-2023 and one from 2016-2017 were analysed using SSCT. The analysis was carried out blinded for the samples in the MEmE project since the PCR results were not made available until after the SSCT analysis was completed. Intestinal investigation was only carried out on the PCR positive foxes in the MapEm project.

The small intestine was divided into four equal length segments and a 20 % subsample of the intestinal content of each segment screened for the presence of adult *Em* after sieving and sedimentation steps (Umhang et al., 2011). If *Em* was detected in at least one of the four intestinal segments then the total volume of liquid and sediment was analysed from each segment to assess the total worm burden, regardless of initial segment infection status after screening the first subsample.

### 2.4.3 Phylogeographic typing – whole genome sequencing

Adult worms were preserved in 70 % ethanol and frozen at -80 °C for later molecular analysis. The findings from three of these samples were analysed as part of the project MEmE and published in Santoro et al. (2024).

### 2.4.4 Statistical analyses

The data set was analysed using JMP statistical software (14.0.0 SAS Institute Inc) to calculate prevalence, mean, median and confidence intervals (CIs). Pairwise contingency analysis was used to compare prevalence data by sex, age, trapping year, trapping month, body weight, fat index and geographic origin including distance to the core vole habitat area. Agreement analysis was used to compare the rt-PCR and SSCT results (Dohoo et al., 2003). A significance level of $p<0.05$ was selected for all statistical analyses.

## 2.5 Results

A total of 31 (N=700; prevalence 4.5 % [CI = 3.1-6.2 %]) faecal samples were rt-PCR positive whilst 9 (N=380; prevalence 2.4 % [CI = 1.3-4.4 %]) of the intestinal samples had adult *Em* nematodes in their small intestines. There were no significant differences in *Em* prevalence with age (rt-PCR p=0.6; SSCT p=0.3), sex (rt-PCR p=0.13; SSCT p=0.2), or weight (rt-PCR p=0.06; SSCT p=0.5). A significant trend was seen between *Em* rt-PCR prevalence and fat index (p=0.04) with a similar trend for SSCT prevalence, but the significance level was not reached (p=0.06). The trend showed that a higher proportion of positive foxes had lower fat index than the rt-PCR negative foxes.

The *Em* prevalence differed significantly between the different trapping seasons (PCR p<0.01; SSCT p=0.01; Figure 4). However, there were also significant differences in the geographic distribution of the foxes (trapping location, data not shown) between trapping seasons which could partially account for this.

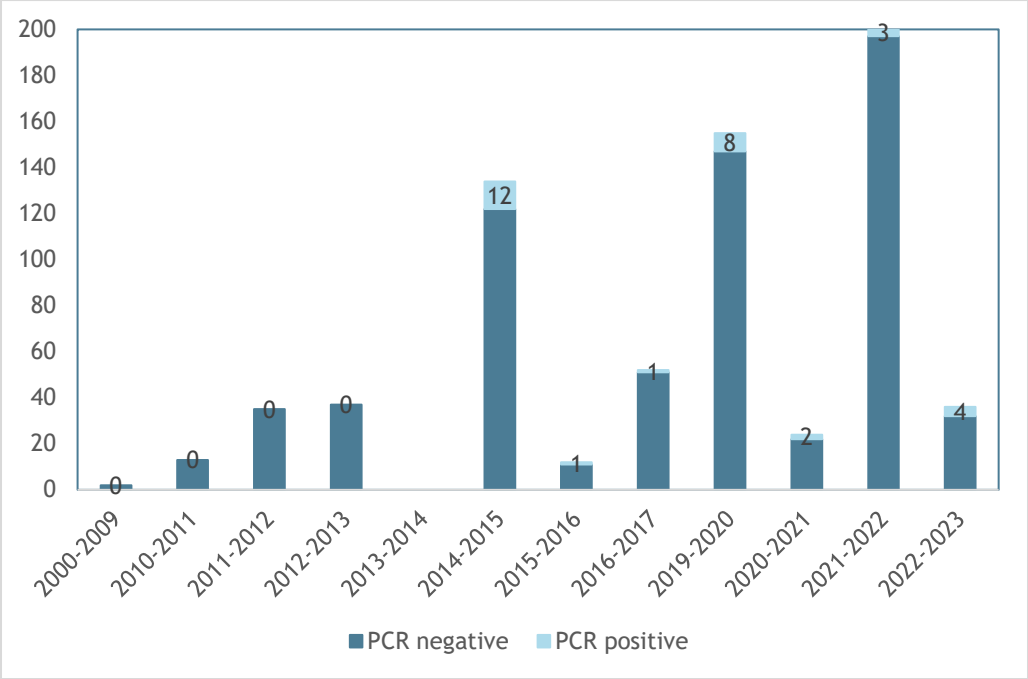


*Figure 4. The magnetic capture rt-PCR results for the detection of* Echinococcus multilocularis *in arctic fox faeces from Svalbard, by trapping season. The total number of positive samples is shown for each season.*

With the SSCT method nine of the foxes had *Em* adults detected in the small intestine, but the worm burdens were not evenly distributed (Table 3). Segment 4 had the highest burdens in five of the foxes, segment 3 in two and segment 2 in one. No adults were detected in the first segment in any of the foxes. One fox had nine adults detected in segment 4 whilst the other segments were negative for the parasite. This sample had been analysed immediately after a sample with over 75 000 adults in segment 4 with reuse of a hand washed glass measuring cylinder. Given that the rt-PCR result in this animal was negative and that the measuring cylinder had been reused after hand washing, we cannot exclude the possibility that this individual was a false positive. Subsequent to this potential false positive detection the method was adapted to use single-use plastic conical tubes for the final sedimentation steps.

| Intestinal segment | No. positive foxes (N=9) | Mean (Median) count | Range of intensity |
|---|---|---|---|
| S1 – anterior SI including duodenum | 0 | 0 (0) | 0 |
| S2 – anterior to mid jejunum | 3 | 59,3 (0) | 56-291 |
| S3 – mid to posterior jejunum | 8 | 1096,3 (78) | 16-5256 |
| S4 – posterior jejunum and ileum | 9 | 9143,8 (402) | 1-75400 |
| Entire small intestine | 9 | 10299,4 (480) | 9-80656 |

*Table 3. A summary of where adult* Echinococcus multilocularis *worms were found in the small intestine of arctic foxes, and the burdens found with the SSCT method.*

Agreement analysis gave a kappa value of 0.58 (moderate agreement) between the two detection methods but with significant differences ($p<0.01$). The faecal rt-PCR method detected twice as many positive samples as the intestinal (SSCT) method (Table 4). The intestinal method had one positive fox that was PCR negative. This sample, as discussed previously, was in all likelihood a false positive.

| SSCT | MT-CO1 rt-PCR | | Total |
|---|---|---|---|
| | 0 | 1 | |
| 0 | 360 | 10 | *370* |
| 1 | 1 | 8 | *9* |
| Total | ***361*** | ***18*** | ***379*** |

*Table 4. Comparison the* Echinococcus multilocularis *results (detected 1; not detected 0) between the molecular analysis of the faeces (rt-PCR) and the microscopic analysis of the small intestine (SSCT).*

A comparison of the geographic location of the positive arctic foxes (Table 5, Figure 5) showed a wide distribution of infected individuals across Nordenskiöld Land as well as one individual in Hopen. A trend of decreasing prevalence was seen with increasing distance from the core vole area (Figure 5), however, given the sample sizes the significance level was not reached ($p=0.29$).

| Trapping location | No. PCR positive | Total no. fox faecal samples examined by rt-PCR | No. SSCT positive | Total no. fox intestines analysed | Total no. Adult *Em* in intestine | Distance category (km) |
|---|---|---|---|---|---|---|
| Grumant | 2 | 15 | 2 | 7 | 406-480 | 0-5 |
| Bødalen | 2 | 26 | 1 | 20 | 6048 | 5-15 |
| Colesbukta | 4 | 35 | 2 | 7 | 206-361 | 5-15 |
| Fardalen | 1 | 10 | | | | 5-15 |
| Istjørndalen | 1 | 9 | | | | 5-15 |
| Synndalen | 2 | 6 | 1 | 6 | 495 | 5-15 |
| De Geerdalen Sør | 2 | 4 | | | | 15-30 |
| Foxdalen | 1 | 7 | | | | 15-30 |
| Gangdalen | 2 | 23 | 1* | 16 | 9* | 15-30 |
| Mälardalen | 2 | 16 | | | | 15-30 |
| Semmeldalen | 1 | 11 | | | | 15-30 |
| Skardalen | 1 | 2 | | | | 15-30 |
| Bellsund | 1 | 157 | | | | 30-50 |
| Blåhuken | 2 | 19 | | | | 30-50 |
| Isfjordflya | 1 | 26 | | | | 30-50 |
| Ispallen | 1 | 4 | 1 | 3 | 80656 | 30-50 |
| Kapp Schollin | 1 | 15 | | | | 30-50 |
| Kollfjellet | 1 | 14 | | | | 30-50 |
| Sassendalen | 1 | 32 | | | | 30-50 |
| Vendomdalen | 1 | 13 | 1 | 4 | 4032 | 30-50 |
| Hopen | 1 | 1 | | | | >50 |

