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Present status, actions taken and future considerations due to the findings of *E. multilocularis* in two Scandinavian countries

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ABSTRACT

When *Echinococcus* (*E.*) *multilocularis* was first detected in mainland Scandinavia in Denmark in 2000, surveillance was initiated/intensified in Sweden, mainland Norway and Finland. After 10 years of surveillance these countries all fulfilled the requirements of freedom from *E. multilocularis* as defined by the EU, i.e. a prevalence in final hosts <1% with 95% confidence level. However, in 2011 *E. multilocularis* was detected in Sweden for the first time and surveillance was increased in all four countries. Finland and mainland Norway are currently considered free from *E. multilocularis*, whereas the prevalence in foxes in Sweden and Denmark is approximately 0.1% and 1.0%, respectively. *E. multilocularis* has been found in foxes from three different areas in Denmark: Copenhagen (2000), Højer (2012–14) and Grindsted (2014). Unlike Sweden, Norway and Finland, human alveolar echinococcosis (AE) is not notifiable in Denmark, and the number of human cases is therefore unknown. In Sweden, *E. multilocularis* has been found in foxes in four counties, Västra Götaland, Södermanland, Dalarna (2011) and Småland (2014). *E. multilocularis* has also been found in an intermediate host in Södermanland (2014). Two cases of AE have been reported in humans (2012), both infected abroad. No cases of *E. multilocularis* or AE have been reported in Finland and Norway. Recommendations and future considerations are discussed further.

1. Background

Apart from the finding in 1999 of *Echinococcus multilocularis* in the high-arctic island of Svalbard (Henttonen et al., 2001) the first case of *E. multilocularis* in the Nordic countries was found in a traffic-killed fox from Tåstrup, a western suburb of Copenhagen in 2000 (Kapel and Saeed, 2000). In 1994, serological investigations using the metacestode stage Em2 antigen (Gottstein et al., 1991) had indicated, but not definitively proved, the presence of the parasite. Following the Danish find in 2000, surveillance was initiated in Norway and Sweden and was intensified in Finland (Madslien et al., 2014; Wahlström et al., 2011) whereas no further Danish studies were conducted until 2011. After 10 years of surveillance in Sweden, the first positive fox was detected (Osterman Lind et al., 2011). The parasite has not been found in Norway, apart from on Svalbard, or in Finland (Henttonen et al., 2001; EFSA, 2013; Madslien et al., 2014).

Detection of *Echinococcus* spp. in animals is notifiable in all Scandinavian countries which is not the case for alveolar echinococcosis (AE) in humans. In Denmark, AE in humans is not notifiable. However, data concerning serological detection of *Echinococcus* spp. are available from the National Public Health Laboratory upon request (Henrik Vedel Nielsen, Statens Seruminstitut, pers. comm. 2014). In Sweden, Norway and Finland, human AE has been notifiable since 2004, 2003 and 1995, respectively (Anonymous, 2013; Folkehelseinstituttet, 2014) yet, information on species level is not required. In Sweden, notification from laboratories has been in place on a voluntary basis since 1994 (Anonymous, 2002). Species information based on laboratory results, clinical and radiological findings and epidemiology has when available been summarised (Anonymous, 2014a).

Import requirements to prevent introduction of *E. multilocularis* by dogs entering from EU-countries not free from the infection have been in place in most Scandinavian countries. However, since Denmark has never been officially free of *E. multilocularis* such requirements have never been in place in this country. Yet, from

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February 2013, the Danish Veterinary and Food Administration has recommended that dogs are dewormed prior to entering Denmark if they are imported from, or have been visiting, high endemic areas in central Europe. In Sweden, prior to 1994 all imported dogs were dewormed in quarantine. In 1994, quarantine for dogs from EU countries was replaced with import requirements including that a veterinary deworming certificate should be presented at the border. Since this year, the number of imported dogs has increased substantially. In 1995, border control was relaxed and it is considered that compliance with import requirements consequently decreased (Maria Cedersmyg, Swedish Board of Agriculture, pers. com, 2014). In 2012, the requirement to deworm pets prior to entering Sweden was abolished as a consequence of the finding of *E. multilocularis* in foxes (Anonymous, 2014c). Norway also had strict quarantine regulations requiring deworming of dogs whilst in quarantine. In 1997, quarantine for pets travelling from EU countries was replaced by a requirement to show a veterinary deworming certificate at the border (exemption if travelling from *E. multilocularis*-free countries) and a recommendation that a second deworming should be done after entry. Nevertheless, the border control of travelling pets is limited. Since March 2011, after the findings of *E. multilocularis* in Sweden, deworming was also required of dogs entering from Sweden, but no veterinary certificate was required to certify that deworming had been done. In 2012, the recommendation of a second deworming after entry was abolished. Finland had quarantine requirements until 1994 but, since 2001 there is a requirement that, for dogs imported from EU countries that are not "*E. multilocularis*-free", a veterinary deworming certificate should be shown at the border. Since 1995, as in Sweden, border controls were relaxed due to the principle of free movement in the EU. From 2012 onwards, the deworming requirement also applies to dogs imported from Sweden (Virva Valle, Finnish Food Safety Authority Evira, pers. comm, 2014).

Current legislation is believed to have resulted in an increased risk of importing *E. multilocularis* to countries considered free from this parasite (Defra, 2010). Furthermore, spot checks by the Norwegian Food Safety Authority and a study by the Norwegian Veterinary Institute have revealed lack of compliance with the anthelmintic treatment requirements (Davidson and Robertson, 2012; Hamnes et al., 2013; VKM, 2012).

