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Annual assessment of *Echinococcus multilocularis* surveillance reports submitted in 2022 in the context of Commission Delegated Regulation (EU) 2018/772

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Abstract

This report is part of the *Echinococcus multilocularis surveillance* scientific reports which are presented annually by EFSA to the European Commission and are intended to assess the sampling strategy, data collection and detection methods used by Finland, Ireland, United Kingdom (Northern Ireland) and Norway in their respective surveillance programmes. The surveillance programmes of these four countries were evaluated by checking the information submitted by each of them and verifying that the technical requirements were fulfilled as laid down in Commission Delegated Regulation (EU) 2018/772 of 21 November 2017 supplementing Regulation (EU) No 576/2013 of the European Parliament and of the Council with regard to preventive health measures for the control of *Echinococcus multilocularis* infection in dogs, and repealing Delegated Regulation (EU) No 1152/2011. The information was divided into four different categories for assessment: the type and sensitivity of the detection method, the selection of the target population, the sampling strategy and the methodology. For each category, the main aspects that need to be considered in order to accomplish the technical requirements of the legislation were checked against compliance of several criteria. Three of the countries participating in this surveillance (Finland, Ireland and Norway (mainland)) succeeded in the fulfilment of the technical legal requirements foreseen in Commission Delegated Regulation (EU) 2018/772 concerning these four different categories. United Kingdom (Northern Ireland) fulfils those requirements, only assuming a diagnostic test sensitivity value of 0.99 (value provided by the national reference laboratory, higher than the conservative sensitivity value suggested by EFSA, i.e. 0.78). None of the four countries recorded positive samples in the 12-month reporting period.

Keywords: *Echinococcus multilocularis*, absence of infection, freedom from disease, surveillance

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Summary

Following a request from the European Commission and, indirectly, from the European Free Trade Association (EFTA) Surveillance Authority, the Biological Hazards & Animal Health and Welfare Unit (BIOHAW) at EFSA was asked – in the context of Article 31 of Regulation (EC) No 178/2002 – to annually evaluate the surveillance programme on *Echinococcus multilocularis* infection in animals carried on by the Member States listed in the Annex to Commission Implementing Regulation (EU) 2018/878 (as amended by the Commission Implementing Regulation (EU) 2020/2017 of 9 December 2020): Malta, Finland, United Kingdom (Northern Ireland),[1] Ireland and Norway.

In order to be included in the Annex to Commission Implementing Regulation (EU) 2018/878, Member States must comply with the rules laid down in Article 2 of Commission Delegated Regulation (EU) 2018/772 on 'rules for categorisation of Member States in view of their eligibility for preventive health measures for the control of *Echinococcus multilocularis* infection in dogs entering their territory'.

In accordance with this Article, Malta falls under the category described in paragraph 2, i.e. it is in the position of demonstrating that the infection with *Echinococcus multilocularis* parasite has not been established because of the absence of wild red foxes in the whole of its territory. Article 4(1) provides details on the conditions to be fulfilled in order to remain eligible for preventive health measures. For Member States like Malta, in the absence of a definitive host, the conditions to be met are:

a) having a national observation programme in place to detect the presence of wild red foxes;
b) immediate notification to the Commission and the other Member States of the detection of the presence of wild red foxes during each 12-month observation period;
c) report to the Commission on the results of the national programme referred to in point (a) by 31 May following the end of each 12-month observation period. The evaluation of the observation programme and its results are out of the remit of the mandate received by EFSA and this related scientific report.

Also, in accordance with Article 2, Ireland, Finland and the United Kingdom (Northern Ireland) fall under the category described in paragraph 3, i.e. they are in the position to demonstrate that the occurrence of the infection with this parasite has not been recorded in wild definitive host animals. Article 4(2) provides details on the conditions to be fulfilled in order to remain eligible for preventive health measures.

In this report, EFSA assesses the pathogen-specific surveillance programmes implemented by the three concerned Member States and by Norway.

The surveillance programmes performed in 2021 by Finland, Ireland, United Kingdom (Northern Ireland) and Norway as reported in 2022 were assessed by checking the reports for completeness against relevant elements that need to be addressed when performing an *E. multilocularis* surveillance in the context of Commission Delegated Regulation (EU) 2018/772 and analysing the raw data collected by these countries. In order to facilitate the assessment, the information given by the different countries was divided into four different categories corresponding to the critical points that are addressed in the legislation in the 'requirements for the pathogen-specific surveillance programme provided for in point (c) of Article 4(2): (i) the type and sensitivity of the detection method, (ii) the selection of the target population, (iii) the sampling strategy and (iv) the methodology'.

The three Member States and Norway (i) used appropriate techniques for the detection of *E. multilocularis* in intestinal contents or faeces, (ii) performed a 12-month surveillance period of data collection and (iii) designed an appropriate sampling strategy for the detection of the parasite, if present in any part of the Member State, at the design prevalence of less than 1% (0.01), with a 95% confidence level.

All the countries selected adequate wild definitive hosts in order to perform the surveillance.

United Kingdom (Northern Ireland) does fulfil the requirements of Commission Delegated Regulation (EU) 2018/772 related to the desired confidence level of 95% only if assuming a test sensitivity of 0.99, provided by the national reference laboratory, i.e. a value higher than the one recommended by EFSA in 2015 (0.78).

None of the three Member States nor Norway recorded positive samples in the 12-month surveillance period.

[1] In accordance with the Agreement on the withdrawal of the United Kingdom of Great Britain and Northern Ireland from the European Union and the European Atomic Energy Community, and in particular Article 5(4) of the Protocol on Ireland/Northern Ireland in conjunction with Annex 2 to that Protocol, for the purposes of this Annex, references to Member States include the United Kingdom in respect of Northern Ireland.

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1. Introduction

Overall, at any time, more than 1 million people are affected by one of the four human echinococcosis diseases: alveolar (caused by *Echinococcus multilocularis*), cystic (caused by *Echinococcus granulosus sensu lato*), neotropical (caused by *Echinococcus vogeli*, *Echinococcus oligarthrus*). The WHO assists countries to develop and implement pilot projects leading to the validation of effective cystic echinococcosis control strategies.[2]

Human alveolar echinococcosis (AE), caused by the larval stage of the fox tapeworm *E. multilocularis*, is a serious parasitic zoonosis (Torgerson et al., 2010; EFSA AHAW Panel, 2015; EFSA and ECDC, 2017). Alveolar echinococcosis is confined to the northern hemisphere, in particular to regions of Asia (around 95% of the burden), Europe (< 5%) and North America (< 0.05%). Table 1 reports the number of cases and notification rates in the EU/EFTA by country and year. *E. multilocularis* is considered an emerging parasite in Europe. In fact, human AE has been recently detected in Hungary and Croatia; thus, differential diagnosis and therapy of AE is a new challenge in clinical practice in these countries (Dezsényi et al., 2019; Dušek et al., 2020).

Table 1: Reported human cases of cystic and alveolar echinococcosis and notification rates per 100,000 population in the EU/EFTA, by country and year, 2015–2019 (EFSA and ECDC, 2021)

Country	2019					2018		2017		2016		2015	
	National coverage[a]	Data format[a]	Total cases	Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates	
				Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
Austria	Y	C	36	36	0.41	46	0.52	50	0.57	26	0.30	8	0.09
Belgium	Y	A	20	20	0.17	14	0.12	12	0.11	17	0.15	9	0.08
Bulgaria	Y	A	193	193	2.76	206	2.92	218	3.07	269	3.76	313	4.35
Croatia	Y	C	4	3	0.07	4	0.10	15	0.36	9	0.21	7	0.17
Cyprus	Y	C	0	0	0.00	0	0.00	0	0.00	0	0.00	2	0.24
Czechia	Y	C	1	1	0.01	4	0.04	1	0.01	4	0.04	3	0.03
Denmark[b]	–	–	–	–	–	–	–	–	–	–	–	–	–
Estonia	Y	C	2	2	0.15	0	0.00	1	0.08	0	0.00	0	0.00
Finland[c]	Y	C	8	8	0.14	1	0.02	5	0.09	4	0.07	2	0.04
France	Y	C	45	45	0.07	62	0.09	53	0.08	38	0.06	48	0.07
Germany	Y	C	134	134	0.16	172	0.20	141	0.17	181	0.22	157	0.19
Greece	Y	C	7	7	0.07	11	0.10	15	0.14	18	0.17	13	0.12
Hungary	Y	C	10	10	0.10	9	0.09	14	0.14	5	0.05	2	0.02
Ireland[c]	Y	C	0	0	0.00	2	0.04	0	0.00	2	0.04	0	0.00
Italy[b]	–	–	–	–	–	–	–	–	–	–	–	–	–
Latvia	Y	C	6	6	0.31	10	0.52	6	0.31	11	0.56	10	0.50
Lithuania	Y	C	81	81	2.90	50	1.78	53	1.86	26	0.90	33	1.13
Luxembourg	Y	C	1	1	0.16	0	0.00	2	0.34	0	0.00	0	0.00
Malta[c]	Y	C	0	0	0.00	0	0.00	0	0.00	1	0.22	0	0.00
Netherlands	Y	A	48	48	0.28	42	0.24	38	0.22	33	0.19	64	0.00
Poland	Y	C	70	70	0.18	51	0.13	75	0.20	64	0.17	47	0.12
Portugal	Y	C	5	5	0.05	9	0.09	2	0.02	2	0.02	4	0.04
Romania	Y	C	1	1	0.01	4	0.02	14	0.07	13	0.07	18	0.09
Slovakia	Y	C	11	11	0.20	10	0.18	7	0.13	4	0.07	5	0.09
Slovenia	Y	C	6	6	0.29	6	0.29	7	0.34	3	0.15	7	0.34
Spain[d]	Y	C	34	34	–	68	0.15	83	0.18	87	0.19	83	0.18
Sweden	Y	C	26	26	0.25	29	0.29	34	0.34	27	0.27	26	0.27

[2] https://www.who.int/news-room/fact-sheets/detail/echinococcosis

Country	2019					2018		2017		2016		2015	
	National coverage[(a)]	Data format[(a)]	Total cases	Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates		Confirmed cases and rates	
				Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
United Kingdom[(c)]	Y	C	3	3	0.00	–	–	4	0.01	–	–	26	0.04
EU Total			**752**	**751**	**0.18**	**810**	**0.21**	**850**	**0.19**	**844**	**0.22**	**887**	**0.20**
Iceland	Y	C	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Norway	Y	C	7	7	0.13	7	0.13	5	0.10	5	0.10	3	0.06
Switzerland	–	–	–	–	–	–	–	–	–	–	–	–	–

–: Data no reported.
(a): Y: yes; N: no; A: aggregated data; C: case-based data.
(b): No surveillance system.
(c): Finland, Ireland, Malta, the United Kingdom and mainland Norway have been declared free of *E. multilocularis*.
(d): Data not complete for 2019, rate not calculated.

Affected humans show clinical signs that include fatigue, loss of weight, abdominal pain, general malaise and signs of hepatitis or hepatomegaly. In untreated patients, the disease can develop to a severe form associated with liver failure, splenomegaly, portal hypertension and acidosis which can be fatal: before the advent of medical benzimidazoles treatment, the fatality rate exceeded 90% of AE cases within 10–15 years from diagnosis (Wilson et al., 1992). Even treated patients can experience a reduction in their quality of life (Mihmanli et al., 2016; WHO, 2017). Indeed, AE is thought to be responsible for about 666,434 disability-adjusted life-years (DALYs) globally per year (Torgerson et al., 2010).

The transmission cycle of *E. multilocularis* occurs when the adult worm (sexual stage) of the cestode residing in the small intestine of the definitive hosts (canids) release viable eggs into the environment via faeces (Peregrine et al., 2012; EFSA AHAW Panel, 2015). The infective eggs are ingested by an intermediate host (rodents) and the oncosphere migrates inside them until reaching target organs such as the liver (CDC, online; Peregrine et al., 2012). In the liver, the oncosphere develops into larval vesicles (metacestode asexual stage) which resembles a malignancy in appearance and behaviour, because it proliferates indefinitely by exogenous budding and invades the surrounding tissues. In rodents, parasitic vesicles contain numerous protoscoleces (infective stages), while in humans, protoscoleces are rarely observed (Moro and Schantz, 2009). The cycle continues when the definitive host consumes an infected intermediate host (Torgerson et al., 2010). Humans may be infected through the ingestion of viable eggs of the parasite by close contact with the definitive host, hand-to-mouth transmission or ingestion of food or water (Torgerson et al., 2010). There is an increasing concern on hand-to-mouth transmission of *Echinococcus* spp. eggs (Tamarozzi et al., 2020).

Few species (fox-Arvicolinae) maintain the cycle in Europe. Several species can be infected by *E. multilocularis* in nature. A scientific opinion on *E. multilocularis* performed by EFSA in 2015, revised the potential hosts (definitive and intermediate) of the parasite for this continent (Table 1; See EFSA AHAW Panel, 2015 for more detailed information).