*Table 5. The geographic location of the* Echinococcus multilocularis *rt-PCR and SSCT positive foxes, including the distance category (range in km) from the core Eastern European vole area around Grumant/Fuglefjella. The number*

*of adult Em found in the small intestines is also shown. The suspected SSCT false positive sample is shown with an asterix (*).*

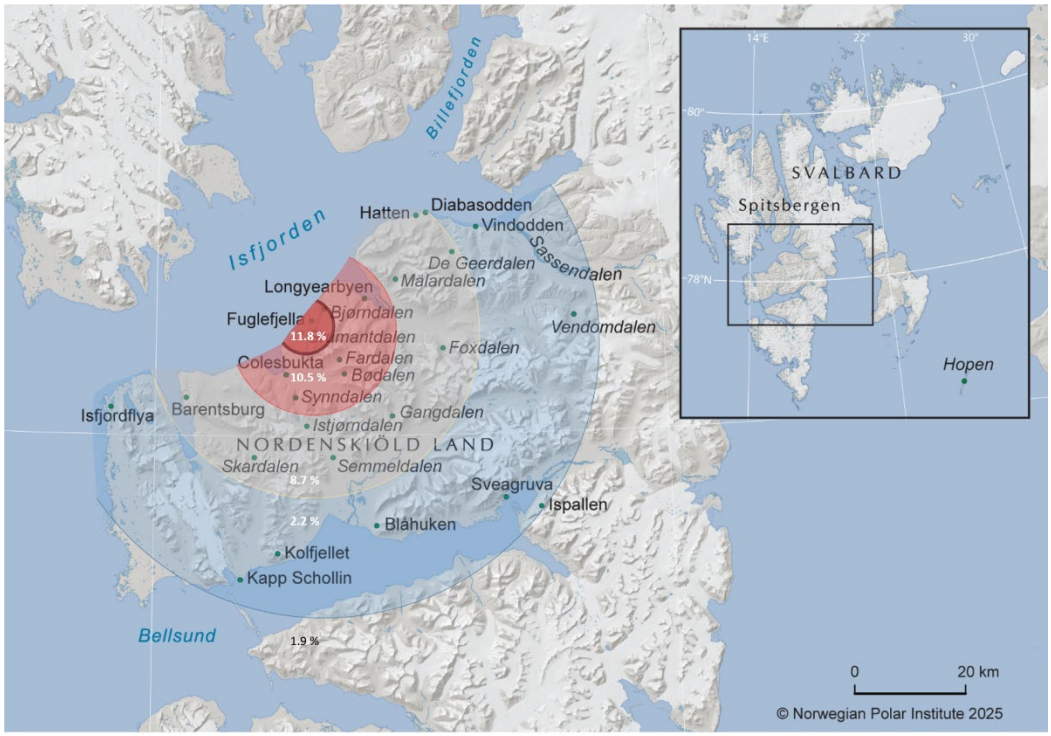


*Figure 5. A map showing the trend of decreasing* Echinococcus multilocularis *in rt-PCR prevalence in arctic fox faeces with distance to Grumant (p=0.29). Dark red: 0-5 km, n = 17, 11.8 % [CI = 1.5 - 36.4]; pale red: 5-15 km, n = 95, 10.5 % [CI = 5.2-18.5]; pale orange grey: 15-30 km n = 103, 8.7 % [CI = 4.1-15.9]; blue: 30 -50 km, n = 407, 2.2 % [CI =1-4.2]; no colour overlay: >50km, n = 54, 1.9 % [CI = 0 - 9.9].*

The geographic trapping locations of all the arctic foxes that were analysed for *Em* using faecal rt-PCR are listed in Table 6.

| Trapping location | *Em* rt-PCR positive | Total no. foxes | Distance category to Grumant- Fuglefjella (km) | Total no. rt-PCR positive for distance category | Total. no. analysed | *Em* prevalence (%) | 95 % CI for prevalence |
|---|---|---|---|---|---|---|---|
| **Bjørndalen** | 0 | 1 | 0-5 | 2 | 17 | 11.8 | 1.5-36.4 |
| **Gronberget** | 0 | 1 | | | | | |
| **Grumant** | 2 | 15 | | | | | |
| **Colesdalen** | 0 | 3 | 5-15 | 10 | 95 | 10.5 | 5.2-18.5 |
| **Gruve 3** | 0 | 1 | | | | | |
| **Hotellneset** | 0 | 1 | | | | | |
| **Longyearbyen** | 0 | 2 | | | | | |
| **Svalbard lufthavn** | 0 | 1 | | | | | |
| **Fardalen** | 1 | 11 | | | | | |

| Trapping location | *Em* rt-PCR positive | Total no. foxes | Distance category to Grumant- Fuglefjella (km) | Total no. rt-PCR positive for distance category | Total. no. analysed | *Em* prevalence (%) | 95 % CI for prevalence |
|---|---|---|---|---|---|---|---|
| Istjørndalen | 1 | 9 | | | | | |
| Bodalen | 2 | 26 | | | | | |
| Synndalen | 2 | 6 | | | | | |
| Colesbukta | 4 | 35 | | | | | |
| Berzeliusdalen | 0 | 1 | 15-30 | 9 | 103 | 8.7 | 4.1-15.9 |
| De Geerdalen | 0 | 5 | | | | | |
| Diabasodden | 0 | 13 | | | | | |
| Grøndalspasset | 0 | 11 | | | | | |
| Janssondalen | 0 | 4 | | | | | |
| Revneset | 0 | 1 | | | | | |
| Tverrdalen | 0 | 5 | | | | | |
| Foxdalen | 1 | 7 | | | | | |
| Semmeldalen | 1 | 11 | | | | | |
| Skardalen | 1 | 2 | | | | | |
| De Geerdalen Sor | 2 | 4 | | | | | |
| Gangdalen | 2 | 23 | | | | | |
| Mälardalen | 2 | 16 | | | | | |
| Brentskarhaugen | 0 | 2 | 30-50 | 9 | 407 | 2.2 | 1.0-4.2 |
| Bromelldalen | 0 | 2 | | | | | |
| Camp Morton | 0 | 3 | | | | | |
| Collinderodden | 0 | 0 | | | | | |
| Fredheim | 0 | 2 | | | | | |
| Gustavdalen | 0 | 1 | | | | | |
| Höganäsbreen | 0 | 16 | | | | | |
| Hyperittfossen | 0 | 12 | | | | | |
| Ingeborgfjellet | 0 | 8 | | | | | |
| Kaldbukta | 0 | 1 | | | | | |
| Orustdalen | 0 | 2 | | | | | |
| Raneodden | 0 | 1 | | | | | |
| Slettvika | 0 | 5 | | | | | |
| Svartodden | 0 | 1 | | | | | |
| Sveagruva | 0 | 47 | | | | | |
| Tempelfjorden | 0 | 20 | | | | | |
| Van Mijenfjorden | 0 | 4 | | | | | |
| Bellsund | 1 | 157 | | | | | |
| Isfjordflya | 1 | 26 | | | | | |
| Ispallen | 1 | 4 | | | | | |
| Kapp Schollin | 1 | 15 | | | | | |
| Kolfjellet | 1 | 14 | | | | | |
| Sassendalen | 1 | 32 | | | | | |

| Trapping location | *Em* rt-PCR positive | Total no. foxes | Distance category to Grumant- Fuglefjella (km) | Total no. rt-PCR positive for distance category | Total. no. analysed | *Em* prevalence (%) | 95 % CI for prevalence |
|---|---|---|---|---|---|---|---|
| **Vendomdalen** | 1 | 13 | | | | | |
| **Blåhuken** | 2 | 19 | | | | | |
| **Austfjordnes** | 0 | 15 | >50 | 1 | 54 | 1.9 | 0.0-9.9 |
| **Bjørnøya** | 0 | 1 | | | | | |
| **Engelskbukta** | 0 | 31 | | | | | |
| **Sarsøyra** | 0 | 5 | | | | | |
| **Farmhamna** | 0 | 1 | | | | | |
| **Hopen** | 1 | 1 | | | | | |

*Table 6. The geographic trapping location, including distance category to the core vole area in Grumant-Fuglefjella of all the fox faecal samples analysed for* Echinococcus multilocularis *using the MC CO1 rt-PCR.*

## 2.6 Conclusion

This work confirms that *Em* in arctic foxes can be found over a much larger geographic area well beyond the Grumant area in which infected Eastern European voles (also called sibling voles) have their highest densities in Svalbard (Fauteux et al., 2021). A higher *Em* prevalence was seen in foxes from around the Grumant area compared to further afield, albeit without the significance level being reached. These results are supported by the earlier findings of Stien et al. (2009) and Fuglei et al. (2008).