The aim of this article is to summarise the present veterinary situation concerning *E. multilocularis* in Denmark, Sweden, Norway and Finland and to discuss differences in surveillance, costs for surveillance, actions taken and future considerations including human AE in the four countries.

Table 1
Number of foxes/fox scats collected and anlaysed for *Echinococcus multilocularis* between 2000 and 2013 in Denmark, Sweden, Norway and Finland and type of analysis performed. References to methods used are given in the main text.

	Denmark	Sweden	Norway	Finland
2000	0	11[b]	0	9[f]
2001	0	442[b]	0	13[f]
2002	1040[a]	313[b]	85[d]	116[f]
2003	0	400[b]	119[d]	164[f]
2004	0	400[b]	105[d,e]	348[f]
2005	0	200[b]	5[d]	281[f]
2006	0	402[b]	31[e]	209[f]
2007	0	245[b]	539[e]	264[f]
2008	0	244[b]	455[e]	411[f]
2009	0	305[b]	280[e]	184[f]
2010	0	304[b,c]	0	144[f]
2011	287[a]	3775[c]	533[e]	128[f]
2012	262[a]	661[c]	614[e]	234[f]
2013	214[a]	1537[c]	625[c]	254[c,f]

[a] Sedimentation and counting technique.
[b] Coproantigen ELISA (CoA) and the segmental sedimentation and counting technique (SSCT as confirmatory test.
[c] MC-PCR.
[d] CoA and egg PCR as confirmatory test.
[e] Egg PCR.
[f] Coproantigen ELISA (CoA) and SCT as confirmatory test.

Table 2
Number of raccoon dogs collected and anlaysed for *Echinococcus multilocularis* between 2000 and 2013 in Denmark, Sweden, Norway and Finland and type of analysis performed. References to methods used are given in the main text.

	Denmark	Sweden	Norway	Finland
2000	0	0	0	0
2001	0	0	0	2[c]
2002	0	0	0	3[c]
2003	0	0	0	98[c]
2004	0	0	0	239[c]
2005	0	0	0	219[c]
2006	0	0	0	193[c]
2007	0	0	1[b]	227[c]
2008	0	21[a]	0	148[d]
2009	0	28[a]	0	177[d]
2010	0	0	0	166[d]
2011	85[a]	0	1[b]	204[d]
2012	49[a]	0	0	259[d]
2013	70[a]	0	0	418[e]

[a] Sedimentation and counting technique.
[b] Egg PCR.
[c] Coproantigen ELISA (CoA) and SCT as confirmatory test.
[d] CoA and egg PCR as confirmatory test.
[e] MC-PCR.

2. Methods

2.1. DENMARK

2.1.1. Surveillance in animals

Until the national surveillance of *E. multilocularis* in wild carnivores was initiated in 2011, prevalence studies of *E. multilocularis* were few. During the period 1997–2002, a total of 1040 red foxes (*Vulpes vulpes*) were examined from all regions of the country. Of these animals, 340 foxes originated from the greater Copenhagen area. A national surveillance program was initiated in autumn 2011 including approximately 300 wild carnivores each year, mainly foxes and raccoon dogs (*Nyctereutes procyonoides*) collected throughout the country (Tables 1 and 2). Until now (November 2014), a total of 1500 carnivores have been analysed. Studies of foxes and other wild carnivores in Denmark have so far used the sedimentation and counting technique (SCT) (Eckert et al., 2001) to detect *E. multilocularis*. Following morphological identification of *E. multilocularis*, the worms are further characterised by PCR (Knapp et al., 2007; Stefanic et al., 2004). Taeniid infections in intermediate hosts were studied in 719 small mammals trapped in and around the metropolitan area of Copenhagen in 2005–2009 (Al-Sabi et al., 2013). During autopsy visible lesions in the peritoneal cavity and liver underwent morphological analysis, and were subsequently analysed by PCR and sequencing (Al-Sabi and Kapel, 2011). No proper prevalence studies have been performed in Danish pets but faecal samples from Danish dogs ($n = 517$) and cats ($n = 169$) submitted to a diagnostic German laboratory in 2004–05 underwent PCR analysis for *E. multilocularis* as part of the routine diagnostic procedure (Dyachenko et al., 2008).

2.1.2. Risk assessment

The risk of introducing *E. multilocularis* to Sweden by Danish dogs and cats in transit was assessed by the National Veterinary Institute in Denmark in 2006 on the request of the Danish Veterinary and Food Administration (Bødker et al., 2007).

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2.1.3. Costs

The total costs related to the ongoing surveillance (November 2011 to December 2014) of *E. multilocularis* in wild carnivores were calculated. Other expenses linked to e.g. studies of *E. multilocularis* in rodents and anthelmintic treatment of dogs etc., have not been estimated.

2.1.4. Surveillance in humans

Information on the surveillance in humans was obtained from official statistics and via contact with Statens Seruminstitut, Denmark.