Table 2: Potential definitive and intermediate hosts of *E. multilocularis* in Europe (EFSA AHAW Panel, 2015)

Definitive hosts	
Red fox (*Vulpes vulpes*)	Considered the main DH
Arctic fox (*Vulpes lagopus*)	In Europe, only relevant in Svalbard (Norway)
Raccoon dog (*Nyctereutes procyonoides*), Wolf (*Canis lupus*), Golden jackal (*Canis aureus*)	In the presence of the red fox, they can act as DHs. There is no evidence supporting their ability to maintain the lifecycle in the absence of the red fox
Domestic dog and wild cat (*Felis s. silvestris*)	Overall, prevalence of dogs with the parasite is low. However, in experimental surveys, they become infected easily. On the contrary, cats hardly get infected experimentally, but their natural infection has been reported in numerous occasions. For both species, further information is needed.

Intermediate hosts	
Common vole (*Microtus arvalis*), field vole (*Microtus agrestis*), common pine vole (*Microtus subterraneus*), sibling vole (*Microtus levis*), bank voles (*Myodes* spp.), water voles (*Arvicola* spp.), snow vole (*Chionomys nivalis*), lemming (*Lemmus lemmus*)	Various species of voles are confirmed as suitable hosts. However, factors such as their population densities and predation rates may influence in their role in the cycle.
Muridae (*Apodemus* spp., *Mus* spp., *Rattus* spp.), brown hare (*Lepus europaeus*), shrew (*Sorex* sp.)	Although some murid rodents, hares and shrews are susceptible, natural infections occur only sporadically
Muskrat (*Ondatra zibethicus*), beaver (*Castor* spp.), nutria (*Myocastor coypu*), Alpine marmot (*Marmota marmota*)	Large rodents are susceptible hosts. Their role seems to be related to the dispersion of the parasite; e.g. through translocations (beaver)
Suids, horses and domestic dogs	Only accidental or refractory intermediate hosts

The distribution of the parasite seems to expand over time. The uncertainty is linked to the fact that no baseline study has ever been performed at European level. The data relate to scientific literature. Until the 1980s, only four countries (France, Germany, Switzerland and Austria) were known to be endemic for the disease (Eckert and Deplazes, 1999). Since then, EM infections in animals have been increasingly reported in countries previously thought to be free (Davidson et al., 2012).

In total, 23 MS and two non-MS provided 2019 monitoring data on *Echinococcus* in animals. Thirteen MS and two non-MS reported data on, respectively, 6,326 and 621 foxes that were examined for *E. multilocularis*. Seven MS and one non-MS reported positive findings with an overall proportion of test positives of 12.9%. In 2019, 751 confirmed human echinococcosis cases were reported in the EU. The EU notification rate was 0.18 cases per 100,000 population, the lowest in the last 5 years (EFSA and ECDC, 2021). See Table 2 for more details. Furthermore, recent studies suggest that other species may play an important role in the epidemiology of the disease. For example, *E. multilocularis* infections are present in golden jackal populations in the south-western part of Hungary, with a prevalence of 15.6% and mean intensity of 664 worms (Balog et al., 2021).

Table 3: Summary of echinococcosis in humans, of *Echinococcus multilocularis* and of *Echinococcus granulosus sensu lato* in most important definitive and intermediate animal hosts in the EU, 2015–2019 (EFSA and ECDC, 2021)

	2019	***2018***	***2017***	***2016***	***2015***	***Data source***
Humans						
Total number of confirmed cases	*751*	*810*	*850*	*844*	*887*	*ECDC*
Total number of confirmed cases/100,000 population (notification rates)	*0.18*	*0.21*	*0.19*	*0.22*	*0.20*	*ECDC*
Number of reporting MS	*26*	*25*	*26*	*25*	*26*	*ECDC*
Infection acquired in the EU	*173*	*149*	*169*	*122*	*172*	*ECDC*
Infection acquired outside the EU	*89*	*89*	*77*	*112*	*84*	*ECDC*
Unknown travel status or unknown country of infection	*489*	*572*	*604*	*610*	*631*	*ECDC*
Animals						
Echinococcus multilocularis in red foxes						
Number of animals tested	*6,326*	*6,566*	*7,148*	*4,561*	*5,371*	*EFSA*
% positive animals	*13.6*	*17.6*	*16.9*	*19.4*	*9.0*	*EFSA*
Number of reporting MS	*13*	*13*	*11*	*12*	*10*	*EFSA*
Echinococcus granulosus sensu lato in dogs						
Number of animals tested	*2,113*	*2,605*	*2,538*	*2,183*	*3,416*	*EFSA*
% positive animals	*0.2*	*0.1*	*0*	*0.4*	*0.2*	*EFSA*
Number of reporting MS	*6*	*6*	*7*	*5*	*7*	*EFSA*

	2019	2018	2017	2016	2015	Data source
Echinococcus granulosus sensu lato in cattle						
Number of animals tested	*10,956,692*	*9,920,338*	*9,834,374*	*7,746,553*	*6,539,857*	*EFSA*
% positive animals	*0.1*	*0.2*	*0.2*	*0.2*	*0.1*	*EFSA*
Number of reporting MS	*16*	*17*	*15*	*19*	*17*	*EFSA*
Echinococcus granulosus sensu lato in sheep and goats						
Number of animals tested	*36,891,061*	*38,870,644*	*38,278,897*	*12,159,745*	*7,067,952*	*EFSA*
% positive animals	*0.03*	*0.2*	*0.4*	*0.9*	*1.0*	*EFSA*
Number of reporting MS	*15*	*15*	*14*	*13*	*13*	*EFSA*

The prevalence of the parasite is not homogeneous and may vary depending on multiple elements such as e.g. microclimatic conditions, geographical location, host population dynamics and amount of intermediate hosts (Casulli et al., 2015; EFSA AHAW Panel, 2015). A systematic review of the geographical distribution of *E. multilocularis* in definitive and intermediate hosts in the European Union and adjacent countries found differences between countries (Oksanen et al., 2016; Table 3). The prevalence has been reported to range from 0 to more than 50% (EFSA AHAW Panel, 2015) (Table 4).

Table 4: Table based on Oksanen's suggested prevalence classes (Oksanen et al., 2016) of countries in which *E. multilocularis* has been reported in foxes (see also EFSA AHAW Panel, 2015; ECDC, 2016; Lalošević et al., 2016)

Countries	Prevalence in foxes
Finland, Ireland, Malta, United Kingdom, Norway[(a)]	0
Denmark, Slovenia and Sweden	≤ 1%
Austria, Belarus, Belgium, Croatia, Hungary, Italy, the Netherlands, Romania and the Ukraine	> 1% to < 10%
Czech Republic, Estonia, France, Germany, Latvia, Lithuania, Luxembourg, Poland, Serbia, Slovakia, Liechtenstein and Switzerland	> 10%

(a): excluding Svalbard.

The European Union adopted Commission Delegated Regulation (EU) 2018/772 supplementing Regulation (EU) No 576/2013 of the European Parliament and of the Council with regard to preventive health measures for the control of *E. multilocularis* infection in dogs, and repealing Delegated Regulation (EU) No 1152/2011. Article 2 lays down the pathways for a Member State to become eligible for the implementation of preventive health measures for the prevention of introduction of *E. multilocularis* through dogs in Member states, or parts thereof. The concerned Member State may (i) demonstrate that the infection with the *E. multilocularis* parasite has not been established because of the absence of wild red foxes in the whole of its territory; (ii) demonstrate that wild definitive host animals likely to harbour the *E. multilocularis* parasite are present in the whole or parts of its territory and that occurrence of the infection with this parasite has not been recorded in those animals during the ongoing surveillance activities; or (iii) is implementing a compulsory eradication programme.

On the one hand, this Regulation gives to those Member States (or parts thereof) the right to apply preventive health measures (see Article 6) to dogs intended for non-commercial movements prior to their introduction. It should be noted that the same preventive health measures are to be implemented for the import and commercial trade of dogs. On the other hand, this Regulation entails certain obligations for those Member States if they wish to remain eligible for preventive health measures (see Art.4), including the implementation of pathogen-specific surveillance programmes, in accordance with Annex I, to provide evidence for the absence of *E. multilocularis* infection. The requirements for the pathogen-specific surveillance programme are reported and summarised below:

1) The pathogen-specific surveillance programme, using appropriate risk-based or representative sampling, shall be designed to detect, per epidemiologically relevant geographical unit in the Member State or part thereof, the *Echinococcus multilocularis* parasite in the wild definitive host population, if present in any part of the Member State at a prevalence of not more than 1% at confidence level of at least 95%;

2) The pathogen-specific surveillance programme shall describe the target wild definitive host population, including density, age structure, geographical and gender distribution, taking into account the relative risk of infection with the *E. multilocularis* parasite in different species and subpopulation of the target wild definitive host population;
3) The pathogen-specific surveillance programme shall consist in the ongoing collection, during the 12-month surveillance period, of samples from wild definitive hosts, to be analysed using:
    a) the sedimentation and counting technique (SCT), or a technique of equivalent sensitivity and specificity, by examination of intestinal contents for the detection of the *Echinococcus multilocularis* parasite; or
    b) polymerase chain reaction (PCR) methods, or a technique of equivalent sensitivity and specificity, by examination of intestinal contents or faeces for the detection of species-specific deoxyribonucleic acid (DNA) from tissue or eggs of the *Echinococcus multilocularis* parasite.

The outcomes of the pathogen-specific surveillance programme of each Member State and of Norway need to be annually submitted to the Commission by the 31 of May.

At the moment, only four Member States (Finland, Ireland, Malta and the United Kingdom (Northern Ireland)) are listed in the Annex to Commission Implementing Regulation (EU) 2018/878 (as amended by the Commission Implementing Regulation (EU) 2020/2017 of 9 December 2020) as complying with the rules for categorisation laid down either in Article 2(2) or (3) of Commission Delegated Regulation (EU) 2018/772. The Decision of the EEA Joint Committee No 183/2019 of 10 July 2019 also added the whole territory of Norway to the list of countries mentioned in the Annex to Commission Delegated Regulation (EU) 2018/878 (as amended by the Commission Implementing Regulation (EU) 2020/2017 of 9 December 2020) as complying with the rules for categorisation laid down in Article 2(3) of Commission Delegated Regulation (EU) 2018/772.

This report follows previous annual reports (EFSA, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2021) presented by EFSA to the European Commission and aims to analyse and assess the sampling strategy, data collection and detection methods used by these five countries in the context of Commission Delegated Regulation (EU) 2018/772 in their respective *E. multilocularis* (pathogen-specific) surveillance programmes, and verify that the requirements laid down in this regulation are being complied with.

Based on the 'rules for categorisation of Member States in view of their eligibility for preventive health measures' (Art.2), Malta falls under the category described in paragraph 2 of the same article, i.e. it is in the position of demonstrating that the infection with *E. multilocularis* parasite has not been established because of the absence of wild red foxes in the whole of its territory. Article 4 provides details on the conditions to be fulfilled in order to remain eligible for preventive health measures. For Member States like Malta, in the absence of definitive host, the conditions to be met are: (a) having a national observation programme in place to detect the presence of wild red foxes; (b) immediate notification to the Commission and the other Member States of the detection of the presence of wild red foxes during each 12-month observation period; (c) report to the Commission on the results of the national programme referred to in point (a) by 31 May following the end of each 12-month observation period. The evaluation of the observation programme and its results is out of the remit of this assessment.

1.1. Background and terms of reference as provided by the European Commission and the EFTA surveillance authority

The Commission adopted Commission Regulation (EU) No 1152/2011 of 14 July 2011, as regards preventive health measures for the control of *Echinococcus multilocularis* infection in dogs. This was in order to ensure continuous protection of Finland, Ireland, Malta and the United Kingdom that claim to have remained free of the parasite *E. multilocularis* as a result of applying national rules until 31 December 2011. The Decision of the EEA Joint Committee No 103/2012 of 15 June 2012 added the whole territory of Norway[2] to the list of countries complying with the conditions of Article 3 of the Regulation. For the purposes of Norway's obligations under the EEA Agreement, including those under Regulation (EU) No 1152/2011, the territory of Norway does not include Svalbard, cf. Protocol 40 to the EEA Agreement.

This Regulation includes certain obligations for these Member States and Norway in order to implement a pathogen-specific surveillance programme aimed at detecting the parasite, if present in any part of those Member States, in accordance with certain requirements regarding the sampling, the detection techniques and the reporting.

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EFSA is asked, in the context of Article 31 of Regulation (EC) No 178/2002, to provide the following scientific and technical assistance to the Commission:

1) Regular follow-up of the literature regarding *E. multilocularis* infection in animals in the European Union and adjacent countries, including its geographical distribution and prevalence;
2) Analysis and critical assessment, in the context of Regulation (EU) No 1152/2011, of (i) the sampling strategy considered for the programmes of the countries concerned; (ii) the data collected in the framework of these programmes; (iii) the detection methods used.