The overall prevalence of infection in fox faeces was low (<5%) compared to studies from other high Arctic ecosystems where prevalence has been found to fluctuate between 40 % and 100 % (Kokolova et al., 2023; Rausch et al., 1990a). It was also lower, but not significantly so, than the intestinal prevalence found in arctic foxes in Svalbard using the intestinal scraping technique (Stien et al., 2009). Interestingly the intestinal scraping method is not considered to be the most sensitive method with a relatively high limit of detection (Karamon et al., 2012). The SSCT method is considered as having better sensitivity than intestinal scraping and thus has been considered the “recommended standard” (Karamon et al., 2012; WOAH, 2022) but it is less sensitive than PCR analysis. It has been estimated that the SSCT limit of detection lies around ten adults with a 60 % probability of detection (Karamon et al., 2010). We found an overall significantly lower intestinal prevalence (2%), using SSCT, than the study from 1999-2004 using the intestinal scraping method (Stien et al., 2009). The commercially produced copro-ELISA test (coprological ELISA) used by Fuglei et al. (2008) is no longer available. Recent comparison of a number of the currently available copro-ELISA tests showed variable sensitivity and specificity, especially when intestinal worm burdens were low (Wang et al., 2021). Given that each study has utilised different diagnostic tests to evaluate prevalence, combined with

the large geographic and seasonal variations between the studies, direct comparison is challenging.

The fox faecal samples were analysed using the same method throughout the three studies. Twice as many foxes tested *Em* positive by rt-PCR (N=18) compared to investigation of the intestines (SSCT; N=9). Although the direct detection of adult *Em* provides unequivocal confirmation of patent infections, the time taken between time of death and sampling may impact the fragile worms reducing the sensitivity of this method (WOAH, 2022). This approach may be too laborious for the screening of larger samples sizes. The MC CO1 rt-PCR is a sensitive and scalable method capable of detecting parasite DNA from as little as one egg per gram of faeces (Øines et al., 2014).

The SSCT method was based on the initial screening of a 20 % subsample of each intestinal segment content to try and limit the amount of time to be spent on each sample. It is therefore possible that individuals with very low worm burdens could have been missed (Karamon et al., 2010). The multiple freezing and thawing events also impacted the quality of the parasites with many of the worms being fragmented, potentially resulting in an inferior (reduced) positive predictive value (PPV) of the SSCT method for those samples (WOAH, 2022). Our material experienced at least three freeze-thaw cycles prior to intestinal investigation. In the 2022-2023 samples, the entire small intestinal content, and not just a sub-sample, was examined from the four rt-PCR positive foxes. Despite this no adult *Em* worms were detected in these animals. It is possible that some of the worms either remained attached to the intestine during the mucosal stripping step or were degraded by the multiple freeze-thaw cycles. The positive rt-PCR results could also reflect foxes recently infected with protoscoleces prior to the development of adult worms with patent infections.

The distribution of the adults in the small intestine in infected individuals was uneven. To save time and resources it could therefore be possible to only investigate one or two segments of the small intestine rather than all four segments. No one method is perfect and the choice of method for *Em* detection will vary depending on the goals of the study and hosts being studied.

The MC CO-1 rt-PCR method provided a relatively quick and easy means of carrying out sensitive targeted disease surveillance on a large number of samples to provide an estimate of parasite prevalence. However, the evaluation of intestinal worm burden (parasite abundance) for rt-PCR positive individuals provides important additional data with regard to the potential for the spread of infective eggs in the environment. This is hard to decipher from the rt-PCR results. Despite the low prevalence of infection in foxes, the level of environmental contamination with *Em* eggs may be considerable given that the burden of infection in the intestines ranged from 200 to over 80 000 adults. Each adult worm can, over the course of its lifetime, produce multiple gravid proglottid segments each containing around 200 eggs (Fay, 1973). These eggs can survive for a considerable period in the environment given their tolerance to different environmental conditions, including extreme freeze tolerance (Barosi and Umhang, 2024) and patent infections have been recorded in arctic foxes for more than a month (Fay, 1973). There are fewer reports on the burden of infection in the intestines of arctic foxes. A high burden, as reported in one fox with over 80 000 adults in the intestine has also been seen in earlier studies on St. Lawrence Island Alaska, where it was recorded that arctic foxes commonly had burdens between 10 000 and 25 000 adult *Em* (Fay, 1973).

The two haplotypes of *Em* detected in Svalbard (Santoro et al., 2024) group together, and are in some cases identical, with those detected in arctic foxes and rodents from Yakutia, Russia, and Alaska, USA (Lallemand et al., 2024; Santoro et al., 2024). Human alveolar echinococcosis cases, with liver cysts, have previously been reported from St Lawrence Island in Alaska, indicating that transmission of this Arctic strain to humans is possible (Fay, 1973; Rausch et al., 1990a, 1990b). Although there have been no reports to date to suggest any human alveolar echinococcosis cases from Svalbard, a number of people have tested seropositive subsequent to carrying out field work in the Grumant area (Henttonen et al., 2001). Visitors and Svalbard residents should be made aware of the potentially high zoonotic infection risk, especially with regard to drinking surface water and ensuring good hand hygiene when in the core vole areas where higher levels of *Em* contaminated fox faeces have been found.

The impact of climate change on vole distribution remains of concern given the potential for spread of voles into more populated areas, thus increasing the risk of *Em* transmission to domestic dogs and giving a higher risk for spill over to humans. There is, however, a risk of *Em* infection throughout the archipelago given that foxes are not spatially limited in their distribution (Eide et al., 2004; Frafjord and Prestrud, 1992; Fuglei and Tarroux, 2019).

Unfortunately, there is still no routine surveillance for this parasite in arctic foxes and future surveillance is reliant on new research projects being developed. Our main recommendation is therefore to establish an annual surveillance program for the screening of arctic foxes for *Em*. Our experience with the faecal samples and small intestines from arctic foxes in Svalbard leads us to propose the following: first use a sensitive molecular method (like the MC CO1 rt-PCR) for screening faecal samples, followed by more in depth analysis of the intestines of the PCR positive individuals (using SSCT) rather than all the individuals, as an optimal compromise for estimating *Em* prevalence and abundance in this population.

Photo: A sibling vole with extensive alveolar echinococcosis of the liver. Normal liver tissue has been completely replaced by hundreds of white parasitic cysts (Nigel Yaccoz)

# 3 *Echinococcus multilocularis* in Eastern European voles (*Microtus levis*)

## 3.1 Eastern European voles

The Eastern European vole (formerly sibling vole) was accidentally introduced to the former Russian mining settlements in Grumantbyen, Svalbard in the first half of the 20th century (Fredga et al., 1990). They spread to the nearby guano fertilized and productive grassy vegetation associated with the large sea bird colonies at Fuglefjella approximately 10-15 km west of Longyearbyen. The population has so far had a highly restricted distribution (Figure 1) (Henttonen et al., 2001). Eastern European voles are graminivorous (grass-eating) with multivoltine life histories which means that they can produce multiple generations per year. Females reproduce quickly and can start to reproduce as 17 days old (Yoccoz et al., 1993), which provides the possibility for quick population increases. The population dynamic of the vole population was monitored carefully with live trapping between 1990 and 2007 (Ims and Yoccoz, 1999; Stien et al., 2012; Yoccoz et al., 1990; Yoccoz and Ims, 1999) and showed large annual variations in population density and spatial distribution related to direct negative density dependence and climate effects (Fauteux et al., 2021; Hansen et al., 2013; Stien et al., 2012). Especially rain-on-snow (ROS) events, that gave ice-locked vegetation during climatically unstable winters, caused vole population crashes. In dry and cold winters, with no ROS, voles spread out from the core area in Grumant and Fuglefjella and established in small populations eastwards to Longyearbyen and Revneset, and westwards to Colesbukta and Barentsburg (Figure 1; Figure 6) (Fuglei et al., 2021). However, these populations showed little ability to survive winters with ROS and subsequently died out.