2.2. SWEDEN

2.2.1. Surveillance in animals

During 2000–2010, a total of 3266 foxes, 49 raccoon dogs (Tables 1 and 2) and 3000 rodents were analysed. Due to the positive finding in 2011, in a fox shot in 2010, surveillance was initiated with the aim to obtain 3000 hunter shot foxes from different parts of the country. The foxes were examined with the segmental sedimentation and counting technique (SSCT) (Umhang et al., 2011). The sampling intensity was higher in the south west parts of Sweden as it was considered probable that the parasite was introduced by infected dogs in this region. In addition, 119 faecal samples from hunting dogs, collected in the region of the first positive finding, were examined by egg flotation and an in-house real-time PCR. In addition, 236 rodents were trapped and autopsied (Wahlström et al., 2012). To obtain a baseline prevalence to be used for comparison with future studies and to obtain more information on the geographical distribution, a second national monitoring was initiated in 2012 where fox scats instead of fox carcasses were collected (Anonymous, 2014d) and analysed with a semi-automated magnetic capture probe based DNA extraction and real-time PCR (MC-PCR) (Isaksson et al., 2014). The aim was to detect a prevalence of 0.1% with 95% confidence. However, as the fox population is much lower in the northern parts of Sweden, the sampling intensity was also lower there. In addition, a baseline study was performed in 2011, in an infected area (Södermanlands County), where 790 fox scats were collected within a circle with a diameter of 50 km (Anonymous, 2014d) and analysed with the MC-PCR. For subtyping of the parasite, hunters were requested, in 2012, to submit 30 foxes from each of the three known infected areas. In 2014 this requirement was extended to include the fourth known infected area (Småland). Foxes were analysed either with SSCT or with MC-PCR followed by SSCT if positive. As part of an ongoing research project at the Swedish University of Agricultural Science (SLU) and in cooperation with the environmental monitoring and assessment performed at SLU, collection of rodent and fox faecal samples was initiated in 2012 in four different areas: two areas where infected foxes were known to be present and two areas with unknown status. Fox faecal samples were analysed by sieving followed by PCR and sequencing (Mathis et al., 1996; Trachsel et al., 2007) and rodents were autopsied.

2.2.2. Risk assessment

In March 2011, a government mandate was given to the Swedish Board of Agriculture and the National Board of Health and Welfare to clarify the necessary actions to protect public health as a consequence of finding *E. multilocularis*. Within this mandate a qualitative risk assessment was performed by the National Food Agency and the Public Health Agency of Sweden (former Swedish Institute for Communicable Disease Control) to elucidate the risk of human infection in Sweden (Anonymous, 2011).

2.2.3. Costs

The costs for the surveillance/monitoring activities after the positive findings in 2011 were summarised. This included costs for sample collection and analysis in the national screening of foxes in 2011 as well as the collection of fox scats in Södermanlands County in 2011. Costs for the monitoring of dogs and rodents were not included.

2.2.4. Surveillance in humans

Information on the surveillance in humans was obtained from official statistics and by contact with Folkhälsomyndigheten, Sweden.

2.3. NORWAY

2.3.1. Surveillance in animals

Surveillance in red foxes was initiated in 2006 when samples from earlier years (2002–2005) were analysed for *E. multilocularis* (Tables 1 and 2). Since 2007, active surveillance of red foxes has been in place. Surveillance was intensified in 2011, after positive findings in Sweden, approximately 80 km from the Norwegian border. Apart from the 2009–2010 hunting season when budget cuts resulted in the program being temporarily halted, surveillance has been carried out since the 2010–2011 hunting season (Hofshagen et al., 2013; Madslien et al., 2011, 2014; Wahlström et al., 2011). In addition, faecal samples from red foxes, and occasionally wolves and raccoon dogs, were collected by hunters (or pathologists during post-mortem examination) and sent to the Norwegian Veterinary Institute for examination for *E. multilocularis*. Before 2007, samples from red foxes were analysed using copro-ELISA and confirmatory PCR (Davidson, 2011). Since 2007, two different methods have been used for analysis of faecal samples from hunted red foxes. Egg isolation via flotation and sieving coupled to a multiplex PCR was used until the 2012 hunting season (Davidson et al., 2009). Collaboration between the Norwegian and Swedish National Veterinary Institutes revealed that the method used in the Norwegian surveillance program was not as sensitive as previously estimated (Øines et al., 2014). Since 2012 the Norwegian surveillance program has used the same MC-DNA extraction method as in the Swedish program coupled with a different real-time PCR (Øines et al., 2014).

2.3.2. Risk assessment

In 2012, the Norwegian Scientific Committee for Food Safety published an opinion regarding the risk of introducing *E. multilocularis* into mainland Norway in the next decade (VKM, 2012). Furthermore, in 2012 a sudden increase in the number of rescued stray dogs being imported into Norway, probably caused by changes in EU pet travel legislation in 2012, was observed. This prompted the Norwegian Food Safety Authority to request a risk assessment regarding the import of pathogens with “stray” dogs from Eastern Europe (Høgåsen et al., 2012).

2.3.3. Costs

Costs for surveillance, including collection, submission and analysis of samples were summarised.

2.3.4. Surveillance in humans

Information on the surveillance in humans was obtained from official statistics and information provided by the Norwegian Public Health Institute (Folkehelseinstituttet).

2.4. FINLAND

2.4.1. Surveillance in animals

In 2000, surveillance of foxes and raccoon dogs was initiated at the Finnish Food Safety Authority (Evira), although rodent scien-

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tists at the Finnish Forest Research Institute (Metla) have routinely performed parasitological necropsies on arvicolid rodents since the 1970s (Haukisalmi et al., 1987). During 2000–2009 a total of 1995 foxes, 1306 raccoon dogs (Tables 1 and 2) and 19700 arvicolid rodents were examined for *E. multilocularis* (Wahlström et al., 2011). In 2010–2013, between 128 and 254 foxes and 165–418 raccoon dogs were tested annually, as well as between 280 and 3500 rodents (Anonymous, 2014b). The fox and raccoon dog surveillance (2000–2014) has been a hybrid between simple representative sampling aiming at all animals in the country having an equal chance to be sampled, and risk-based sampling targeting the supposed high risk area at the southeastern border. As rabies vaccination follow-up is done along the southeastern Russian border, which is also regarded as risk area for *E. multilocularis*, this area has been convenient to sample. Foxes and raccoon dogs were analysed with coproantigen ELISA, first at Evira using commercial tests, and after the commercial tests were discontinued, at the University of Zurich, Switzerland. Coproantigen positive animals were tested by SCT or egg PCR (Mathis and Deplazes, 2006). In 2013, most foxes were tested at the Friedrich-Loeffler-Institut, the German Federal Research Institute for Animal Health (FLI) using SCT. Raccoon dogs and a small number of foxes were tested using MC-PCR (Øines et al., 2014). In 1993, 1999 and 2001, restricted local faecal surveys of dogs were performed in the north-eastern part of the country. In the first one, faecal samples from 93 dogs were examined using the Telemann method (ether-acetic acid sedimentation) (Oksanen and Laaksonen, 1995), and in the next two samplings, faeces from 169 and 183 dogs were examined using both faecal flotation and coproantigen ELISA (Echinotest, Bommeli Diagnostics, Liebefeld-Bern, Switzerland) (Hirvela-Koski et al., 2003).