1.2. Interpretation of the terms of reference

This report addresses ToR 2 of the mandates M-2012-0200 and M-2014-0287 submitted to EFSA by the European Commission and the EFTA Surveillance Authority, respectively, and applies the principles and procedures established in the EFSA reports 'Scientific and technical assistance on *E. multilocularis* infection in animals' (EFSA, 2012a) and 'A framework to substantiate absence of disease: the risk-based estimate of system sensitivity tool (RiBESS) using data collated according to the EFSA Standard Sample Description - An example on *Echinococcus multilocularis'* (EFSA, 2012b).

Commission Delegated Regulation (EU) 2018/772, repealing Regulation (EU) No 1152/2011, gives a description of the requirements for the surveillance programme (Annex I). The methodology adopted by EFSA for the previous assessments does not require changes to fit the new requirements which remain the same in their substantial traits.

1.3. Additional information (if appropriate)

Following an update of the relevant regulation, Malta has been exempted by the obligation of running a surveillance exercise on the domestic dog population. For this reason, in this report, the data of Malta are not presented.

In accordance with Article 2 of Regulation EU 2018/878 of 18 June 2018, as amended by the Commission Implementing Regulation 2020/2017 of 9 December 2020, United Kingdom in respect of Northern Ireland is referenced as 'Member State'. In this report, only data from Northern Ireland are presented and assessed.

2. Data and methodologies

To address ToR 2, EFSA developed a scientific and a technical report in 2012 (EFSA, 2012a,b). The principles and procedures that were established there have been applied in the assessment of each of the subsequent annual national surveillance reports submitted to the Commission, including this report.

As a **first step**, the quality of the 2019 surveillance reports of the three Member States and Norway was assessed by checking the description of the surveillance system for completeness against the relevant elements that need to be addressed in the context of Commission Delegated Regulation (EU) 2018/772.

In order to facilitate the assessment, we divided the information into four different categories (see Table 3) corresponding to the critical points of the three paragraphs addressed in the legislation in the 'requirements for the pathogen-specific surveillance programme' (Annex I).

Table 5: Assessment categories and their equivalence in the Commission Delegated Regulation (EU) 2018/772 (Annex I)

Information category	Main points considered in the assessment	Delegated Regulation (EU) 2018/772
1	**The type and sensitivity** of the detection method was evaluated to ensure the fulfilment of the technical legal requirements regarding appropriate techniques for the detection of *E. multilocularis* in intestinal contents (sedimentation and counting technique (SCT) or a technique of equivalent sensitivity and specificity) or intestinal contents/faeces (detection of species-specific DNA from tissue or eggs of the *E. multilocularis* parasite by polymerase chain reaction (PCR), or a technique of equivalent sensitivity and specificity).	Annex I – Point 3
2	The selection of the target population was evaluated to ensure the fulfilment of the technical legal requirements regarding the collection of samples from wild definitive hosts or domestic definitive hosts in the absence of the first.	Annex I – Point 2
3	**The sampling strategy** was evaluated to ensure the fulfilment of the technical legal requirements regarding appropriate sampling for detection of the *E. multilocularis* parasite, if present in any part of the Member State, at the design prevalence of less than 1% (0.01).	Annex I – Point 1
	The sampling strategy was also evaluated to ensure the fulfilment of the technical legal requirements regarding the 12-month surveillance period of data collection.	Annex I – Point 3
4	**The methodology** was evaluated to ensure the fulfilment of the technical legal requirements regarding a confidence level of at least 0.95 against a design prevalence of 1% (0.01).	Annex I – Point 1, 2, 3

For each of the four evaluation parts, the most relevant elements were extracted from the reports submitted by the MS and checked against the criteria described below (Table 6).

Table 6: Relevant elements checked for compliance of the technical requirements of Annex I of Commission Delegated Regulation (EU) 2018/772

Points addressed in the Annex II	Element	Description of element
Type and sensitivity of the detection method	**Type of test**	The diagnostic test used for the detection of EM must be defined. Modifications of the original method should be indicated.
	Test sensitivity	The sensitivity and specificity of the test used in the surveillance system must be reported. This would ideally be estimates from each participating laboratory reported as a point estimate (average) of the values across the country with minimum and maximum values or a probability distribution. Alternatively, a value of 0.78, as recommended by EFSA, 2015, shall be used.
Selection of the target population	**Definition of susceptible host population targeted by the system**	The susceptible wild definitive host population(s) (red foxes, raccoon dogs) targeted by the surveillance system should be described and the choice justified. If domestic host species (dogs or cats) are sampled, evidence for the absence of wild definitive hosts and for these domestic animals having had access to outdoors should be provided.
	Size of susceptible host population targeted by the system	The size of the targeted (wildlife) population should be reported, together with the evidence for this. Historical population data should be updated since these may not reflect current populations.

Points addressed in the Annex II	Element	Description of element
Sampling strategy	**Epidemiological unit**	It should be clearly defined if individual animals or individual faecal samples collected from the environment constitute the epidemiological unit. If individual faecal samples are collected from the environment, the method applied to establish the species from which the faeces originated has to be reported.
	Sample size calculation	The applied survey design should be fully documented, including considerations regarding potential biases inherent in the survey design. The method and the formula used to calculate the sample size should be fully documented.
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be representative.
Methodology	**Design Prevalence (DP)**	DP is specified in Annex I to Regulation (EU) No 2018/772 and must be 1% (0.01) or lower.
	Geographic epidemiological unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification.
	Methodology for calculation of area sensitivity	For the calculation of the area sensitivity, the diagnostic sensitivity should be set conservatively to the lowest value, excluding the lowest 20th percentile, from the ones reported in the scientific literature and related to the diagnostic tests implemented by the countries listed in Annex I of the Commission Delegated Regulation (EU) No 2018/772. In this case, is 78% (EFSA AHAW Panel, 2015).

A summary of the assessment of the relative elements of the different countries is given at the end of the document (see Annex A–D). As a **second step**, the raw data on individual samples submitted by the five countries via the EFSA Data Collection Framework (DCF) were analysed. For the purpose, the software R was used to compute descriptive statistics. Table 5 lists and describes all the parameters that were extracted from the data submitted (Table 7).

Table 7: List of the parameters extracted from the raw data submitted by the Member States via the Data Collection Framework

	Parameter	Description
1	Theoretical Sampling period	The 12-month reporting period. It may go from January to December, but this is not a restriction: The reporting period can also include 12 contiguous months over 2 years.
2	Actual Sampling Period	Range. Date of the first sampling date and date of the last sampling within the theoretical sampling period
3	Summary dates	Descriptive statistics of the sampling period
4	Sampling period	Total number of days sampled within the actual sampling period
5	Number of samples	Total number of samples collected during the theoretical sampling period
6	Number of test results	Total number of test results. If the number of test results is equal to the number of samples, none of the latter required further investigations (i.e. were negative at the first test).

	Parameter	Description
7	Laboratory test completion	Comparison between the year when the samples are collected and the year when the test was completed
8	Sensitivity	Sensitivity of the diagnostic test
9	Host	Target population size (N); additional information on the host species
10	Animal sample	Type of sample collected
11	Sampling Strategy and Design	As reported (e.g. representative sample, risk-based)
12	Sampling point	Activity adopted for the sample collection (e.g. hunting, veterinary activity, . . .)

3. Information as submitted in the report by the involved countries

3.1. Diagnostic test

3.1.1. Finland

The Finnish Food Authority, which was formed in 2019 when the former Finnish Food Safety Authority Evira merged with two other governmental organisations, used a PCR method (PCR 12 S rRNA) for the detection of *E. multilocularis* eggs in rectal content. The PCR method was described by Isaksson et al. (2014), with a modification in the magnetic beads washing step (manual instead of automatic).

To estimate the actual sensitivity of the test developed by Isaksson et al. (2014), internal validations were performed yearly in Evira/Finnish Food Authority from 2014 to 2020. In 2021, 47 positive control samples (10–15 Em eggs/3 mL rectal content) were examined with sample batches and 45 of them (95.7%) were found positive by the real-time PCR test. In this validation procedure, positive (spiked) samples were tested blindly. As positive control in DNA isolation, own spiked specimens have been used: 10 inactivated (−80°C) *E. multilocularis* eggs/3 mL of intestinal content. Negative control is water sample in PCR. In routine analyses, a positive control was always analysed parallel to actual samples. If a positive control was found negative, the analysis of the whole batch of samples was repeated.

The latest (and so far, the only) proficiency test on detection of *E. multilocularis* in faeces (PCR) was conducted in May 2015. The results of the Finnish Food Authority were correct. The report of the results was provided to the European Commission.

3.1.2. Ireland

Rectal contents from foxes were examined according to the method of Trachsel et al. (2007) referred to as PCR Cest1-Cest2 NAD1. The DNA nucleotide sequences of primers were Cest1 = TGCTGATTTGTTAAAGTTAGTGATC and Cest2 = CATAAATCAATGGAAACAACAACAAG. The positive control that was used was an extract of DNA from adult *E. multilocularis* worms which was supplied by the EU Reference Laboratory for Parasites (EURPL). The negative control used was sterile saline solution. The estimation of the test sensitivity (of 0.78) was based on the most recent advice arising from scientific opinion by EFSA (EFSA AHAW Panel, 2015). In addition, the Irish National Reference Laboratory for Parasites is amenable to participating in any study in order to re-evaluate the test sensitivity estimate, provided a sufficient number of *E. multilocularis* positive samples are supplied by the EURLP or a similar laboratory.

The Irish National Reference Laboratory for Parasites successfully passed both *Echinococcus*-related proficiency tests that it participated in this year. These proficiency tests were organised by the European Union Reference Laboratory for Parasites (ISS, Rome) and were titled as follows; 'Detection of *Echinococcus* spp. worms in the intestinal mucosa of the definitive host' and 'Molecular identification of *Echinococcus* at the species level'.

3.1.3. United Kingdom (Northern Ireland)

In Northern Ireland (NI), a Sedimentation and Counting Technique (SCT) test was used to detect E. multilocularis from individual intestinal content (Eckert, 2003). The analyses were performed at the Agri-Food and Biosciences Institute (AFBI) which is the national reference laboratory for the Department of Agriculture, Environment and Rural Affairs (DAERA).

The counting method sensitivity varies between laboratories. Eckert's suggestion to consider an Se of 99% was used (Eckert, 2003).

In Northern Ireland, AFBI participated in the last proficiency testing in 2021.

3.1.4. Norway

In the Norwegian *E. multilocularis* surveillance system, a DNA-fishing technique was used (Isaksson et al., 2014), referred to as PCR 12 S rRNA, which involves magnetic capture mtDNA extraction from samples applying specific DNA hybridisation (Isaksson et al., 2014), followed by real-time PCR (CO1rtPCR) (Øines et al., 2014). Samples are also analysed in duplicates in the detection step to increase sensitivity, and to reduce the risk of errors introduced by the operator. Results from samples with very low target DNA have shown some false negative which are minimised by running detection in duplicates (Øines et al., 2014).Primers were 'EMrtCO1F' (5'-TGGTATAAAGGTGTTTACTTGG-3'),'EMrtCO1Rew' (5'-ACGTAAACAACACTATAAAAGA-3') and 'Zen probe' (5'-56-FAM/TCTAGTGTA/Zen/AATAAGAGTGATCCTATTTTGTGGTGGGT/3IABkFq/−3'). Following a positive signal, samples are verified by PCR/sequencing confirmation of NAD1 (Trachsel et al., 2007) and an independent real-time PCR (Taq PCR/12 S rDNA real-time by Isaksson et al., 2014) (Table 8 on results of spiked samples).

Table 8: Table extracted from Øines et al. (2014) on the results of the molecular analysis of the spiked fox faeces batches using four detection methods

DNA extraction method	PCR method	Whole data set						Samples with ≤ 15 eggs					
		N	Se	Sp	PPV	NPV	AUC [95% CI]	N	Se	Sp	PPV	NPV	AUC [95% CI]
Egg sieving	Taq PCR	72	0.47	0.92	0.97	0.26	0.66 [0.57–0.79]	57	0.1	0.92	0.75	0.29	0.50 [0.40–0.60]
	EVA PCR		0.32	0.92	0.98	0.37	0.61 [0.51–0.71]		0.03	0.92	0.50	0.28	0.47 [0.38–0.56]
	mPCR		0.3	0.92	0.95	0.21	0.61 [0.51–0.70]		0.07	0.92	0.67	0.28	0.49 [0.39–0.58]
	CO1rtPCR		0.43	1	1	0.26	0.71 [0.65–0.77]		0.03	1	1	0.29	0.51 [0.48–0.54]
DNA Fishing	Taq PCR	72	0.65	1	1	0.37	0.85 [0.76–0.88]	61	0.40	1	1	0.40	0.70 [0.61–0.78]
	EVA PCR		0.77	1	1	0.46	0.88 [0.82–0.93]		0.60	1	1	0.50	0.80 [0.71–0.88]
	mPCR		0.02	1	1	0.17	0.50 [0.49–0.52]		0.0	1	0.0	0.29	0.5 [0.5–0.5]
	CO1rtPCR*		0.63	1	1	0.35	0.81 [0.75–0.87]		0.3	1	1	0.37	0.65 [0.56–0.73]

Prior to analysis of the surveillance samples, the new reagents are tested each year by spiking faeces or water with known numbers of *E. multilocularis* eggs or worms. The results are listed in the table below. The sensitivity is positively correlated with the amount of DNA in the samples. In samples with ≥ 10 eggs, the sensitivity is 0.85. For samples with ≥ 5 eggs, the sensitivity is 0.75 (see Table 9).