Since 2007, after the vole monitoring project in Grumant and Fuglefjella ended, scattered observations indicated that the restricted distribution of the voles in Svalbard was evolving. There were more frequent reports of voles being seen in and around Longyearbyen, as voles were observed by locals every year, indicating more permanent populations (Fuglei et al., 2021). Observations of vole tracks from 2017, at Hatten, Diabas and Vindodden (see Figure 7 showing the geographic location of these places), also indicated that the voles were in the process of spreading outside the core area in Isfjorden, in addition to regular reports of finding traces of rodent activity, including tracks, in Barentsburg (Fuglei et al., 2021). This changing area of distribution can be related to the recent significant climate warming in Svalbard, in that warmer winters reduce the amount of ice-locked vegetation enabling colonisation in new locations. Increased temperature may also have an effect on the vegetation, providing richer more abundant vegetation for the voles to eat, which means larger areas with favourable vole habitat, and thus the potential for a larger permanent distribution of voles (Fuglei et al., 2021). These observations underpinned the recommendation to establish a monitoring system for the spread potential of the voles in Svalbard (Thomassen et al., 2017). In 2020, this monitoring system was established, financed by COAT and the Governor of Svalbard. The establishment of this monitoring system documents the possible spread of voles from their core area in Grumant and Fuglefjella to new areas in the Isfjorden area. In 2020, the project established 32 camera trap boxes specialised for small mammals (Figure 6). The camera traps consist of a metal box (working as a vole tunnel) and a camera with a movement sensor attached to the lid of the metal box (Kleiven et al., 2023; Soininen et al., 2015). The camera takes pictures of all animals running through the box (Figure 7).

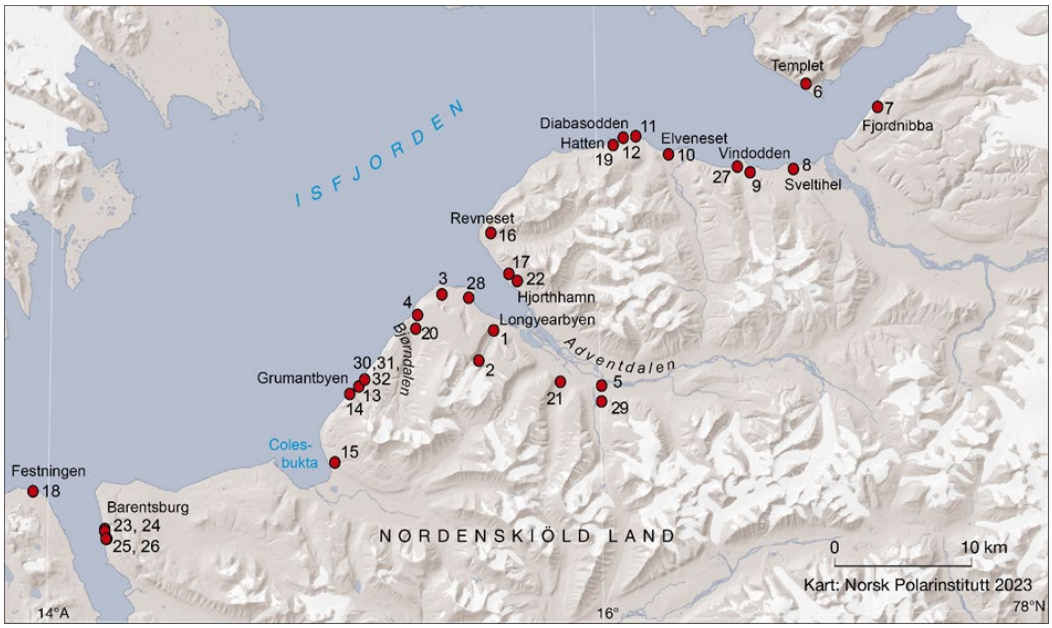


*Figure 6. Map showing the 32 localities where camera trap boxes were established to document the spreading of the voles from the core area in Grumantbyen and Fuglefjella. Map: Oddveig Øien Ørvoll/NP.*

This monitoring system covers both localities with a known presence of voles in the Isfjorden area, and potential new areas that voles may invade. Should signs of increased vole numbers be reported in and around the Longyearbyen settlement, COAT and NP initiate an action plan, especially in autumn (September to November) when vole densities are often at their highest. Large numbers of snap traps are then distributed to volunteer vole trappers in Longyearbyen. The action plan was triggered in 2020 when voles were reported in Longyearbyen and Adventdalen. However, vole densities in subsequent years have been comparatively low so have not triggered new trapping campaigns (Fuglei et al., 2021).

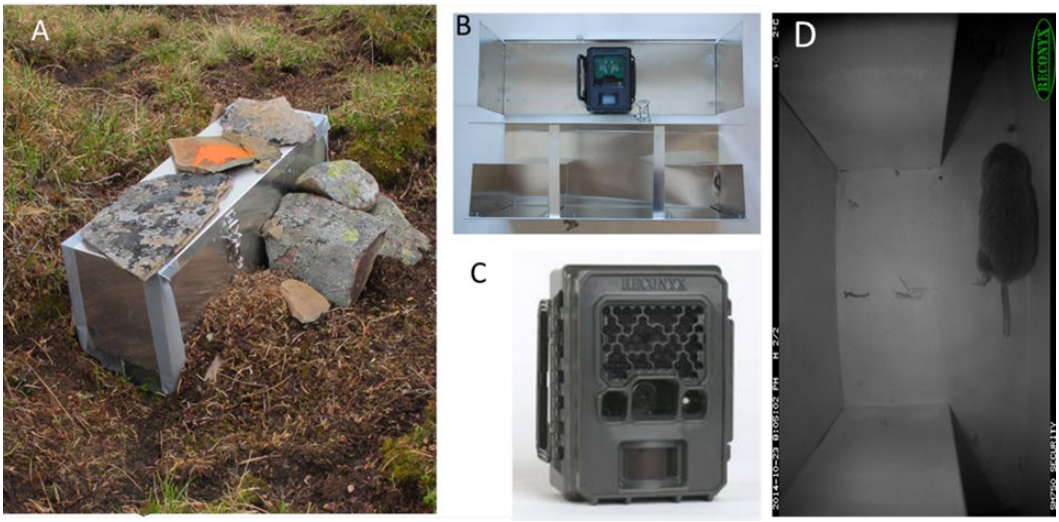


*Figure 7. Camera trap box for small mammals developed by Soininen et al. (2015) used in the monitoring system for voles in Svalbard established in 2020. A: Camera trap box location. B: The camera (Reconyx camera) attached to the lid inside the metal box. C: Reconyx-camera with a custom modified special wide-angel and faster trigger speed lens. D: A vole passing through the camera trap trigging the motion sensor so that a photo is taken.*

The arctic fox is the only terrestrial predator for voles in Svalbard, but foxes breeding close to Fuglefjella, and the core area for voles, mainly feed on sea birds and eggs in the large sea bird colony (Frafjord, 2002). Eastern European voles were considered to be only a minor component of their diet.

## 3.2 *Echinococcus multilocularis* in intermediate hosts

*Echinococcus multilocularis* was first detected in Svalbard in Eastern European voles in 1999 (Henttonen et al., 2001; Mysková et al., 2019), prior to being detected in arctic foxes in subsequent studies. The infected voles were localised on the steep slopes surrounding the old Russian mining settlement at Grumantbyen, which has been abandoned since the 1960's.

The initial study in 1999 and 2000 found almost 59% (N=79) of overwintering adults to have *Em* cysts in their livers (Henttonen et al. 2001). They also found a slight difference, albeit not significant, in prevalence between the sexes (69% males N=32; 53% females N=47). This study also found *Em* infection in one "adult of the year" (N=84) but not in any of the sub-adult or juvenile voles trapped (N=44). The overall prevalence in the population during the first sampling year was 15% (1999; N=179) whilst in the second it was 51% (2000; N=45). They also trapped 24 voles from dog yards around Longyearbyen without detecting the parasite (Henttonen et al., 2001). The heaviest infections (size and number of alveolar hydatid cysts) were seen in the older overwintered age class whilst the lightest infection was in the youngest age class. The low prevalence and low infection intensity in the younger age classes suggests that the infection pressure from eggs in the environment in the cliffs around Grumantbyen was not high. Henttonen et al. (2001) also highlighted that predation on the voles was probably highest in the autumn after the sea-birds have left. This could indicate an autumn/winter window for parasite transmission and explain the higher prevalence in overwintered adults.

The time taken for an intermediate host to show visible lesions, from point of infection, has been estimated to be around 10-12 days (Rausch and Schiller, 1956). The impact of the parasite on the intermediate host will depend on the number and size of the cysts present. The presence of all levels of infection in overwintered Eastern European voles from light to massive, including finding heavily infected older female voles that were both lactating and pregnant, was reported from Svalbard (Henttonen et al. 2001). Massive infections are thought to eventually cause mortalities (Rausch and Schiller, 1956) and would also impact on how easily the vole can move around. The abdomen can become visibly distended as the cysts expand and destroy the liver tissue. Voles that have reduced mobility would be easier prey than uninfected individuals. A recent study by Martini et al. (2024) investigated whether infection with *Em* could result in other behavioural changes in rodents. They evaluated the behaviour of mice 12 weeks post infection, before the lesions reached sizes that could impact mobility. They found no significant differences between infected and uninfected individuals during formalised behaviour tests, but they did observe significant differences in spontaneous behaviour patterns between the two groups. Infected rodents spent more time feeding and more time outside the nest even when they were not feeding. This type of behavioural modification would increase the risk of predation in a natural setting.