2.4.2. Risk assessment

A risk assessment on the risk of introduction of *E. multilocularis* into Finland and spread in Finland was performed in 2001 (Maijala et al., 2001).

2.4.3. Costs of surveillance

The costs of surveillance were calculated taking into account both transport of the foxes and raccoon dogs to the Evira laboratory, labor costs, including Evira overhead, and reagent costs. Foxes and raccoon dogs were delivered to Evira by volunteer hunters who did not receive monetary compensation.

2.4.4. Surveillance in humans

Information on the surveillance in humans was obtained from official statistics and through contact with Helsinki University.

3. Results

3.1. DENMARK

3.1.1. Surveillance in animals

Among the 1040 foxes examined during 1997–2002, three (0.03%) foxes were found to be infected with *E. multilocularis*. However, all infected foxes were from the Greater Copenhagen area (Zealand) which corresponded to a local prevalence of 0.9% (three of 340 foxes) in the year 2000 (Saeed et al., 2006). Since then, no positive foxes have been detected on the island of Zealand, but a German study on clinical samples collected from Danish cats in 2004 demonstrated one *E. multilocularis* positive cat (one of 169 = 0.6%) from Zealand (Dyachenko et al., 2008), whereas no *E. multilocularis* positive rodents have yet been detected in Denmark. The ongoing national surveillance has so far (September 2011–November 2014) included a total of 1500 carnivores: 1169 foxes, 265 raccoon dogs and 66 other wild carnivores. The first positive fox from Jutland was shot in November 2011 near Højer, 8 km north of the border to Germany, and tested positive in April 2012 (Enemark and Nielsen, 2012). The following year, another three *E. multilocularis* positive foxes were detected in the same area corresponding to a local prevalence of 30.8% (4 of 13 foxes) (Enemark et al., 2013). Later analyses confirmed this high local prevalence. A total of 28 foxes have been tested from this area, and nine of these (32.1%) were positive for *E. multilocularis*. In addition, two raccoon dogs, shot in February 2014 in the Højer area, tested positive for *E. multilocularis* corresponding to a local prevalence of 25.0% in this animal species. In January 2014, an *E. multilocularis* positive fox was detected near Grindsted, approximately 100 km north of the prior findings, and subsequent analyses revealed another three positive foxes from this area (four positive out of 97 foxes from Grindsted = 4.1%) (Fig.1). The current national prevalences are 1.2% (14 out of 1169) and 0.75% (2 out of 265) in foxes and raccoon dogs, respectively.

The worm burdens in the first detected foxes on the island of Zealand were between one and 53. Later, during the surveillance in 2011, the worm burdens were also found to be low (<50). However, in 2013 two foxes shot near the border to Germany had worm burdens of 596 and 1527, respectively (Enemark et al., unpublished) and subsequent findings have revealed six animals with worm burdens of several hundred.

3.1.2. Risk assessment and risk management

No risk assessment for human AE has been conducted in Denmark. Based on *E. multilocularis* prevalence in foxes (Saeed et al., 2006) the risk assessment conducted by Bødker et al. (2007) suggested a prevalence in Danish dogs of around 0.003%. It was concluded that export of Danish dogs to Sweden entailed a negligible risk compared to import of dogs from high-endemic areas in central Europe to Sweden, which was considered to be the highest risk of introducing *E. multilocularis* to the country.

Detection of *E. multilocularis* in 2000 had no direct implications for the Danish legislation, but following detection of the high-endemic focus of *E. multilocularis* in red foxes in southern Denmark in January 2013 (Enemark et al., 2013) the Danish Veterinary and Food Administration has recommended, from 18th February 2013, that dogs in the Tønder municipality (i.e. southern Denmark) which are allowed to roam freely in the countryside are dewormed regularly with praziquantel every fourth week. This is in contrast to the general Danish legislation which does not allow preventive anthelmintic treatment or treatment without a clinical or laboratory diagnosis. On February 11th 2014, a few days after the detection of *E. multilocularis* in a fox from Grindsted, the Danish Veterinary and Food Administration extended the recommendations concerning prophylactic treatment of dogs at risk of echinococcosis to the whole country. Thus, today Danish dogs may routinely be treated preventively against this zoonotic parasite similarly to the situation in most other European countries. Additional specific measures to prevent the transmission of *E. multilocularis* to humans include the following recommendations: the public is advised to prevent dogs from eating rodents; to wash free roaming dogs regularly and to rinse raw fruits and vegetables thoroughly before consumption.