Table 9: Table reporting the results from testing spiked samples (2015–2021 data)

Results of spiked samples 2015–2021															
Year	**1 egg**			**5 eggs**			**10 eggs**			**50 eggs**			**One whole worm**		
	Nr. Test	**Nr. Pos**	**Se***	**Nr. Test**	**Nr. Pos**	**Se***	**Nr. Test**	**Nr. Pos**	**Se***	**Nr. Test**	**Nr. Pos**	**Se***	**Nr. Test**	**Nr. Pos**	**Se***
2015	4	2	0.50				4	4	1.00	2	2	1.00			
2016	10	10	1.00				10	10	1.00				2	2	1.00
2017	8	2	0.25				8	6	0.75				8	6	0.75
2018	2	0	0.00				2	2	1.00				10	10	1.00
2019	6	1	0.17	6	4	0.67	4	3	0.75				7	7	1.00
2020	8	1	0.13	6	3	0.50	8	5	0.63				8	6	0.75
2021	16	14	0.88	16	14	0.88	16	14	0.88				16	16	1.00
overall	**54**	**30**	**0.56**	**28**	**21**	**0.75**	**52**	**44**	**0.85**	**2**	**2**	**1.00**	**51**	**47**	**0.92**

*: sensitivity.

Specificity: Negative controls (MQ water) were included for all reactions. None were positive by RT-PCR. The results of the *Echinococcus* spp. PT from EURLP 2021 both on identification of *Echinococcus* worms in the intestinal mucosa of the definitive host and identification to species level were positive.

3.2. Target population (size & distribution & age structure)

3.2.1. Finland

For the whole country of Finland, the entire wild small canid population(s) of the country was defined as the geographical epidemiological unit (even though the population is a continuum of the north-western taiga population). The epidemiological and sampling unit was defined as the individual animal (red fox or raccoon dog). The targeted host species were the raccoon dog (*Nyctereutes procyonoides*) and red fox (*Vulpes vulpes*). The justifications reported for choosing these target species were the facts that the red fox is the primary host of *E. multilocularis* in Europe (Deplazes, 2006), and that raccoon dogs have been shown to be good definitive hosts for *E. multilocularis* (Kapel et al., 2006).

Population size estimates are based on hunting bag statistics provided by the Natural Resources Institute Finland (available online: http://statdb.luke.fi/PXWeb/pxweb/en/). Kauhala (2007) estimated that annual hunting bag is ca. 50% of the autumn population of the raccoon dog and ca. 40% of the autumn population of the red fox. The average annual hunting bag in the 5-year period 2016–2020 (latest available data) was 169,820 raccoon dogs and 46,840 red foxes. Therefore, FI estimated the population sizes of the raccoon dog and the red fox to be $2 \times 169{,}820 = 339{,}640$ individuals and $2.5 \times 46{,}840 = 117{,}100$ individuals, respectively. The estimated size of the susceptible population is therefore 456,740 (Figures 1 and 2).

The population densities for both species are highest in the southern part of the country. See maps in Figure 3. These maps are from year 2007, but the relative densities most probably still apply: Population densities of the raccoon dog are highest in the southern part, especially in the south-eastern part, of the country and decrease towards the north.

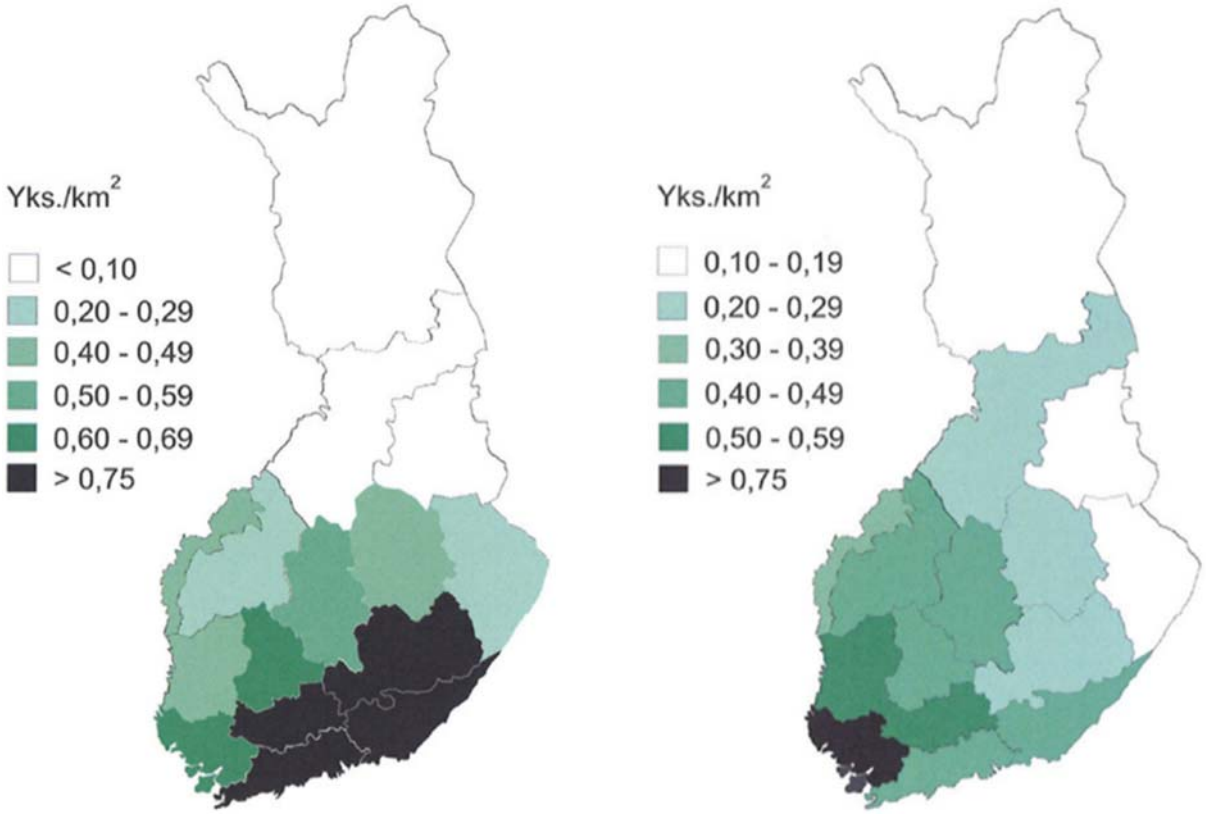

Figure 1: Finland–Raccoon dog densities (left) and red fox densities (right) according to Kauhala (2007) (Yks./km^2 = individuals/km^2)

Most of the hunting bag of the raccoon dog has come from southern part of Finland in 2016–2020. In 2019–2020, the fox bag has decreased markedly in the northernmost Lapland. In other parts of the country, the fox bag has fluctuated. According to annual snow track counts (systematic method for the monitoring of small game populations) by LUKE,[3] the Finnish fox population has decreased over 50% during the past three decades. The red fox population density is higher in the south-western part of the country. For monitoring of the raccoon dog population, snow track counting is not a feasible method because the species hibernates in winter.

No information on age or gender structure of the target population was available.

3.2.2. Ireland

The epidemiological unit used was the same geographical area as that of the EU member state Ireland. The rationale for selecting this area as the epidemiological unit was in order to comply with the conditions of the Regulation 2018/772 for Member States listed in Annex 1.

The animal level epidemiological unit was the individual animal (i.e. the red fox).

In accordance with the requirements for pathogen-specific surveillance for *E. multilocularis* outlined in Commission Delegated Regulation (EU) 2018/772, the most suitable host species to survey is a wildlife definitive host species. In Ireland, because of the occurrence of red foxes throughout the country and no known occurrence of raccoon dogs (Hayden and Harrington, 2000; Marnell et al., 2009), the former was selected as the wildlife definitive host species to survey for the presence of *E. multilocularis*. The red fox population has been estimated to be between 150,000 and 200,000.[4]

The red fox is a seasonal breeder, whereby cubs are born in the spring and are almost fully grown by 7 months of age (Hayden and Harrington, 2000). Therefore, the age structure of the population between young and adult foxes varies depending on the time of year. There is little published scientific evidence of the gender structure of the Irish red fox population.

[3] https://www.riistakolmiot.fi/raportit/kettu-2021/
[4] https://www.npws.ie/sites/default/files/publications/pdf/RL3.pdf (see p.35).

The red fox is distributed throughout Ireland (Hayden and Harrington, 2000; Marnell et al., 2009). Further information about the distribution of the red fox population within Ireland has been produced in a report by Dr. Tomás Murray from the National Biodiversity Data Centre in 2015. See also Figure 4.

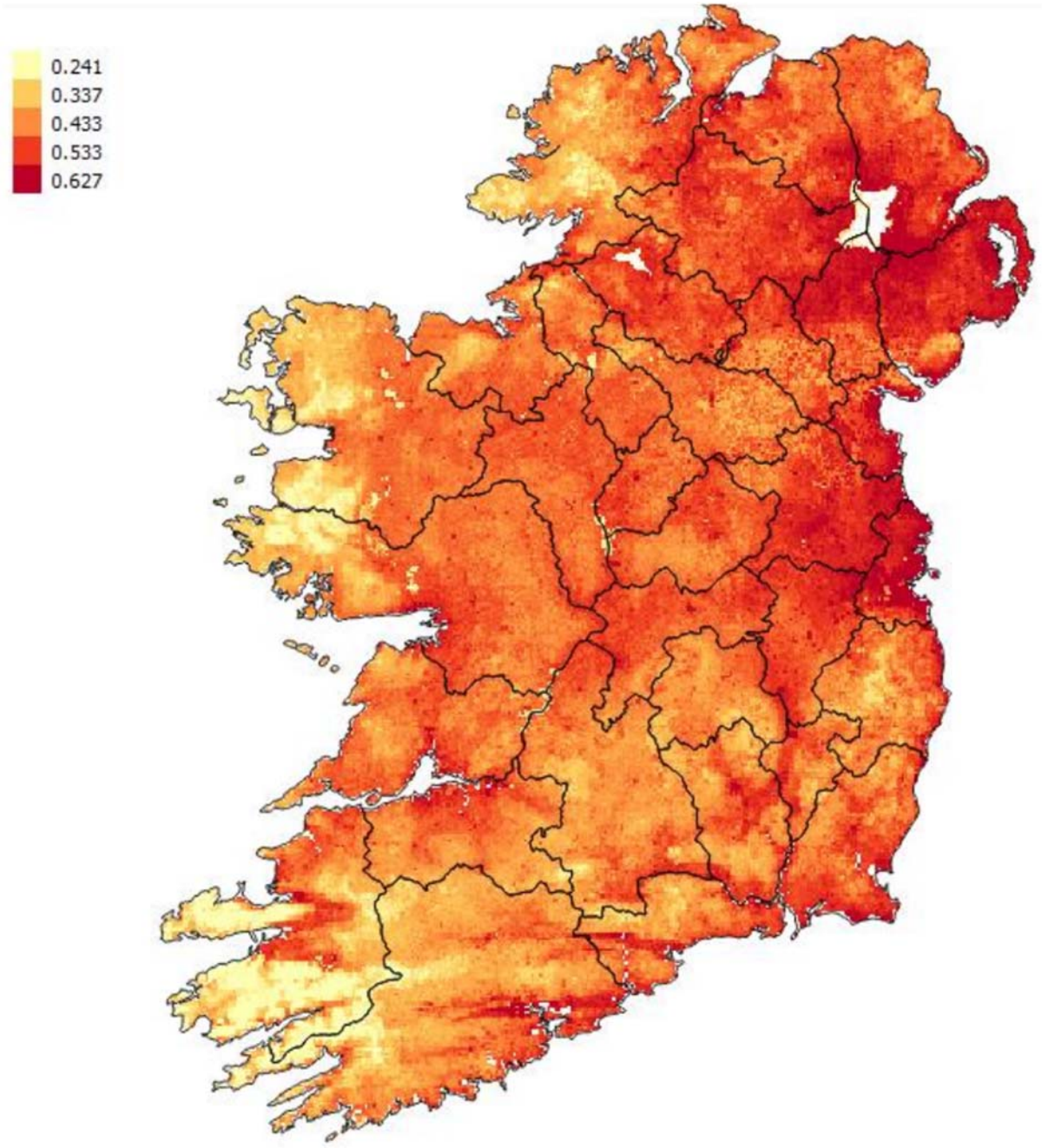

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Figure 2: Probability of presence per 1 km^2 from the final Maxent species distribution model on the island of Ireland. (Phillips et al., 2006) for red fox. Source: data up to 2015 provided by Dr. Tomás Murray, from National Biodiversity Data Centre (Ireland)

3.2.3. United Kingdom (Northern Ireland)

The red fox is the only wild definitive host for *E. multilocularis* in Northern Ireland. No other wild definitive host is present. Northern Ireland is part of an island with no access for other wild carnivores from other parts of Europe.