## 3.3 Study animals

A total of 52 voles in 2018-2019 and 71 voles in 2020-2021 were collected from Longyearbyen and one rodent from Barentsburg in the project "Spredning av østmarkmus og parasitt på

Svalbard" (project #18/53; 2018-2020) financed by the Svalbard Environmental Fund. Thirty three voles trapped in 2016-2017 were also collected, but not analysed, in the Svalbard Environmental fund project "Status of *Echinococcus multilocularis* in Svalbard", and were subsequently investigated and included in our analyses (Figure 8).

The COAT action plan for trapping voles was triggered in autumn 2020 and snap traps were distributed to local inhabitants for vole collection. The collection system consisted of a medium sized Zarges box with one compartment containing free snap traps that vole trappers from Longyearbyen could collect, and a second compartment in which trappers could place the dead voles, along with details on their contact information and the vole's trapping location and date. The Zarges box was stored outside the NP building and monitored at least once a week by NP logistics personnel who collected all the voles and stored them in the -80 °C freezer followed by longer-term storage at -20 °C until *post-mortem* analyses could be carried out. Information about the collection system was circulated through local media and small prizes (badges) were awarded to the trappers for their contributions.

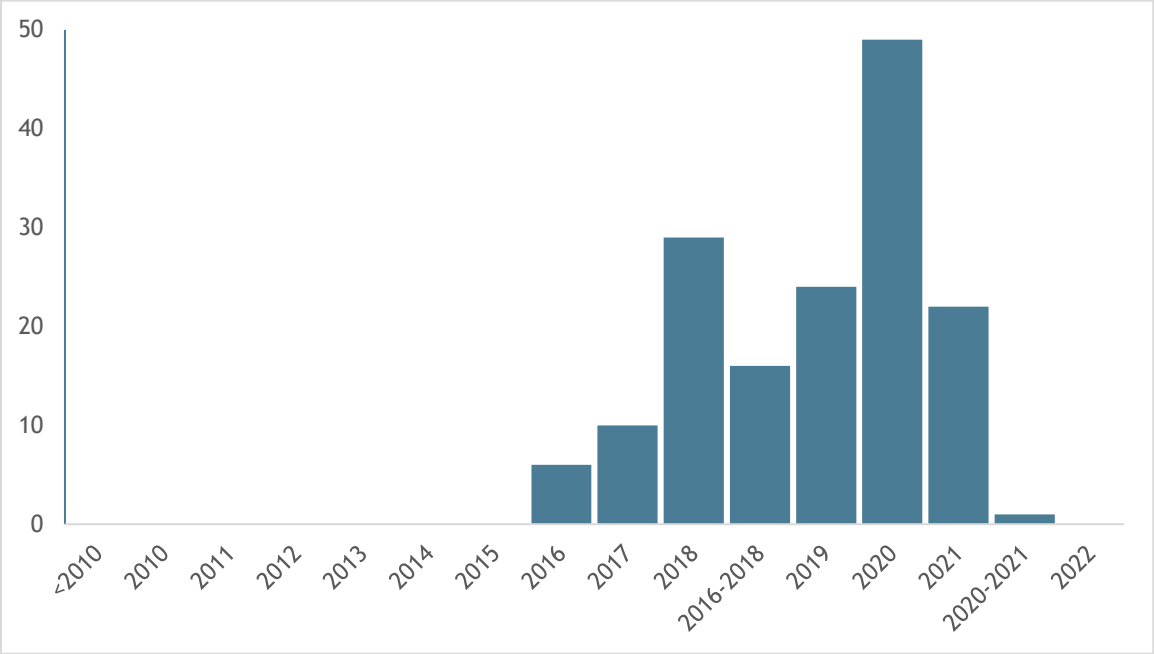


*Figure 8. The number of Eastern European voles (*Microtus levis*) collected by residents in Longyearbyen from 2016-2021 and the one house mouse (*Mus musculus*) from Barentsburg (in 2021). Some of the voles were not labelled with the year of trapping and therefore have been recorded for the period of sampling (either 2016-2018 or 2020-2021) during which they were submitted for* post-mortem *examination.*

## 3.4 Materials and Methods

### 3.4.1 *Post-mortem* examination

The rodents were stored frozen (-20 °C) until 3 days prior to examination when they were transferred once more to the -80 °C to ensure that any surface contamination with *Em* eggs would not be infectious. The voles were allowed to defrost overnight in a refrigerator (2-6 °C) prior to carrying out a simplified necropsy. The geographic origin, sex and degree of sexual maturity, weight (grams), length (nose to tip of tail in centimetres) as well as presence or absence of lesions in the liver or lungs were recorded.

### 3.4.2 Histological examination

The liver and/or lungs were examined macroscopically for lesions. Organs with pathological changes were removed for further histological analysis. Samples with lesions were first fixed in 10% buffered formalin and embedded in paraffin wax. The samples were prepared on slides for staining with haematoxylin and eosin according to standard histological techniques for histological examination. The pathological changes were noted and categorised as being parasitic or non-parasitic in origin.

## 3.5 Results

A total of 157 rodents were trapped in 2016-2021 (Figure 8). Voles were trapped from January to June and September to December. The majority (53%) of the voles were trapped during October and December, with 43% of all the voles trapped during November. The trapping sites ranged from Hiorthamn to the north of Longyearbyen, Barentsburg in the South West and to Bolderdalen in East as well as closer to, and in Longyearbyen, itself (Table 7). The one rodent submitted from Barentsburg (2021) was identified as a house mouse (*Mus musculus*), otherwise all the remaining rodents were identified as Eastern European voles. There were 71 females, 84 males as well as four individuals in which the sex was not recorded. Their mass ranged from 12.3 to 40 grams (mean 22.97 g [CI = 22.15-23.79], median 22.4) and the length ranged from 8 to 21.2 cm (mean 11.35 cm [CI = 10.96-11.74]; median 10.7).

| Location | Distance to Longyearbyen | No. rodents trapped |
|---|---|---|
| Hiorthamn | 3-4 km north across Adventfjorden | 12 |
| Bolterdalen/Gruve 7 area | 10 km east | 45 |
| Todalen | 7 km east | 12 |
| Gruvedalen, dog yards | 1.5 km east | 23 |
| Longyearbyen (including Longyeardalen) | Reference point | 32 |
| Vestpynten/Airport | 5 km west | 8 |
| Bjørndalen | 10 km west | 1 |
| Barentsburg | ca 40 km south west | 1 |
| Location not recorded | | 23 |

*Table 7. The geographic location and distance from Longyearbyen, of the rodents macroscopically investigated for* Echinococcus multilocularis *lesions in 2016-2021 (N=157)*

The initial trapping season of Eastern European voles, prior to the implementation of the COAT action plan, highlighted the need to improve the methodology and ensure that carcasses were as fresh as possible prior to being submitted for *post-mortem* examination. Many of the rodents submitted during this initial sampling were desiccated or severely decomposed (autolysed) making detection of liver and lung pathology challenging.

No lesions were detected in the liver or lungs of the rodents in 2016-2017 and 2018-2019. One rodent had a liver with pathological changes in the 2020-2021 collection season but autolysis complicated further investigation. No parasite specific structures were observed, and this individual was also recorded as being negative for parasites.

## 3.6 Conclusion

The absence of *Em* in Eastern European voles trapped in Longyearbyen in this study is good news. These negative findings can probably be attributed to a combination of low intermediate (Eastern European voles) and definitive host (canids) population densities in these areas of spatial overlap (Raoul et al., 2001). However, the impact of climate change must not be underestimated as a milder climate is leading to locally increased Eastern European vole distribution ranges and densities, thereby increasing the potential risk of *Em* transmission to foxes and dogs with subsequent spillover to humans (Atkinson et al., 2013).

The prevalence of infection in the core Eastern European vole area was not investigated as part of this study. However, earlier studies (2001-2006) were unable to show a direct effect of climate on *Em* epidemiology in the intermediate hosts (Fuglei et al., 2021). The collapse of the rodent population saw *Em* prevalence drop from 70% to 20-30% from one year to the next after the population crash (Stien et al., 2007). In years with high vole density, the spread of *Em* from the core area to further afield is feasible, especially if new permanent populations establish due to more verdant vegetation and milder winters. Higher vole densities over larger areas will increase the potential for interaction with arctic foxes and thus increase the risk of *Em*. However, the contraction of rodent distribution and densities during less favorable winters, especially with ROS events causing icing of the vegetation, will reduce this risk.