3.1.3. Costs

In the period 2011–2014, approximately 50 000€ have been allocated annually for *E. multilocularis* surveillance of wild carnivores, which corresponds to approximately 170€ per animal. This figure includes costs of sample collection, SCT analyses and PCR verification of taeniid positive samples. During the same period analyses of 250 additional carnivores have been financed by the National Veterinary Institute as part of the routine surveillance

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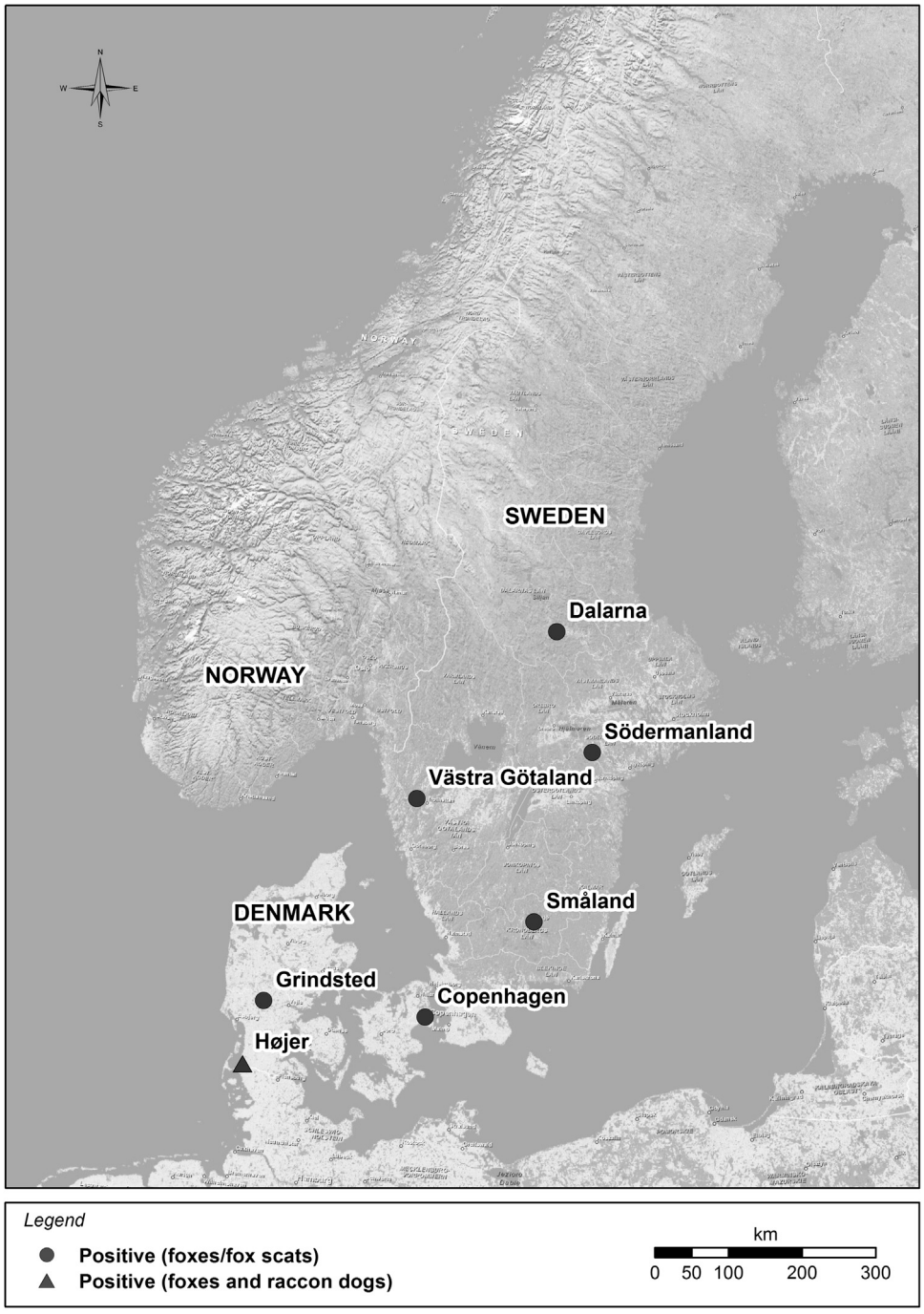

Fig. 1. Location of the three sites in Denmark, where foxes and raccoon dogs positive for *Echinococcus*(*E.*) *multilocularis,* and the four cites in Sweden where foxes or fox scats positive for *E. multilocularis* have been found by December 2014.

of road-killed wildlife; the costs of these analyses have not been estimated separately.

3.1.4. Surveillance in humans

Only one case of human AE has been described in Denmark and this patient was considered to have been infected in central Europe (Samuelsson and Kapel, 2004). Nevertheless, after a review of available data from 2009 to 2013, it was concluded, based on clinical signs and serology, that in 2013, one patient originating from the Baltic countries most probably had AE. (pers. com. Vedel Nielsen, H., Statens Seruminstitut. 2014). So far no autochthonous cases of human AE have been detected in Denmark. During the period 2009–2013, a mean of 86 people (approximately 15/million inhabitants) were tested annually for *E. multilocularis*. Since 2000 an annual average of 1–3 tested positive for *E. multilocularis* by ELISA (Müller et al., 2007) and in 2012 and 2013, 6 people tested positive each year. As the test is not 100% specific and due to increased awareness, all inconclusive and test positive samples have been verified by the *Echinococcus* Western Blot IgG (LDBIO Diagnostics, Lyon, France) since November 2014. Yet, since *E. multilocularis* is not notifiable the actual number of infected people is unknown (pers. com. Vedel Nielsen, H., Statens Seruminstitut).