For Northern Ireland, the fox population size (adults) has been estimated at 14,000 by wildlife experts (Declan O'Mahony (AFBI); pers. comm.) which is equivalent of 1 fox per km^2 and accounts for the large area of rural land in contrast the urban land use (Conserve Ireland, 2009). This probability of presence per 1 km^2 originates from the final Maxent species distribution model (Phillips et al., 2006) for red fox. The input data go up to 2015 and were provided by Dr. Tomás Murray, from National Biodiversity Data Centre (Ireland). The population does fluctuate from year to year, but is believed to be relatively stable, if marginally increasing.

The rapid spread of sarcoptic mange in the red fox population and the population genetic structure according to microsatellite analysis (Atterby et al., 2015) demonstrates that there is considerable mixing of the red fox population within GB and within the island of Ireland, despite the variation in abundance. More in detail, there is a single land border with another EU Member State, which is the Republic of Ireland. This border is porous for wildlife; however, Ireland also has official disease-free status for *E. multilocularis*. The fox is found throughout Ireland, although the density of fox populations is highly variable. They are most abundant in areas that offer a wide variety of food and cover. In contrast, areas of uniform land, such as moorland or open plains, generally carry much lower densities. At high population densities, foxes generally have small home ranges and disperse over short distances. Some foxes become resident in an area and form stable home ranges, whilst others are nomadic and appear to wander from one place to another. Two crucial factors determining the size of a fox territory are the availability of food and the cost of defending the territory.

Regarding the structure of the population, some considerations can be done: breeding season begins in January and the red fox may have up to five cubs in a litter. The cubs stay with the mother for ~ 7 months. Max age is 10–11 years but 3 years is the average. Survival rate depends on availability of food and mortality due to road traffic accidents.

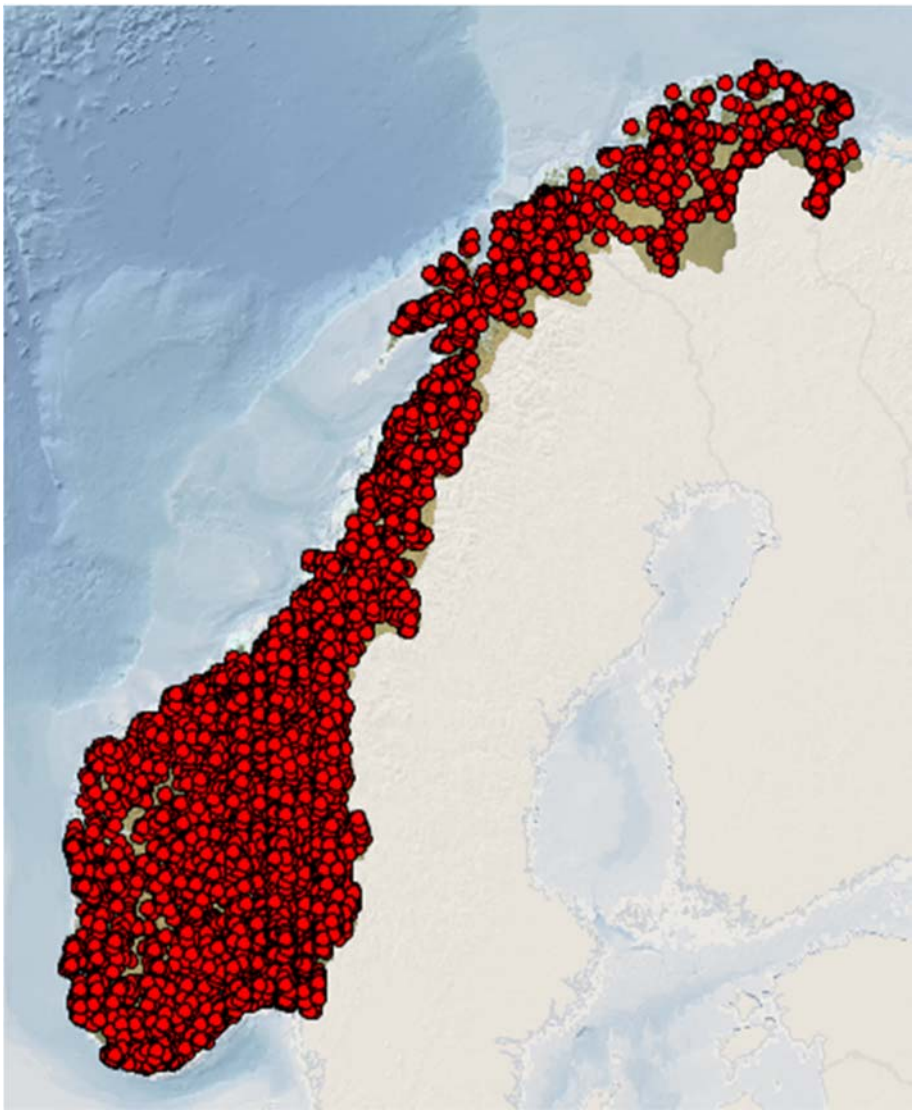
3.2.4. Norway

The red fox is the target species and practically, the only wild definitive host for *E. multilocularis* in Norway. There are only small populations of wolves and arctic foxes, whereas raccoon dogs are only occasionally reported. The arctic fox is a critically endangered species in Mainland Norway. The Norwegian population over the period 2018–2020 was estimated to be between 273 to 338 adult foxes.[5] There is to our knowledge no more recently updated information regarding the estimated number of Arctic fox in mainland Norway. In winter 2021–2022, there were 51–52 wolves registered in Norwegian territories and 74–77 wolves living in territories that are located partly in Norway and partly in Sweden.[6] In addition to the 511 red foxes tested in 2021 as part of our official surveillance programme, samples from small number (i.e. 20) of wolves, submitted for forensic post-mortem examination, were also tested analysed for E. multilocularis; all tested negative.

There are no scientific studies describing the Norwegian red fox population size. However, around 21,000 red foxes are hunted annually in Norway (Statistics Norway) and in the absence of better alternatives, an updated estimated Norwegian red fox population of 151,000 was used in the surveillance programme. This updated population estimate was provided by professor emeritus Olav Hjeljord at the Norwegian University of Life Sciences and was partly based on the spatial distribution of preferred fox habitat and hunting statistics. Prof. Hjeljord confirmed that the estimate of a population size of 151,000 red foxes in Norway is still valid (personal communication, 29/6/2020).

The red fox is geographically distributed all over Norway, but the population densities during spring are (roughly estimated) varying from 1 red fox/10 km^2 (mountain areas), 3 red foxes/10 km^2 (forest/marsh) and 10 red foxes/10 km^2 (urban/agricultural areas; e.g. Akershus, Vestfold, Østfold) (personal communication Prof. Olav Hjeljord, 29/6/2020). See also Figure 5.

[5] https://brage.nina.no/nina-xmlui/handle/11250/2719248
[6] https://rovdata.no/Ulv/Bestandsstatus.aspx



Source: Norwegian Biodiversity Information Centre (open source). https://artsdatabanken.no/Pages/180936

Figure 3: Map showing observations of red fox in Norway. Online service where citizens can logon and register their observations of fauna and flora in Norway

3.3. Sample size (sampling strategy & distribution)

3.3.1. Finland

The sample size was calculated by Finland using an overall sensitivity of the diagnostic approach of 0.78 and the design prevalence (DP) of 1% prescribed in Regulation (EU) No 1152/2011 using the RiBESS tool.

As size for the target population, a fixed value of 456,740 was used. The RiBESS tool returned a sample size equal to 383 to achieve the required confidence.

The samples were collected by hunters on a voluntary basis. Hunters were informed of the sample collection by press releases in the Finnish Food Authority website[7] and e-mails and personal contacts to the Finnish Wildlife Agency which in turn informed local hunting associations. To motivate hunters, they received by post a written report of the results of the health status of the animals they sent in.

[7] www.ruokavirasto.fi

A total of 409 and 244 samples were collected from raccoon dogs and foxes, respectively (N = 653). The majority of the samples (71%) originated from Southeast Finland (Pohjois-Karjala, Etela-Karjala, Etela-Savo, Kymenlaakso) as this is the region where active monitoring of rabies control programme has taken place since 1990. The same area can be considered having an elevated risk of introduction of *E. multilocularis* due to geographical closeness of infected areas in the south. Also, Southeast Finland has the highest density of raccoon dogs in Finland (Kauhala, 2007), but in general, the population densities for both species are highest in the southern part of the country. Hunters in the south-western part of the country (Helsinki-Uusimaa, Varsinais-Suomi, Satakunta, Pirkanmaa) have also submitted samples following a request from the Finnish Food Authority. The red fox inhabits the whole country including the northernmost 'fjeld' regions where densities can be locally high. Active hunting campaign to reduce the red fox population in the fjeld region of northern Lapland seems to have had an effect since the hunting bag of the region has decreased over time. The raccoon dog is continuously spreading northwards, and nowadays, a few hundred individuals are hunted yearly even in southern Lapland.

Gender ratio was unbalanced in foxes (female:male 1:1.23) but nearly even in raccoon dogs (1:0.96). Of the animals that could be classified by age (N-age = 542), 63% were juveniles. The proportion of juveniles was 68% in raccoon dogs and 52% in foxes.

A major sampling area was the bait vaccination zone for rabies control in south-eastern Finland (Pohjois-Karjala, Etelä-Karjala, Etelä-Savo, Kymenlaakso, 71% of the samples). Four south-western regions which were specifically encouraged by FFA to send samples provided 16% of samples. A remarkable sample of foxes (11% of all animals) was received from Lappi (Lapland) where active red fox population reduction to protect the arctic fox was ongoing (see Figures 4 and 6).

Samples were collected throughout 2021 (see Figure 5). Sampling is mostly done in the cold season. Nearly all the foxes from Lapland were hunted in January–March. In May, June and July, the sample sizes decreased since the fox is protected, and consequently, hunting is only focused on diseased or injured individuals. The raccoon dog was recently (1 June 2019) classified in the Finnish law as an alien invasive species with no protection seasons, but hunting and sampling still happen mostly in the cold season.

All 653 samples were negative by PCR. Thus, no sample was found positive for *E. multilocularis*.