Henttonen et al. (2001) argued that the low prevalence and low infection intensity in the younger Eastern European vole age classes, compared to overwintered adults, suggests that the infection pressure from eggs in the environment in the slopes around Grumant was not high. They also highlighted that predation on the voles was probably highest in the autumn after the sea-birds have left. This hypothesis was supported by subsequent dietary studies (Frafjord, 2002). This could indicate an autumn/winter window for parasite transmission and explain the higher prevalence in overwintered adult voles. All the foxes with *Em* PCR positive faeces in our study were trapped between January and March in their respective years. That observation, taken in isolation, might be interpreted as evidence of a predominantly autumn/winter transmission. Given that 90% of all the foxes examined in our study, were trapped during this same period and that there was no significant difference in *Em* prevalence by trapping month, the likely *Em* transmission period between hosts remains uncertain.

The highest infection risk for canids (foxes and dogs) remains therefore the consumption of infected Eastern European voles from the core Eastern European vole area around Grumant and Fuglefjella, just 10-15 km from Longyearbyen. Infected arctic foxes have, however, been detected throughout Nordenskiöld Land and further afield (Stien et al., 2010). The potential for environmental contamination, and therefore human infection risk, is present throughout the archipelago.

The continued monitoring of this Eastern European vole population is therefore more than warranted. The action plan proposed by COAT in years of high rodent density is a vital early warning for the potential spread of this parasite into human settlements and increased risk of infection to both domestic dogs and humans. A secondary benefit to the action plan is the reduction of the rodent population around human settlements thereby helping reduce the potential for parasite transmission.

Photo: Sled-dogs interacting with children (John Davidson)

# 4 *Echinococcus multilocularis* and other intestinal parasites in domestic dogs

## 4.1 Domestic dog population on Svalbard

There are strict regulations regarding the import of domestic animals to Svalbard, as the archipelago resides outside of the EU-/Schengen area. Some Norwegian laws are valid on Svalbard but not all. The Animal welfare law (dyrevelferdsloven, LOV-2009-06-19-97, LOVDATA, 2010) and food safety law (matloven, LOV-2003-12-19-124, LOVDATA, 2003) have been implemented but not the animal health law (dyrehelseforskriften, LOV-2022-04-06-631, LOVDATA, 2022). The laws are regulated and upheld in cooperation with the Norwegian Food Safety Authority (Mattilsynet) and the Governor of Svalbard (Sysselmesteren). In addition to these laws, Svalbard specific legislation has been enacted. A prohibition on the import of animals to Svalbard is tightly regulated (forskrift om innførselsforbud til Svalbard, FOR-1988-08-31-744, LOVDATA, 1988). This details a general rule of prohibition regarding the transfer of animals to Svalbard. Dog owners can, however, apply to government officials for exemptions prior to travel, provided the dog has been vaccinated against rabies and has undergone anthelmintic treatment against internal and external parasites. The logistics of importing a dog to Svalbard, and back to the mainland from Svalbard, are then the same as for the non-commercial import of a dog from a third-party country outside of the EU (kjæledyrforskriften, FOR-2016-05-19-542, LOVDATA, 2016). As soon as the pet is on the archipelago, there is no need to reapplying for exemption, unless the pet is travelling again after the approval has expired. For this project, this means that dogs residing permanently on Svalbard do not have to routinely undergo inspection for deworming treatment. As a rule of thumb, dogs permanently residing on Svalbard are usually restricted to commercially driven dog yards (~50% of the total number of dogs on Svalbard). There are about 1200 dogs registered on Svalbard in the local database governed by the Governor of Svalbard and the Norwegian Food Safety Authority.

## 4.2 Study population

Faecal samples from domestic dogs (*Canis lupus familiaris*) were collected in 2016-2017 (n=89; Figure 9) via invitation to the Longyearbyen dog club (hundeklubben), invitation posters at the local supermarket and collection with help from Svalbard Vet AS which was, at that time, the only veterinary clinic in Longyearbyen. This was done as part of the "Status of *Echinococcus multilocularis* in Svalbard" project. A requirement for participation was that the dogs had not received anthelmintic treatment in the previous three months. Samples were delivered to the veterinary clinic and stored refrigerated until analysis. The samples were collected between 3$^{rd}$ to 6$^{th}$ October 2016 (n=17) and a second collection carried out on 2$^{nd}$ to 4$^{th}$ May 2017 (n=72).

In 2021, Svalbard Dyresykehus AS organised and carried out the sampling (n=315; Figure 9) as a partner in the EU One Health EJP project MEmE. Samples were collected from dog yards as a part of the daily cleaning routine, whereas the privately owned dogs had their faeces collected during their daily walk. A requirement for inclusion was that the dogs had not received anthelmintic treatment during the four weeks prior to sampling. Kits for sampling were distributed to dog owners by their veterinarian along with a questionnaire. Dogs were sampled

from 2nd February to 3rd August 2021. The samples were stored frozen (-20 °C) in Svalbard prior to shipment to NVI in Ås.

Given the limited canine population size in Svalbard the information regarding breed has been grouped for analysis purposes into commercial and private ownership and sled-dog/non-sled dog breed categories to avoid the identification of unique individuals. The sled-dog category includes the following recorded by their owners: Alaskan husky, Alaskan malamute, Samojed, Siberian husky, Polar husky and Greenland husky as well as other mixed breed dogs used for sled dog activities. The non-sled dog category includes the following Border collie, Bernese mountain dog, English setter, Finnish lapphund, Jack Russel terrier, Labrador retriever, Miniature schnauzer, Pomeranian and mixed breed dogs not recorded as being involved in sled-dog activities.

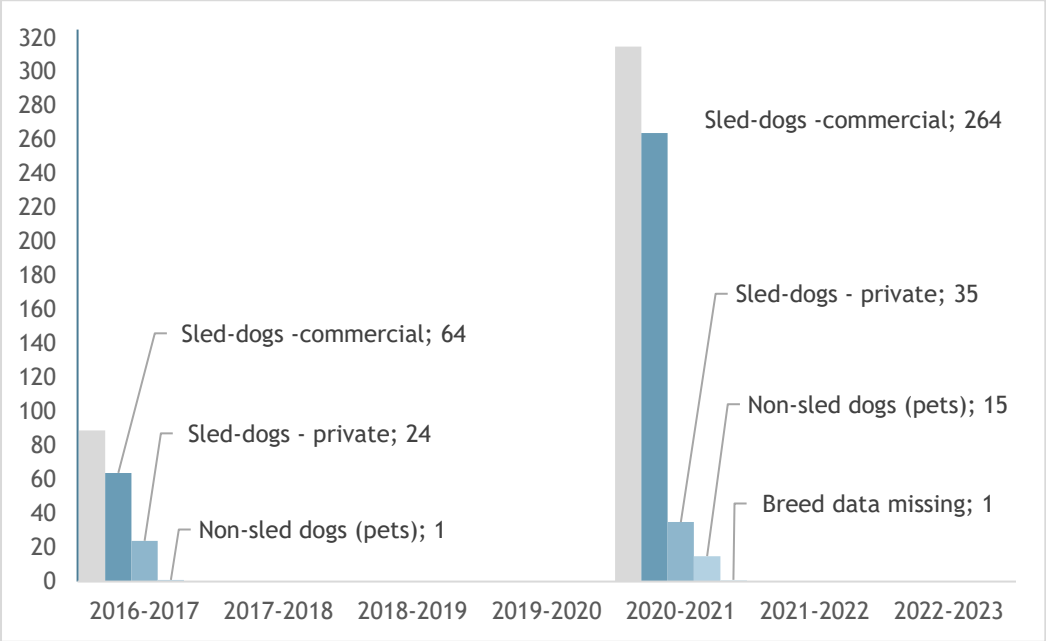


*Figure 9. The number of domestic dogs analysed for faecal parasites by year (the division of the breed group (sled-dog/non-sled dog) and whether private or commercial sled-dogs is shown). A total of 89 and 315 dogs were sampled in 2016-2017 and 2021 respectively. The grey column shows the total number dogs in each period.*

## 4.3 Materials and methods

### 4.3.1 Faecal parasite analysis - 2016-2017

A modified McMaster method with a sensitivity of 5 eggs per gram was used to analyse 4 gram faecal samples (n=89) for the presence of gastrointestinal parasite eggs and oocysts (Henriksen and Aagaard, 1976). The eggs/oocysts were identified and quantified by microscopy (100x magnification) following flotation using a saturated sodium chloride-glucose flotation fluid (specific gravity 1.27). The faeces were also analysed using the magnetic capture MC CO1 rt-PCR method (Øines et al., 2014), i.e. same as with the arctic fox samples.