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3.2. SWEDEN

3.2.1. Surveillance animals

In the national screening in 2011, 2985 foxes were analysed and three positive cases (0.1%) were detected in three different areas (Wahlström et al., 2012). In the surveillance of a known infected area in Södermanlands County, six out of 790 (0.8%) faecal samples were positive. For subtyping of the parasite, a further 73 foxes were shot in known infected areas (December 2014) and three of these animals were found to be infected. However, subtyping results are still pending. Data on worm burden is available from seven of the nine foxes analysed with SSCT. One fox had 1235 worms, one had between 100 and 500 worms and five foxes has between one and 11 worms. Within the EMIRO research project and the FoMA surveillance (EMIRO, 2015; FoMA, 2015), positive fox scats have been found in areas known to be infected and, in 2014 in a new county in southern Sweden (Småland) (Miller, A. unpublished) (Fig. 1). Furthermore, within this project the first infected intermediate host was identified (Miller, A. unpublished). Final results are pending.

3.2.2. Risk assessment and risk management

The risk assessment concluded that the risk for humans was very low. It was estimated that about one person among the nine million Swedes would be infected every fifth year. It also estimated that if the probability of infection in humans became the same as in Switzerland, this figure would increase to 20–30 cases per year (Anonymous, 2011; Wahlström et al., 2012). This figure was compared with other infections considered to be severe such as tick borne encephalitis and tularaemia where about 200 and 200–500 cases are reported annually (Anonymous, 2011; Wahlström et al., 2012). Special hygienic measures were only recommended for hunters when handling fox carcasses. The importance of food and drinking water for the transmission of AE to humans could not be assessed, as there were no documented risk-reducing effects of washing vegetables and berries. Taking the benefits of outdoor activities including harvesting and consuming berries and vegetables into consideration, it was concluded that it was not appropriate to issue any specific recommendations about *E. multilocularis* and food. However, the importance of following general advice on food hygiene was emphasised. For consumers not willing to accept any risk, information was given that boiling food would inactivate *E. multilocularis*. As *E. multilocularis* was considered to be endemic in the country with a low prevalence (0.1%) in foxes, no specific deworming recommendations for dogs were given. For worried owners of dogs at risk of becoming infected with *E. multilocularis*, information was given that monthly deworming would prevent dogs from spreading the infection. It was however recommended to deworm dogs considered at risk which were entering Sweden from countries where *E. multilocularis* is more common, both to protect the owners but also to prevent any additional spread of the disease in Sweden (Anonymous, 2011; Wahlström et al., 2012).

It was concluded that as long as the prevalence of *E. multilocularis* remained low, if infected foxes are mainly found outside cities and densely populated areas and new knowledge does not point out the importance of food or water as a source of AE, no further recommendations would be given to the public. Therefore more knowledge concerning the prevalence of *E. multilocularis* increased and repeated monitoring of *E. multilocularis* in foxes as well as of the fox population is needed. Of special interest is the urban fox population (Anonymous, 2011; Wahlström et al., 2012).

3.2.3. Costs

The total cost for the national screening in the spring 2011 (n = 2,895) was approximately four million SEK (~400 000 €). About 50% of the costs were related to laboratory analysis and the rest were costs for collection, handling, deep freezing, autopsy, and administration including daily reporting of the project. The cost for the intensified surveillance in the infected area of Södermanlands County (n = 790 fox scats) was 659 000 SEK (~66 000 €).

3.2.4. Surveillance in humans

Two cases of AE have been reported in Sweden, both in 2012 and both considered to have been infected abroad based on epidemiological information (Anonymous, 2014a). Before *E. multilocularis* was detected in Sweden, between 2008 and 2010, on average three patients were specifically tested for *E. multilocularis* (Em2+ ELISA, Bordier Affinity products, Crissier, Switzerland) annually (0.3 per million inhabitants). In 2011, 2012 and 2013, 37, 48 and 79 patients were tested respectively, i.e. increasing to eight patients tested per million inhabitants. A total of eight patients tested positive with the Em2+ ELISA method and further investigation including WB (LDBIO Diagnostics, Lyon, France) and EM18 antigen immunoblot (performed in Zurich, Switzerland) showed that two of these were infected with *E. multilocularis* (pers com. Botero-Kleiven, S., Folkehelseinstituttet, 2014).

3.3. NORWAY

3.3.1. Surveillance in animals

A total of 3405 red fox faecal samples from throughout Norway have been examined since 2002 (Madslien et al., 2014). All the samples have tested negative. The estimated true prevalence of *E. multilocularis* for the period 2002–2013 was 0% (95% confidence interval 0–0.2%) (Madslien et al., 2014). No rodents have been examined for *E. multilocularis* in mainland Norway. Prior to 2012 faecal samples from 12 wolves and two raccoon dogs had also been screened for *E. multilocularis* (Davidson, 2011). All were negative.

3.3.2. Risk assessment

The Norwegian Scientific Committee for Food Safety concluded that it was likely that *E. multilocularis* would be detected in mainland Norway during the next decade (VKM, 2012). They also highlighted that with the current surveillance program it was unlikely that *E. multilocularis* would be detected upon first introduction. Nearly 1000 foxes could theoretically have become infected before the first case was detected since the surveillance program was designed to detect a prevalence level of <1% in an estimated red fox population size of 70 000–120 000. They also highlighted the paucity of border control checks as of concern with regard to the risk of importing infected dogs from Europe into mainland Norway. Høgåsen et al. (2012) concurred and considered there was a moderate probability of importing *E. multilocularis* with rehomed stray dogs from Eastern Europe. This was further highlighted by Hamnes et al. (2013) who found that a large number of the rehomed stray dogs examined had not been given the correct anthelmintic treatment prior to and post import.

3.3.3. Costs

It was estimated that the annual costs were around 150 000 € for the active and passive surveillance work (pers comm. Dr.Knut Madslien, Norwegian Veterinary Institute, Norway, 2014).