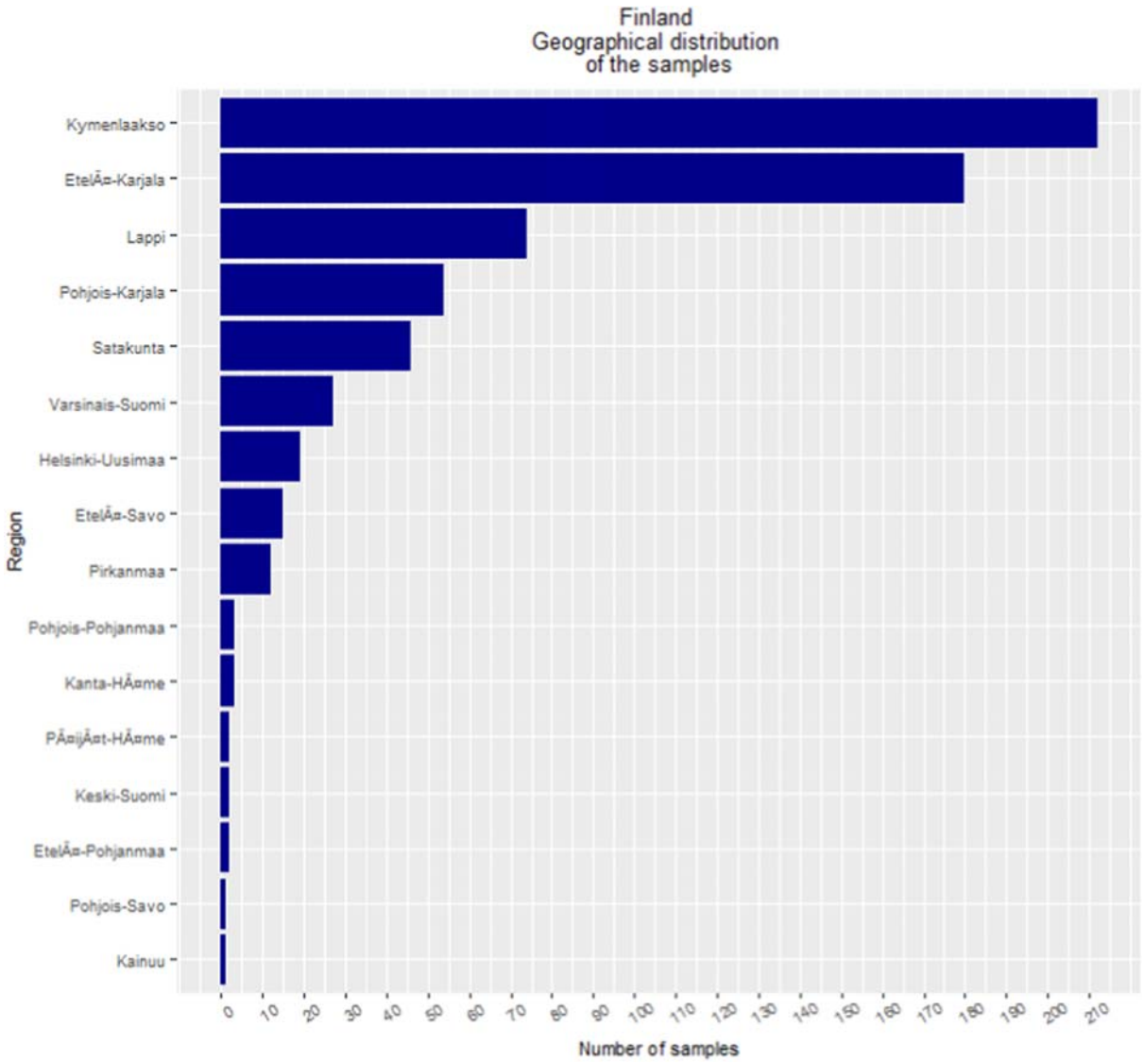


Figure 4: Finland - Geographical distribution of samples

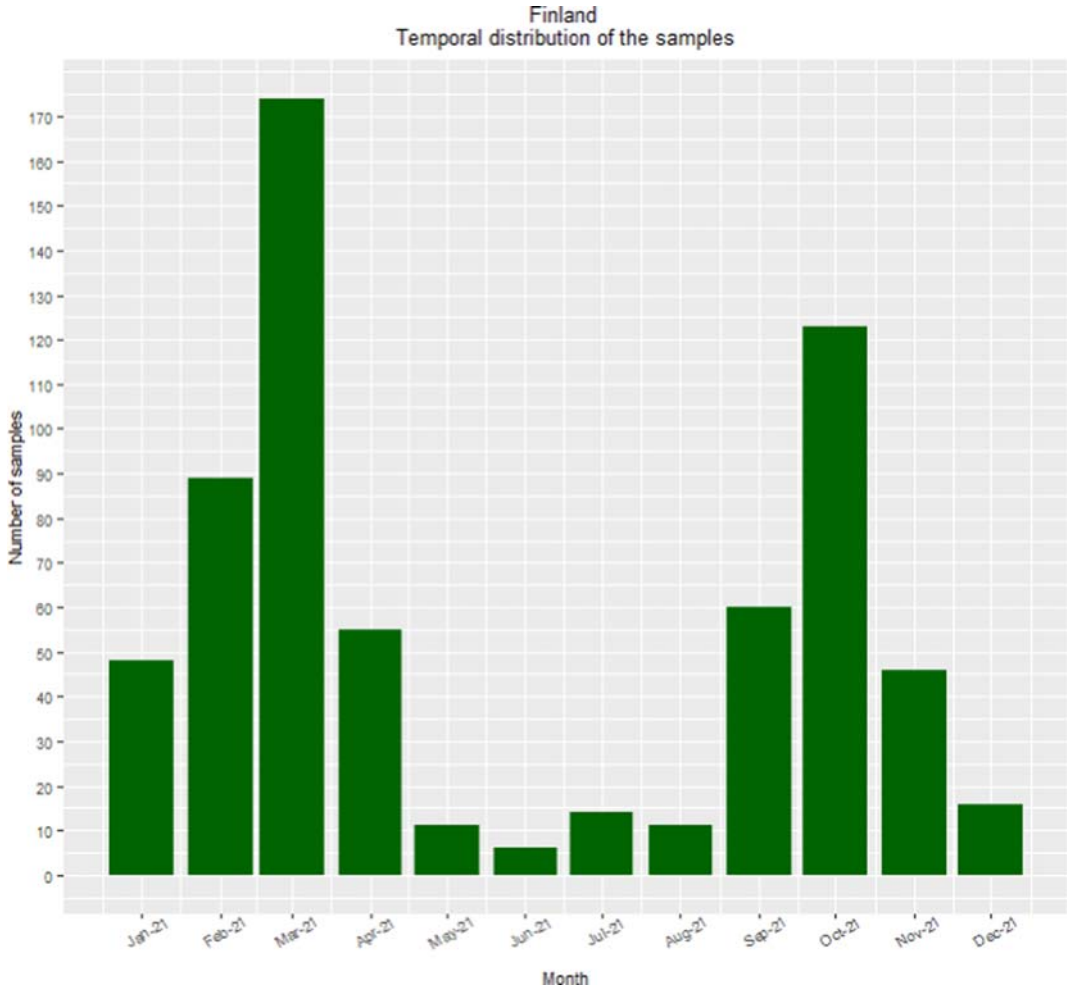


Figure 5: Finland – Temporal distribution of samples

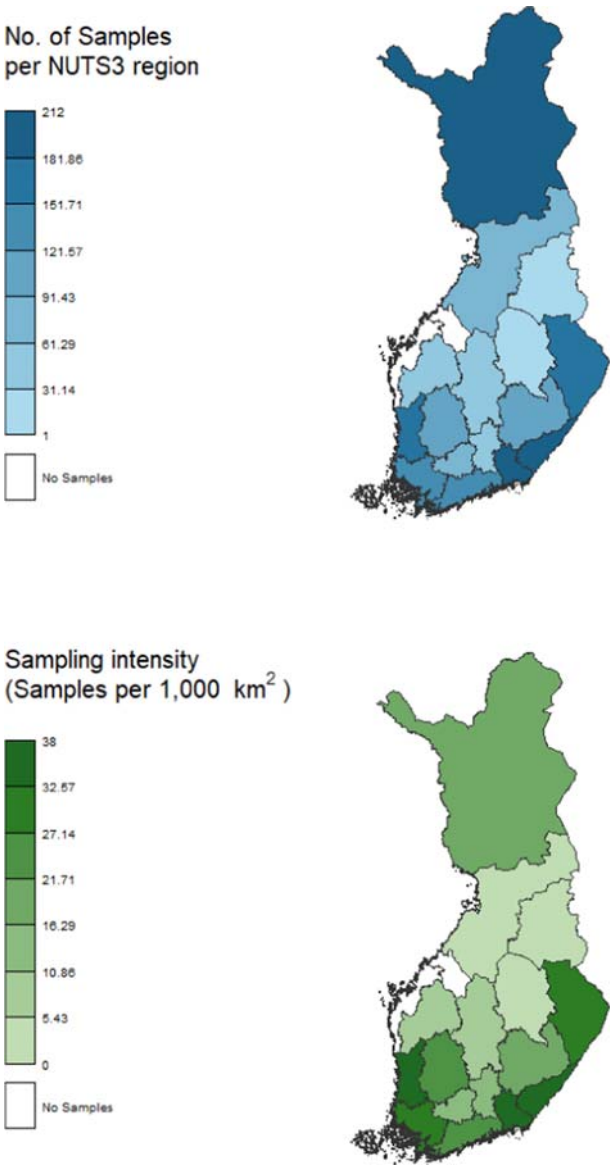


Figure 6: Finland – Sampling activity and intensity by NUTS 3 region

3.3.2. Ireland

The survey was designed to detect *E. multilocularis*, if present, in red foxes in Ireland by taking a representative sample of the red fox population based on a design prevalence of 1%, a target survey sensitivity of 0.95, *fox population size of 150,000 and test sensitivity of 0.78. The animal samples w*ere obtained from foxes which were culled (by shooting) for pest and predator control reasons and foxes that were inadvertently captured in traps set for other wildlife as part of wildlife disease control measures. Each of the 16 Regional Veterinary Offices in Ireland was requested to obtain a number of wild foxes, based on their respective area size and the fox population density to obtain a total number for that region which reflected the number calculated in the 'Red fox (*Vulpes vulpes*) Species Distribution Model' for each area.

Samples were collected through the work of the 16 Regional Veterinary Office personnel and from all eight NUTS3 regions. A slightly greater number than the minimum required to achieve the desired survey sensitivity for the entire survey were tested. In total, a collection of 398 samples was reported by Ireland. The sampling intensity was undertaken to reflect the distribution throughout Ireland and further adjusted to reflect the geographical variation in the density of the fox population distribution (Figures 7 and 9). Samples were obtained during 8 months of the year (see Figure 8). Similar to previous years, a greater number of samples were collected from culling during October and

November, in order to avoid the culling of adult female foxes during the nursing period. Collection of samples predominantly during the winter months should not adversely affect the sensitivity of the survey, based on a study from an endemic urban area in Switzerland, which found a greater prevalence of E. multilocularis in foxes in winter months (Hofer et al., 2000).