### 4.3.2 Faecal parasite analysis - 2021

Upon arrival at NVI in Ås, the faeces was stored at -80 °C for a minimum of 72 hours, as a biosecurity measure, prior to being transferred to -20 °C until analysis. The canine faeces from 2021 were analysed according to Trachsel et al. (2007) using the QIAamp Fast DNA Stool Mini Kit (www.qiagen.com; cat. no. 51604) following the "Isolation of DNA from Larger Volumes of

Stool" protocol, and the "Using Stool Tubes for Isolation of DNA from Stool for Pathogen Detection protocol" (QIAmp Fast DNA Stool Mini Handbook: www.qiagen.com/HB-1764) for DNA extraction. Additional InhibitEx buffer (cat. no. 51306) was needed to allow for the screening of the larger volumes of stool.

#### 4.3.3 Questionnaires to dog owners

Owners were asked to complete a questionnaire, during both collection periods (2016-2017 and 2021) regarding their dog (breed, sex, age), living environment (home, kennel), types of outdoor access and activities (housing indoors, dog yards, kept unsupervised in outdoor areas, sled-dog, walking on lead only etc.), deworming history and frequency as well as information about the diet provided to the dog (commercial, raw meat or fish, game meat). The survey in 2021 also asked owners if they knew if their dog ate rodents.

## 4.4 Results

### 4.4.1 Faecal parasite analysis

#### *4.4.1.1 Faecal egg counts and PCR results*

All the samples tested by the MC CO1 rt-PCR (Øines et al., 2014) for *Em* were negative. Gastrointestinal parasites were detected by McMaster analysis in two dogs: one with coccidia (*Cystoisospora canis* at 6400 oocysts per gram) and one with roundworm (*Toxoascaris leonina* at 10 eggs per gram). Information about the age and breed of these dogs was not available. All other samples were negative for faecal parasite eggs/oocysts.

#### *4.4.1.2 Faecal PCR results 2021*

All the samples tested using the multiplex PCR (Trachsel et al., 2007) were PCR negative for *Em* and *E. granulosus s.l.* whilst one 4 year old privately owned male sled-dog tested PCR positive for *Taenia* sp. Sanger sequencing of the 266 bp product indicated that the sample was either *T. krabbei* or *T. serialis,* as the sequence obtained from the PCR product was identical to the GenBank sequences from both species. The reference sequence of *T. krabbei* (MH843683 & MH843684) was only 225 bp, allowing only partial alignment of the sequence generated for this species. The trimmed sequence product was identical to the *T. serialis* (MF495483) region of the 12S small ribosomal RNA. We concluded that although the PCR product was inadequate for complete differentiation of these two *Taenia* species, earlier publications have described *T. krabbei* from foxes and reindeer in Svalbard (Bye, 1985; Stien et al., 2010), backed by molecular confirmation using other molecular targets (Ø. Øines, unpublished data) making it likely that the sample was *T. krabbei*.

### 4.4.2 Questionnaire results

#### *4.4.2.1 Risk factors dogs 2016*

Completed questionnaires were received with information on 25 privately owned dogs. Dog breeds were reported as sled-dog breeds (n=24) or non-sled dog (n=1). Dogs were owned as either companion animals only (n=4), sled-dogs only (n=19) or as both companion animals and sled-dogs (n=2). Dogs were housed at the Longyearbyen kennel club with an indoor doghouse (n=20), private households (n=2) or at both the kennel club and private residencies (n=3). None of the dogs had spent time alone outside houses in Longyearbyen but all spent time in shared dog yards. Twelve of the dogs had never left Svalbard. Only 1 private owner reported the age of their dogs (n=9) with the mean age 4.52 years (range <1 to 9 years old).

Dogs owners reported deworming their dogs every 6 months (n=20), more than twice a year (n=4) or only when the dog travelling to/from Svalbard (n=1). The last time of treatment was either within the last 3-6 months (n=18) or more than 6 months prior (n=7). As required for inclusion in the study, none of the dogs had been treated within 3 months prior to sampling. Dogs were treated with Drontal vet. (Vétoquinol; praziquantel and pyrantel) (n=11), Droncit vet. (Vétoquinol; praziquantel) (n=9), Pancur vet. (MSD Animal Health; fenbendazole) and Drontal vet. (n=4) and Milbemax vet. (Elanco; milbemycin oxime and praziquantel) (n=1). All of the pharmaceuticals contain praziquantel, with the exception of panacur. No questionnaires were received from the commercial sled dog companies. However, all commercial sled dog companies stated that they dewormed twice yearly.

Owners reported that their dogs were healthy with no symptoms (n=16), had a cough (n=4), had gastrointestinal symptoms (vomiting and/or diarrhoea) alone (2) or combined with a cough (n=3). Dogs were fed commercial food only (n=3), a mix of commercial food, household/industry food waste and raw meat (n=4), or commercial food and raw fish (n=1), commercial food, household/industry food waste and raw fish (n=9) and all food types (n=8).

Further analysis of risk factors was not possible due to the lack of positive samples.

#### *4.4.2.2 Risk factors dogs 2021*

Questionnaires were collected for all the dogs sampled (N=315) although not all questions were answered by every respondent. There were significantly more males (60 % [CI = 54.5-65.7]) than females (40 % [CI = 34.3-45.5]) among the 309 dogs in which sex was recorded in this study population. The mean age of the dogs (n=296) was 4.99 years (range 6 months to 13 years). The dog breed was not surprisingly heavily skewed to sled-dogs (Figure 9).

Six of the dogs were reported to eat rodents whilst the owners of the remaining dogs did not know if their dogs ate rodents. All six rodent eating dogs were privately owned: one was a non-sled dog; whilst the other five were sled-dogs.

Worming was reported to be carried out with varying frequency with the vast majority of dogs wormed twice a year (n=248) (Figure 10). Worryingly, a number of sled-dogs were wormed less than once a year (n=48) or not at all (n=3). Milbemax vet. was the most frequently used wormer (243 dogs), but Droncit vet. (n=3), Drontal comp. vet. (Bayer Animal Health GmbH; febantel, pyrantel and praziquantel) (n=1), Panacur vet. (n=2) and Milpro vet. (Virbac; milbemycin oxime and praziquantel) (n=1) were also used. Information about the anthelmintic drug given was either incomplete or not provided for the remaining 64 dogs. The reported time between deworming and sampling varied from one to 16 months (mean 6.82 months (±1.84SD); median 6 months) which matches the reported deworming frequency for the majority of this population. One of the dogs that was known to eat rodents was dewormed quarterly (every 3 months) whilst the others were either dewormed twice a year (n=3) or less than once a year (n=2).

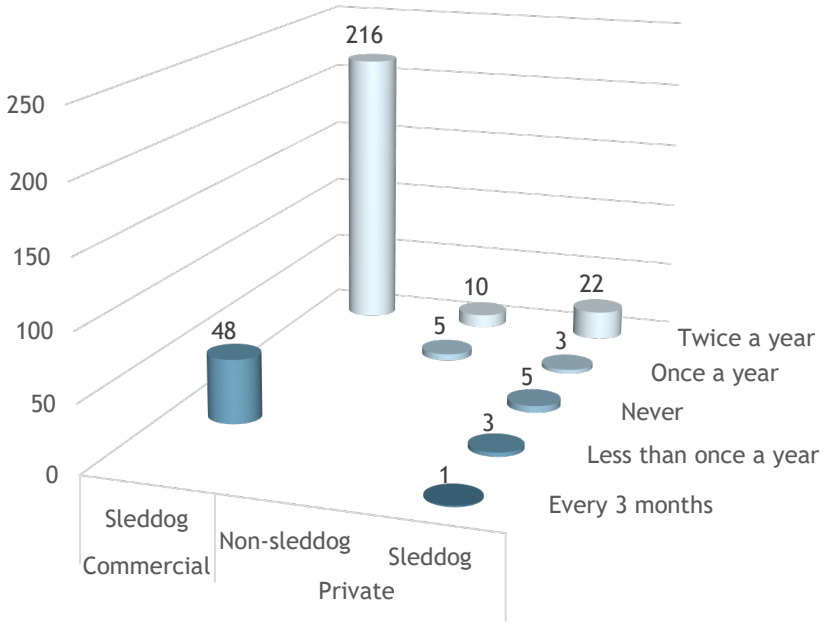


*Figure 10. The reported deworming frequency of domestic dogs in Svalbard (2021) by breed group (sled-dog/non-sled dog) and whether privately owned or part of a commercial sled-dog kennel. Data regarding deworming was missing for one dog.*