3.3.4. Surveillance in humans

A total of 19 echinococcosis cases have been notified in the period 2006–2013. Although not notifiable prior to 2003, 16 cases were recorded in the period 1975–2002. The species involved is not recorded (Folkehelseinstituttet, 2014) but based on gross and histopathological appearance, cystic hydatidosis has been the presumptive diagnosis. In one of the cases *E. granulosus* was confirmed using molecular methods (personal communication Øivind Øines, Norwegian Veterinary Institute, 01.09.14). Two arctic fox

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field researchers from Svalbard were found to have seroconverted to *E. multilocularis*, however lesions have not been reported.

3.4. FINLAND

3.4.1. Surveillance animals.

During the surveillance performed in 2000–2014, when 2,759 foxes and 2,353 raccoon dogs were examined, or any other occasion before that, no *E. multilocularis* infected animal has been found.

3.4.2. Risk assessment

The risk assessment performed in 2001 (Maijala et al., 2001) highlighted the importance of preventing introduction, as the conditions for the spread of *E. multilocularis* appear favorable, with both suitable definitive and intermediate hosts. It was concluded that there was a considerable risk of introduction with wildlife, but the risk was difficult to assess because the information about the *E. multilocularis* situation in northwestern Russia was lacking. Also the risk of introduction with infected pets was regarded as real. To prevent introduction, treatment of imported pets against cestodes was recommended. The control of fox and raccoon dog populations was recommended as the best means to control *E. multilocularis* spread in Finland. This was, however, regarded as practically difficult to perform. It was also recommended to increase surveillance in wild definitive host populations.

3.4.3. Costs

Costs of surveillance as performed in 2014 using the semi-automated magnetic capture probe based DNA extraction and real-time PCR (MC-PCR) (Øines et al., 2014) was calculated to be about 96€ per sample, making the total cost approximately 60 000€. This presents a substantial saving compared to SCT, which alone costs 174€ per fox and 317€ per raccoon dog sample (due to its longer intestines) (Marja Isomursu, and Petra Heikkinen, Evira, pers. comm., 2014). However, SCT was only used as confirmatory test, since coproantigen-ELISA was used as a screening test, and only animals testing positive or inconclusive were tested by SCT. The coproantigen-ELISA testing was carried out by courtesy of Professor Peter Deplazes, University of Zurich.

3.4.4. Surveillance in humans

During 2000–2014 (until September 22nd), 22 cases of echinococcosis were reported (2014). None of the reported cases was considered autochthonous. Most of them were diagnosed as caused by *Echinococcus granulosus* G1. No AE cases have been reported (Antti Lavikainen, Helsinki University, pers. comm., 2014).

4. Discussion

In Denmark and Sweden *E. multilocularis* was first detected in 2000 and 2011, respectively. In Denmark, a serological study of *E. multilocularis* in foxes performed in 1994 indicated that the parasite occurred in the whole country. However, as the serological test cross reacted with other common tapeworms it was not possible to definitively conclude that the foxes were infected with *E. multilocularis*, and it was not until 2000, within a research project, that the presence of the parasite was confirmed (Kapel and Saeed, 2000). Nevertheless, an official national surveillance of *E. multilocularis* was not initiated until several years later following the random finding of a positive cat in a clinical sample collected in 2004 which was analysed by a German laboratory. Although notifiable in Denmark, this finding was not reported to Danish authorities and therefore not recognised until the publication in 2008 (Dyachenko et al., 2008).

In Sweden, the parasite was detected after 10 years of surveillance and examination of about 3000 foxes, which is consistent with the number of samples needed to detect a prevalence of 0.1% (the present prevalence in Sweden) with 95% confidence level. Thus, if surveillance had not been performed, *E. multilocularis* in wildlife would not have been detected.

In Norway and Finland, the sensitivity of the surveillance system has increased in recent years due to an increasing number of samples (Norway) and improved sensitivity of the testing procedure in 2013 (Norway) and 2014 (Finland). It is likely that *E. multilocularis* will also be detected in Norway and Finland in the near future; and it may in fact already be present, although below the detection level of the surveillance system. Norway has a very long common border to Sweden where *E. multilocularis* is present about 80 km from the Norwegian border. Finland has more than 1300 km of land border with Russia, which is open for red foxes and raccoon dogs to cross. Currently, there is very limited information about the *E. multilocularis* situation in adjacent Murmansk Oblast, Republic of Karelia and Leningrad Oblast regions of Russia. However, despite lack of recent information, it is well known that *E. multilocularis* occurs in a wide area of the Russian federation (Eckert et al., 2001). In Estonia, across the 70 km wide Gulf of Finland, infection was found to be widespread and prevalent (Moks et al., 2005). Therefore, a high infection pressure probably exists just beyond the Finnish borders.

No autochthonous cases of AE have been reported in the four countries. Yet, in Denmark two cases have been detected (2004 and 2013) and likewise two cases were notified in Sweden (2012), all four are considered to have been acquired abroad. Interestingly, these human cases were reported just a few years after the first finding of *E. multilocularis* in foxes. It is likely that increased awareness, due to detection of *E. multilocularis* in foxes, i.e. an increase in the sensitivity of the passive surveillance, has increased the probability of an AE case being diagnosed. In Sweden, the number of tests for AE increased from three in 2010 (before the first finding) to 79 in 2013. Corresponding figures from Denmark are not available for the years around the first findings in foxes in 2000. At present, testing intensity is about twice as high in Denmark compared to Sweden where approximately eight tests per million inhabitants were performed in 2013 compared to the Danish average of about 15 during 2009–2013. Other countries also report findings in foxes followed by findings in humans. For example, in Slovakia the first case of *E. multilocularis* in a fox was reported in 1999 immediately followed by the first reported case of AE in a human in 2000 (Miterpakova and Dubinsky, 2011). Furthermore, compulsory notification is required to obtain reliable statistics. For example in Denmark, it is unknown if more AE cases have been present as there is no duty of notification, and clinical confirmation of *E. multilocularis* positive laboratory results are not reported. This deficiency is highlighted by the results presented here revealing the random detection of a probable case of AE in 2013 (Henrik Vedel Nielsen, Statens Seruminstitut, pers. comm. 2014). Another weakness of current notification systems is that many countries do not report cases of echinococcosis to species level. This is clearly a deficiency as cystic echinococcosis caused by *E. granulosus* and AE caused by *E. multilocularis* are different diseases with different epidemiology and diverse clinical symptoms. This is especially important for countries that consider themselves to be free from *E. multilocularis*.