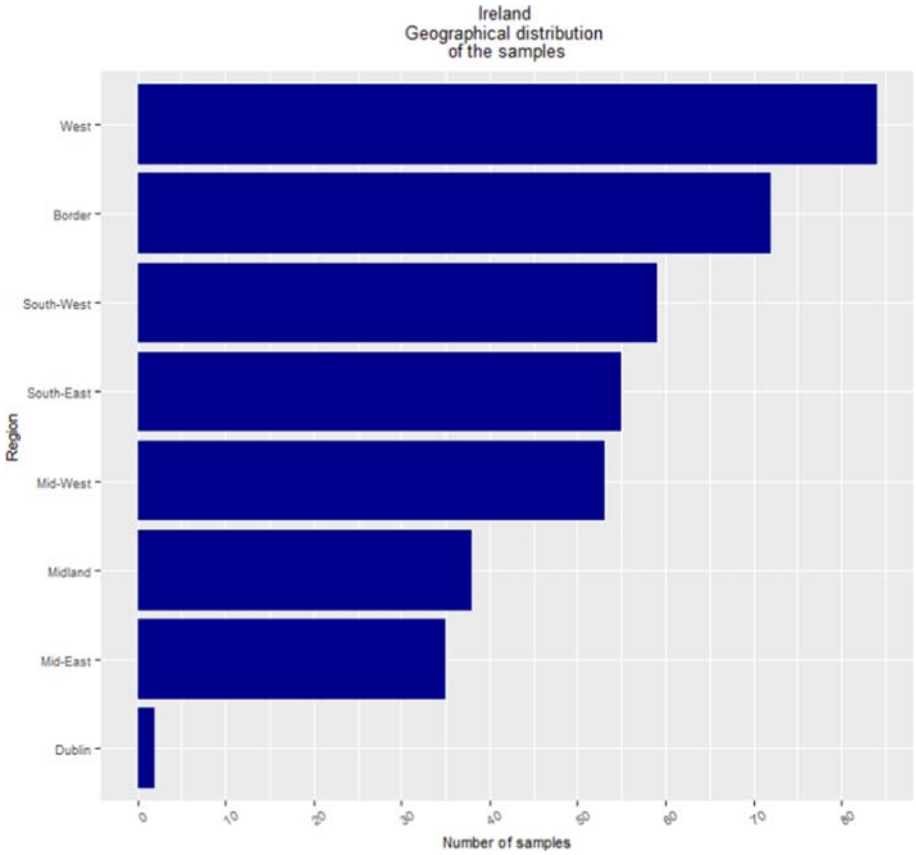


Figure 7: Ireland – Geographical distribution of samples

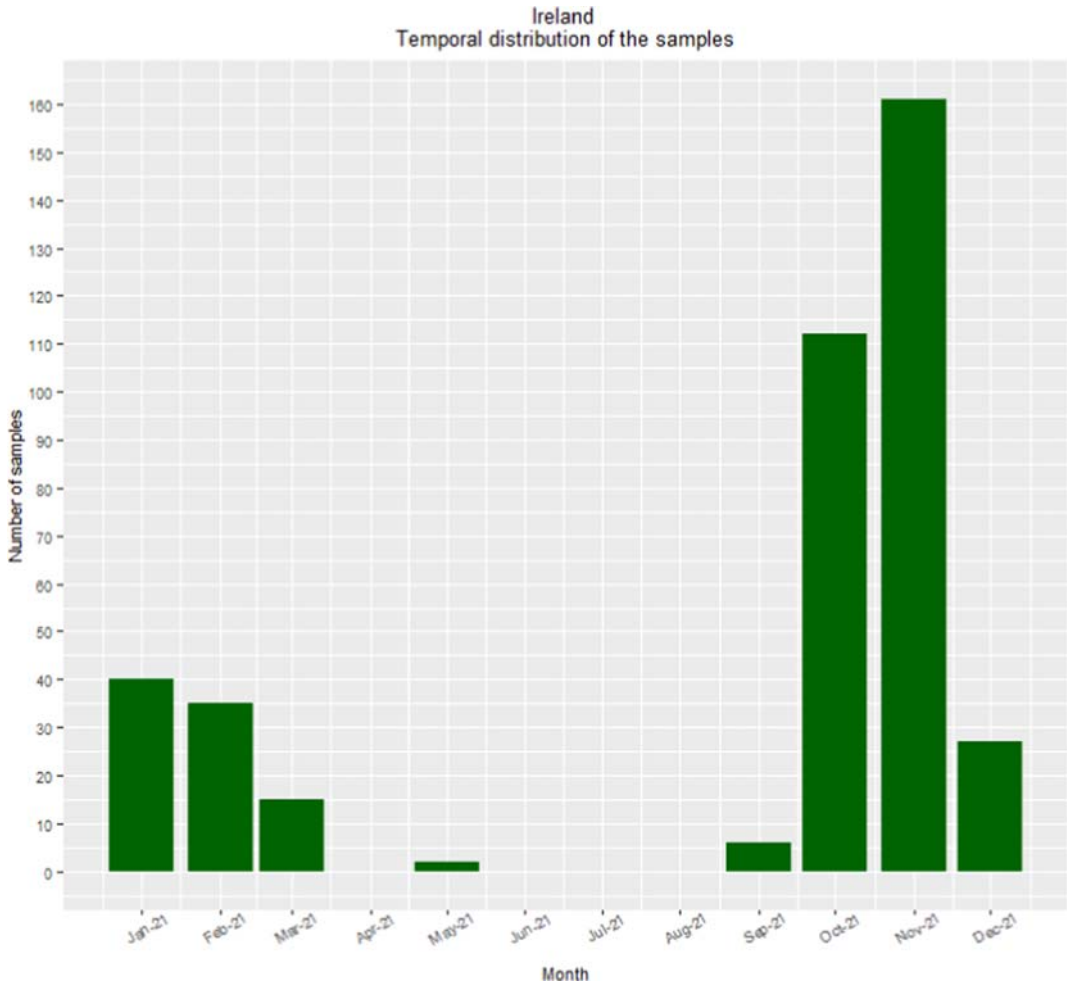


Figure 8: Ireland – Temporal distribution of samples

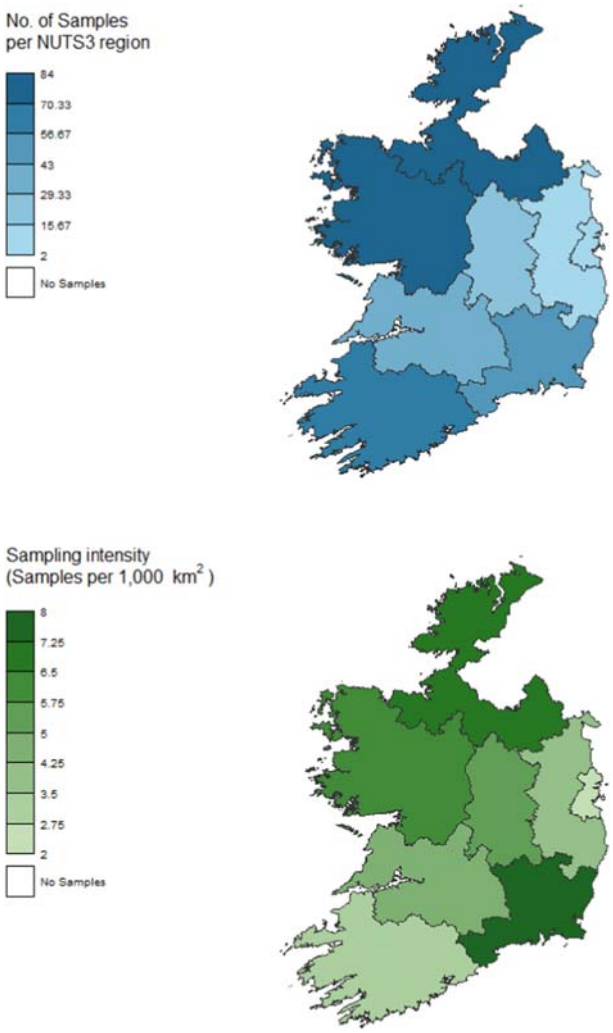


Figure 9: Ireland – Sampling activity and intensity by NUTS 3 region

3.3.3. United Kingdom (Northern Ireland)

The epidemiological unit was the individual animal. As animal carcasses rather than fox intestinal content were collected, the results could be reported at the individual fox level.

The sample size was calculated using the EFSA RiBESS tool (assuming a test sensitivity of 0.99) which returned a value of 298 samples to be tested, over a population of 14,000 individuals, to achieve the target 95% confidence set by the Regulation.

Random sampling – not risk-based – is carried out at certain times of the year – the target is the wild population, and therefore, hunting is not permitted during the breeding season. Wild animal carcasses were collected from hunting or road kills, for which accurate location is available.

Hunters and gamekeepers who shoot foxes as part of pest population control were contracted to collect carcasses. All carcasses were collected by DAERA vans and brought to AFBI. Road kills were only occasionally suitable for testing; therefore, the number was low.

Reports were made at NUTS 3 level (the lowest level of NUTS: districts in Northern Ireland) (See Figures 10 and 12). The NUTS boundaries are only rarely amended, and therefore, comparisons could be made from 1 year to the next in terms of distribution. In NI, 346 samples were collected and tested. The sampling activity was implemented in all regions (See Figure 10). Sampling was carried out at certain times of the year, mainly during the autumn and winter seasons (See Figure 11).

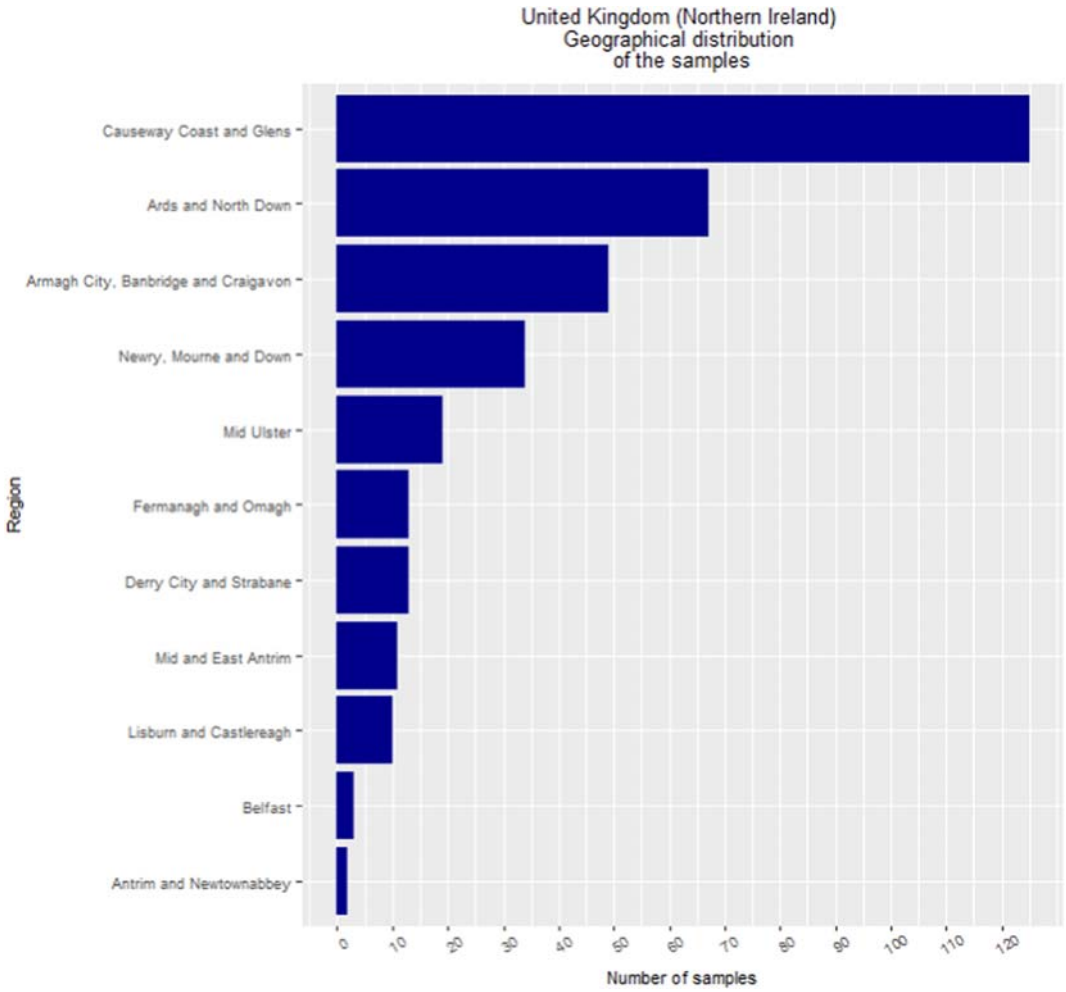


Figure 10: Northern Ireland – Geographical distribution of samples

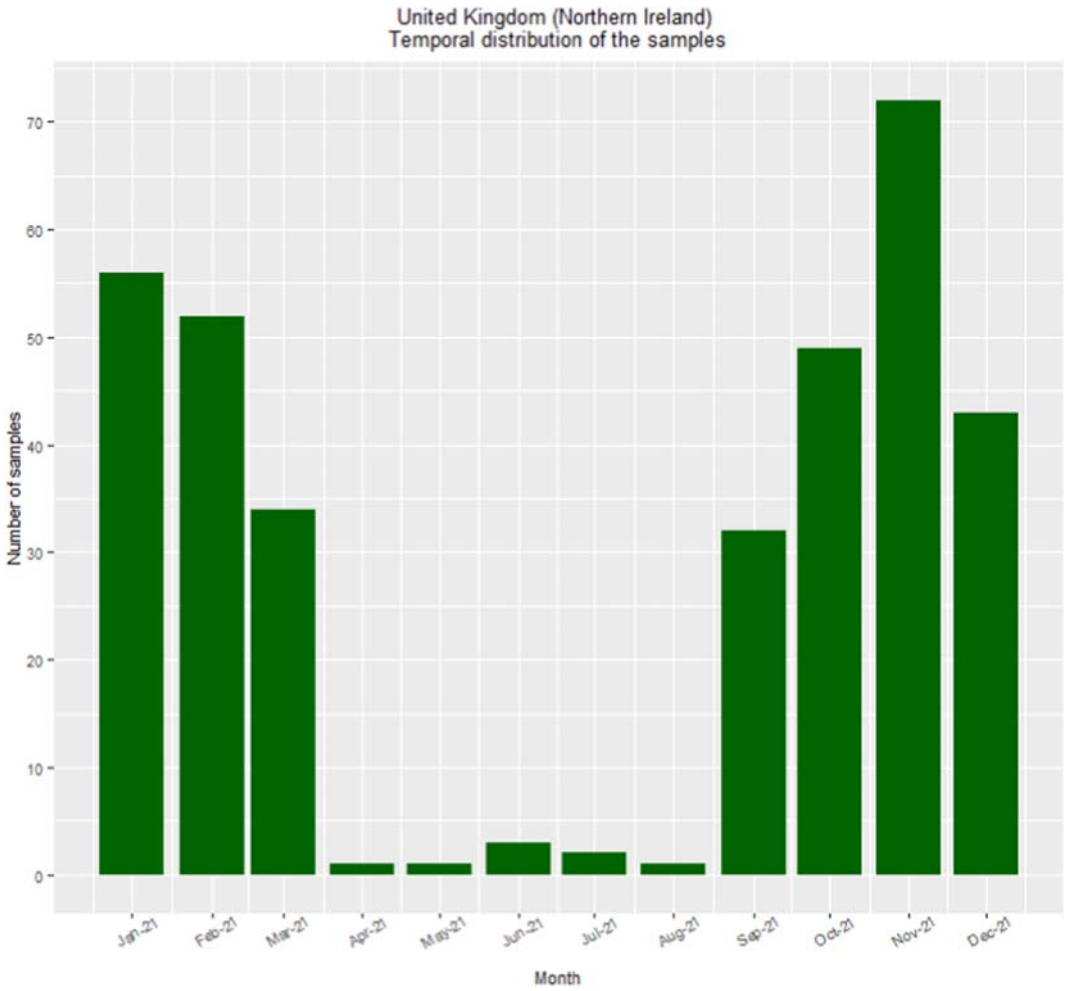


Figure 11: Northern Ireland – Temporal distribution of samples

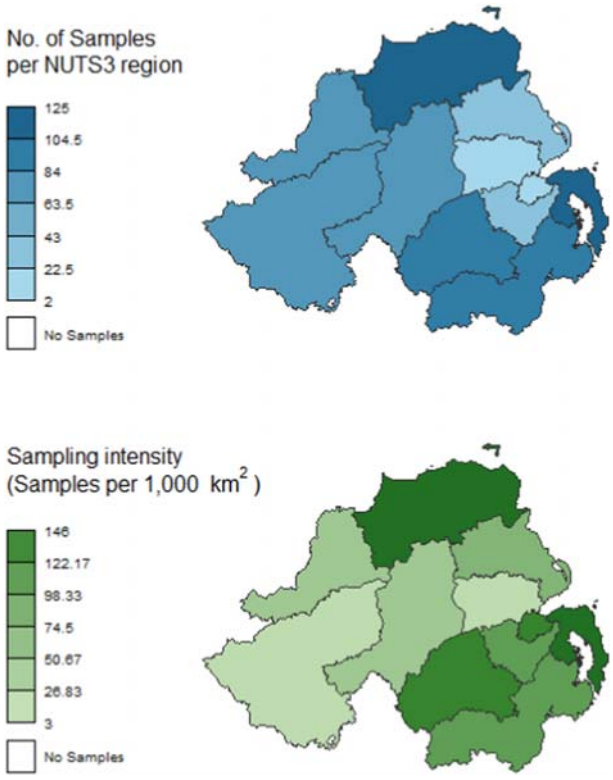


Figure 12: Northern Ireland – Sampling activity and intensity by NUTS 3 region

3.3.4. Norway

The RiBESS tool (https://shiny-efsa.openanalytics.eu/app/ribess) was used to estimate the sample size required to substantiate the absence of the parasite from the target population with a confidence level of 95%. The goal was approximately 474 samples from red foxes in 2021, i.e. the epidemiological unit is the red fox. In the Norwegian neighbouring country, Sweden, the first case of *E. multilocularis* was reported in late 2011 in a red fox from the southern part of the country. Consequently, foxhunters along the Swedish Norwegian border in the south-eastern part of Norway were encouraged to increase their hunting and to submit more samples. The presence of *E. multilocularis* in Sweden may entail an increased risk of introduction of the parasite to Norway via migrating foxes. However, habitat use and extent of migration of red foxes in Sweden are not known. This lack of knowledge makes it complicated to assess the potential threat from Swedish foxes. Additionally, increasing prevalence of *E. multilocularis* has been observed in other nearby regions such as e.g. the Baltics and Denmark. We therefore consider the risk of introduction to be relatively high. Although the parasite is now approaching via migrating wildlife in neighbouring countries (Uddevalla in Sweden is about 80 km from the Norwegian border), lack of compliance with the anthelmintic treatment requirements for pets entering Norway is also a cause for concern. Thus, we have chosen to uphold the simple random sampling of red foxes.