As for diet, all dogs had access to commercial dog food, but only 32 dogs received it exclusively (Table 8). There were 15 reports of home slaughtering (n=309; asked as a separate question) whereas the number of dogs with access to slaughter waste (n=134) and/or game meat (n=41) was considerably higher. It should be noted that slaughter waste is in regard to game like reindeer and not production animals which are not present on the archipelago. A large number of dogs received raw meat (265) in combination with other food types. In two cases it was stated that the raw meat was a commercially prepared raw meat product (Vom) but for the others no additional information on the type of raw or game meat was available.

| Owner | Breed group | Commercial food | Raw meat | Game meat | Access to slaughter waste |
|---|---|---|---|---|---|
| Commercial | Sled-dog | 264 | 247 | 20 | 120 |
| Private | Sled-dog | 35 | 13 | 17 | 13 |
| | Non-sled dog | 15 | 6 | 3 | 0 |
| | Not reported | 1 | 0 | 1 | 1 |

*Table 8. An overview of the reported number of dogs with access to commercial food, raw meat, game meat and slaughter waste in Svalbard in 2021. After butchering game meat dogs may have access to any remaining offal and bones (slaughter waste) or be given meat (game meat)*

The one *Taenia* positive dog ate rodents according to its owner, had access to slaughter waste and game meat, but did not eat raw meat. Further analysis of risk factors was not possible given the lack of positive samples.

## 4.5 Conclusion

None of the domestic dogs tested positive for *Em* in these studies. As discussed in the chapter on *Em* in Eastern European voles, low densities of susceptible intermediate hosts and limited spatial overlap with potentially infected arctic foxes, currently results in extremely low infection risk to rodents and thereby also to dogs. However, many of the dogs do have the potential to have contact with rodents, within their dog yards. Especially, if they have activities in and around the Grumant area this will increase their chances of future infections.

There are currently no standardised deworming guidelines for dogs in Svalbard. Deworming recommendations in Svalbard should, however, take into account risk behaviour, like eating rodents, living predominantly outside in dog yards and/or having access to raw meats/slaughter offal. Ideally anthelmintics (deworming medicine) should only be given to dogs that require it and not prophylactically (except for puppies and pregnant bitches) (ESCAAP, 2021). This can be based off faecal egg counts. However, faecal egg counts are unable to distinguish between taeniid eggs from *Taenia* sp. and *Echinococcus* sp. Nor are faecal egg counts as sensitive as the molecular methods used in this study. As our study shows domestic dogs can be infected with *Taenia* sp. in Svalbard. *Taenia krabbei* has reindeer as its intermediate host (Bye, 1985) and therefore infection with this parasite is a reflection of the dog having access to infected raw or undercooked reindeer meat. *Taenia krabbei* is not zoonotic. Routine screening of canine faeces for *Em* using molecular methods is currently not justified. Should infected rodents become established near human settlements, however, then this situation might change and have to be reconsidered.

The majority of dog owners in Svalbard report worming their dogs twice a year which, given the current low *Em* infection pressure, would seem to be sufficient. However, for dogs with higher risk behaviour, especially if they have activities in and around Grumant and Fuglefjella, more frequent worming would be recommended. The European Scientific Counsel Companion Animal Parasites (ESCAAP, 2021) advise monthly worming of dogs with risk behaviour in areas where *Em* is endemic. Again, this would depend on the risk behaviour of the dog and if it has access to potentially infected rodents. The increased deworming frequency would only be needed during periods with higher risk of infection and not necessarily year-round.

For the accurate detection of parasitic DNA, we used two different molecular approaches due to the harmonized approach adopted with all partners in MEmE for the canine faecal analysis. There are significant methodological differences between these two approaches including differences in template preparation and which parasite species can be detected. The MC CO1 rt-PCR protocol (Øines et al., 2014) uses large sample volumes (10x), compared to the multiplex PCR method (Trachsel et al., 2007), in which column-based DNA-extraction is carried out. The MC CO1 rt-PCR has a targeted approach focusing on *Em* only. Obviously, the targeted approach increases the potential of finding low levels of that specific DNA target, but is less suitable for later down-stream analysis of other targets. In some situations, improved sensitivity, scalability and purer template (less non-target DNA present), may be preferred over potential reuse of a sample downstream. On the other hand, the less specialized approach with the simple QiAamp DNA extraction protocol allows for the detection of other parasites (*Taenia* spp.; *E. granulosus s.l.*) or other genetic elements of interests, thus providing additional data that might outweigh any decreased sensitivity. The choice of optimal extraction method and molecular protocol will ultimately depend on the aims of a study.

The focus of our studies was on the distribution of *Em*, and therefore the use of the *Em* specific and more sensitive MC CO1 rt-PCR method was justified, especially for foxes for which the method was developed (Øines et al., 2014). We also adopted this method for the first study in domestic dogs, despite the prior lack of validation in domestic dog faecal samples. We have experienced, during routine surveillance work in the laboratory, that some faecal samples contain residues (e.g. being high in fat, potentially more common in dogs than foxes) that can exceed reagent capacity. This can result in clogged samples, rendering them unusable, or can exceed the pH buffering capacity, giving poorer DNA extraction results. Further optimization of the MC CO1 rt-PCR method is, therefore, recommended for domestic dog samples.

The second study in dogs utilized the multiplex-PCR method since all partners in the MEmE project were able to carry out this method. The MC CO1 rt-PCR method was only implemented by a handful of partners in the MEmE project, due to the need for specialised equipment, and therefore this method could not be used for screening the domestic dog samples. Here challenges arose due to the short sequence obtained, and subsequent sequencing of the DNA fragment was unable to definitively confirm *T. krabbei* in the one dog. Again, the choice of optimal method will depend on the aims of the study, the sample matrix being screened and methodological limitations with the different protocols.

# 5 Discussion

The main findings of these three studies show that *Em* continues to be endemic in Svalbard, with infected foxes found across a wide geographic area, albeit at relatively low prevalence levels (<10% upper limit of the 95% CI for the overall prevalence). The highest prevalence levels were seen in foxes trapped closer to Grumant/Fuglefjella (over 10 % prevalence closer to core vole area <30km) and decreasing prevalence with increasing distance to this core Eastern European vole area (Figure 5).

Annual variations in prevalence were seen, but long-term trends were not possible to analyse given large annual differences between the trapping locations and the highly variable number of samples analysed annually (from one to 159). The prevalence of *Em* DNA in faeces (2009-2023) was not significantly different to the prevalence estimates made during the first study in arctic foxes in 1996-2004 (Stien et al., 2010) based on the intestinal investigation of trapped foxes during the winter. However, the intestinal scraping technique used in latter study has lower sensitivity than the molecular methods used in the later studies. This could imply a trend of decreasing infection prevalence over time. Our studies were the first to evaluate *Em* abundance in arctic foxes in Svalbard which revealed a different risk situation than prevalence data initially suggested. The high number of adult worms found (from hundreds to tens of thousands), although not unheard of in arctic foxes, is of concern and the main reason that we now recommend additional analysis of the small intestines of the foxes with PCR positive faecal samples.

There were no infected Eastern European voles or domestic dogs detected around Longyearbyen. The source of infection of the one infected fox from Hopen is harder to assess. It may have been infected in the Grumant area or alternatively it may have migrated over the sea-ice from other endemic areas in the Russian arctic. Further investigation is also needed in and around Barentsburg, given that no voles and only one house mouse were submitted from this area. House mice are less suitable intermediate hosts for *Em* than cricetid rodents (Beerli et al., 2017; Guo et al., 2021). The dynamic infection risk situation may change in a warming climate. Milder winters and more abundant vegetation allows permanent populations of Eastern European voles to establish over time in new areas, or voles may become a more important part of the arctic fox diet during certain periods of the year. Graminoid vegetation becoming more abundant and productive, due to fertilization from dog faeces around dog yards in Adventdalen, is also of concern. These may become future hot spots for voles. Visitors and residents of Svalbard should be informed of the zoonotic risk of this parasite and be provided with up-to-date data on the epidemiology of this parasite on Svalbard. The only way to achieve this, is through long-term monitoring programs.

The longer-term monitoring of changing *Em* prevalence and abundance levels, as well as population densities and distributions, in arctic foxes and Eastern European voles would allow local authorities to map infection risk trends using comparable methods. This has not been possible up until now given the different methodological approaches used by each independent study. The analysis method chosen for longer-term monitoring should be sensitive and specific, concurrently scalable for years with high numbers of trapped foxes and allow for the estimation of parasite abundance. The population dynamics and distribution are already monitored for

both Eastern European voles and arctic foxes through the NP/COAT project. However, the monitoring of important zoonoses like *Em* are not included.

# 6 Acknowledgements

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