Several recent studies report increasing prevalence of *E. multilocularis* in Europe, which is believed to be correlated to the increase in fox populations after the eradication of rabies (Schweiger et al., 2007). Similarly, increasing geographical spread of *E. multilocularis* has been described; however, the reasons for this may also be closely linked to more intensive surveillance, as for example reported from Denmark and Sweden. It is likely that infected dogs may have been imported to Denmark as there has been no requirement for deworming of dogs entering this country. In Sweden, import control according to EU regulations has been in place, yet,

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several studies have shown them to be insufficient (Hamnes et al., 2013; Høgåsen et al., 2012; Torgerson and Craig, 2009; Vågsholm, 2008). When the first *E. multilocularis* positive foxes were detected in Denmark and Sweden it was considered likely that the infection had been introduced via dogs imported from endemic areas without proper deworming. Nonetheless it is debatable whether the parasite has been present, but undetected, for a long time. If the parasite was recently introduced, the prevalence of *E. multilocularis* and thereby the risk for humans is expected to increase over time. In order to gain more knowledge on this issue, subtyping using multi-locus microsatellite analysis (Bart et al., 2006) of Danish and Swedish isolates is underway. Furthermore, repeated monitoring is needed to clarify if the prevalence will increase in the future. In Sweden, one regional baseline study has been done in Södermanlands County and one national baseline study will be finalised this year.

Surveillance for subclinical infections with a very low prevalence is difficult. For example in Sweden, with an expected prevalence of 0.1% about 3000 samples are needed (assuming a sensitivity of 100%) to detect the infection. The SCT or SSCT test requires culling of foxes and submission of the whole carcasses which is cumbersome, costly and also presents a potential zoonotic risk for those handling the carcasses. Using the MC-PCR, a test considered to have about the same sensitivity and specificity as the SCT, facilitates the sampling procedure and also reduces the zoonotic risk during handling of samples. In Sweden in 2011, about 466 000€ was allocated by the authorities for surveillance, the first national screening ($n = 2985$) and one screening in an infected area ($n = 790$). The costs for the second national surveillance is expected to be lower as costs for collection and handling of samples (fox scats) are expected to be significantly lower than in the first national surveillance. In Norway and Finland, surveillance is required by EU authorities to document freedom (EFSA, 2013) and the authorities have allocated about 150,000€ in Norway and about 60 000€ in Finland annually for surveillance of *E. multilocularis* in the final hosts. In Denmark, the authorities allocated approximately 50 000€ annually during a four year period (2011–2014) and additional funding has been obtained from the National Veterinary Institute; future funding is decided on a year to year basis. Thus, of the two infected Scandinavian countries, Swedish authorities allocate more funding for surveillance than Denmark, and even in countries currently regarded free of *E. multilocularis*, funding for surveillance is higher than in Denmark. However, increased funding per se is not a goal. Yet, sufficient funds should be available to reliably survey the prevalence of *E. multilocularis* in wildlife to detect sudden dramatic increases in prevalence. This will enable timely information to the public and adaptation of preventive measures.

A risk assessment to clarify the risk that *E. multilocularis* poses to humans has been done in Sweden but not in Denmark. Risk management also differs between the countries. In Sweden, no general recommendations were given concerning washing of vegetables and fruit. Special hygienic recommendations were given to fox hunters but no general recommendations were given concerning dogs at risk of infection. In Denmark, the authorities recommended consumers to exercise good hygiene when preparing raw fruit and vegetables, and with regard to dogs at risk, routine deworming was recommended in addition to preventive measures targeted against dog owners to prevent dogs from eating rodents and to prevent the transmission of *E. multilocularis* eggs via contaminated dogs' fur. A reason for these differences may be that the prevalence in Denmark, particularly in certain areas, is higher compared to Sweden. The Swedish risk assessment also highlighted the need to follow the situation to detect any change in prevalence and to identify hotspots, should they occur in urban areas. However, the National Food Agency clarified that its recommendations will be re-evaluated when new information becomes available and that any changes will be dependent primarily on evidence relating to the role of food in the transmission of the disease or effectiveness of washing rather than on any change of *E. multilocularis* prevalence in domestic foxes. The Agency also highlighted that more data is needed in this field.

5. Conclusion

In conclusion, more monitoring is needed to describe the present geographical distribution and prevalence of *E. multilocularis* in the four Scandinavian countries. Repeated monitoring is necessary to clarify if the prevalence is increasing in Denmark and Sweden. Further, monitoring for *E. multilocularis*, including sample collection as well as analyses, is costly and research is required to lower these costs. In addition, it is important to have reliable statistics on the occurrence of AE in humans. For this purpose, awareness of the infection is needed among clinicians and notification systems should be in place including information on species level. Finally, more knowledge is wanted regarding risk factors for AE in humans, allowing effective risk management in different prevalence situations.

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