Initially, red foxhunters from across the country were invited to participate based on a list obtained from The Norwegian Register of Hunters. After a few years, it became very popular to participate in the surveillance programme. Therefore, the last 5–6 years we have had an online registration at the NVI's Web pages to register as a (potential) hunter for the following years sampling. This registration is usually open for 3–4 weeks in November. The hunters enter their name and municipality via the webpages of the Norwegian Veterinary Institute (https://www.vetinst.no/nyheter/registrering-som-provetaker-av-rodrev). This registration is announced on NVI's web page and at the NVI's Facebook page. Those that have contributed to the programme previous years are invited by e-mail to register, but the registration is also open for new hunters. The selection of foxhunters has then been based on residence and previous quality of their submitted samples. In addition, the selection also includes some hunters that are new to the programme and therefore covers some new regions. Sample containers and detailed instructions for sampling were forwarded to the hunters who participates in the programme. The foxes were mainly killed with firearms (shotgun or rifle), but occasionally caught

in traps or road killed. To secure, that the samples originated from individual animals the hunters also had to submit the tongue from each fox. The samples together with information concerning origin of the fox, date of the hunt, sex (male or female) and estimated age of the animal (juvenile or adult) were submitted to the laboratory in prepaid envelopes. In addition to samples from foxes, samples from wolves killed legally or illegally during 2021 were tested for *E. multilocularis*. For safety reasons, all samples were frozen at −80°C for at least 3 days before analysis.

All counties in Norway were represented in the sampling regime. Five hundred and eleven samples were collected from red foxes in 2021 and all were negative in PCR. The spatial distribution of samples is somewhat uneven since the topography of Norway (large areas with mountains) entails scattered settlements, and hunters do the fox sampling voluntarily in the proximity of their homes (See Figures 13 and 15). The temporal distribution of samples is also somewhat uneven (See Figure 14) due to preferred hunting conditions during winter and banned hunting between 15 April and 15 July (and between 24 and 31 December). In September and October, it is also hunting season for wild cervids such as moose and red deer (and in which many Norwegian hunters participate), which might be an explanation for the low numbers of red fox samples from these months. There samples are recorded in May and June in the data set, a period that is outside the legal hunting period in Norway: One of the foxes was shot after being hit by a car and the other as pest control after killing a number of young lambs.

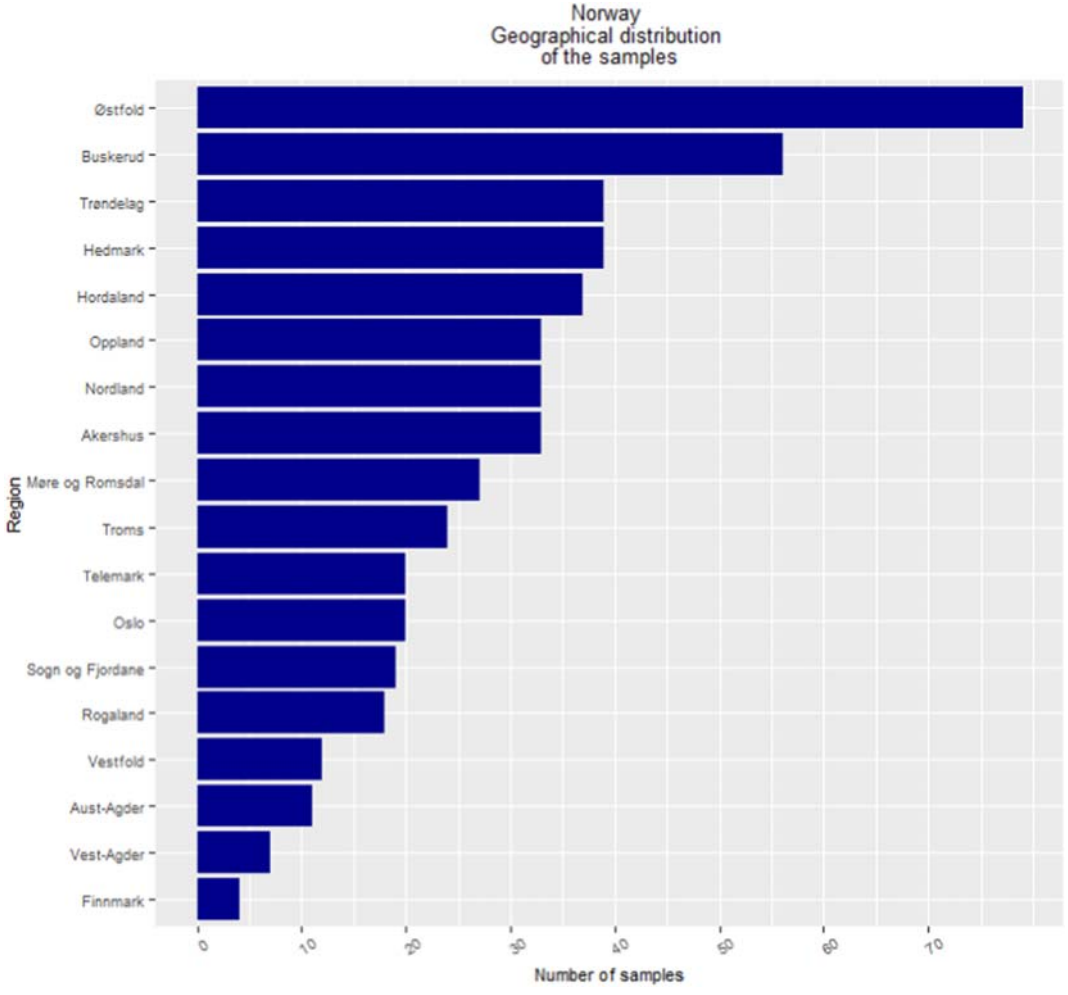


Figure 13: Norway – Geographical distribution of samples

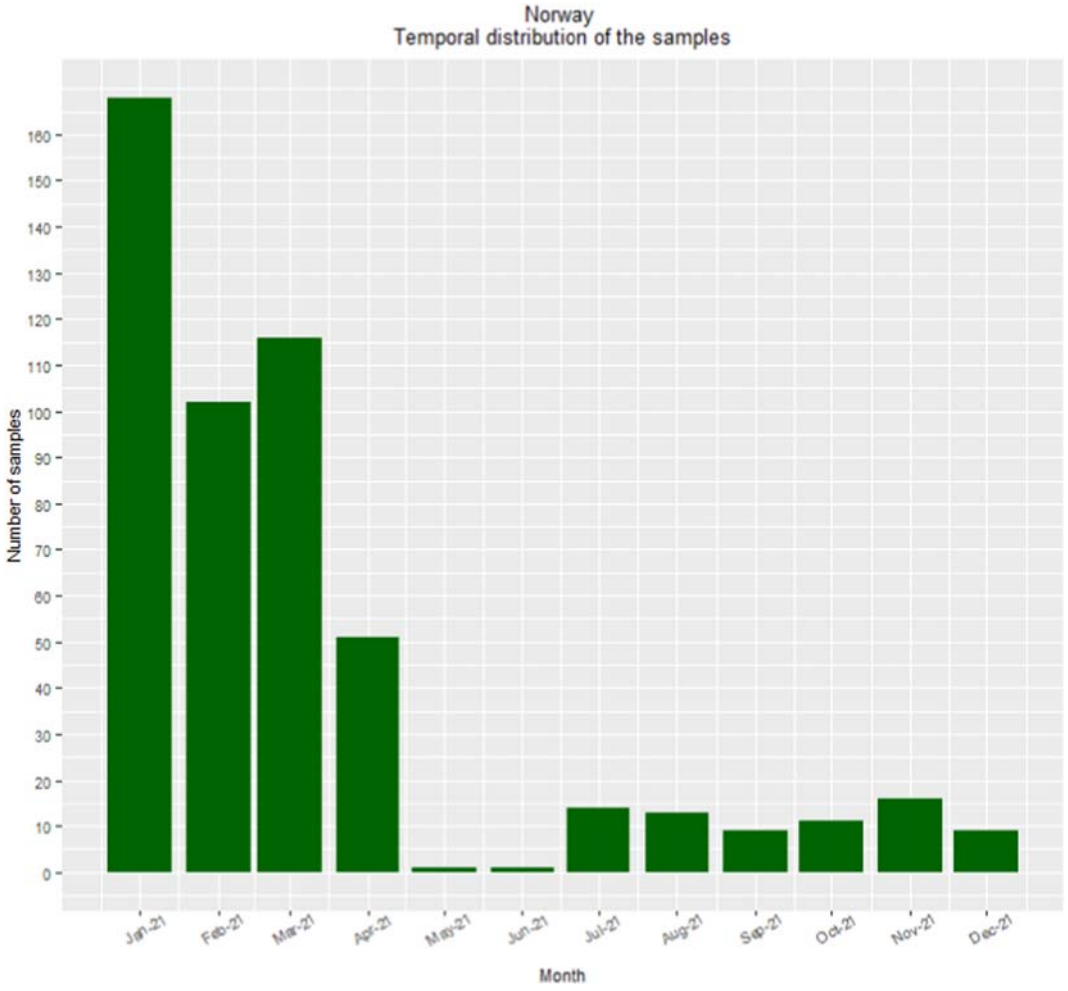


Figure 14: Norway – Temporal distribution of samples

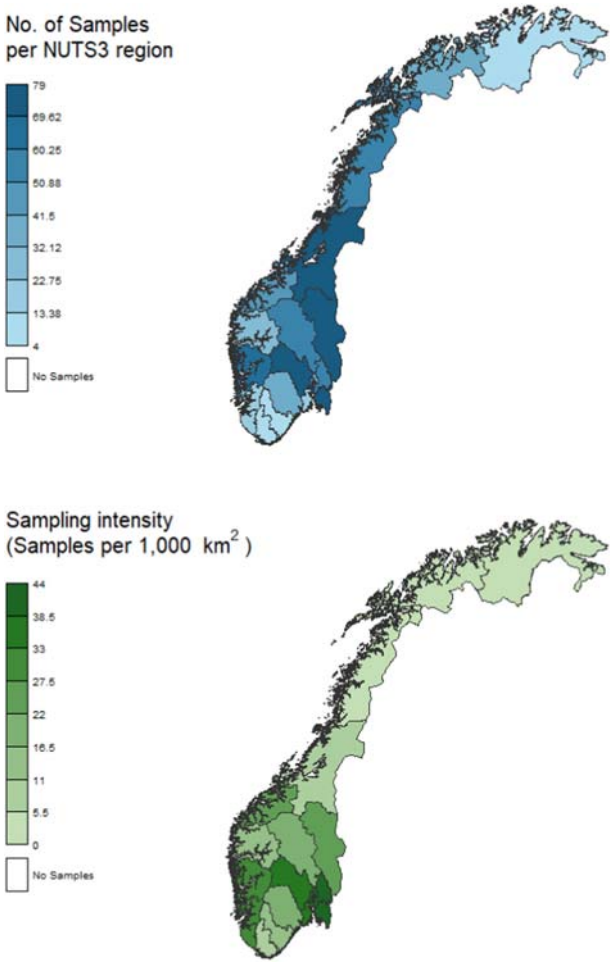


Figure 15: Norway – Sampling activity and intensity by NUTS 3 region

4. EFSA comments and considerations

4.1. Finland

4.1.1. Type and sensitivity of the detection method

Type of the detection method

The diagnostic test used by Finland for the detection of E. multilocularis consists of a PCR method (PCR targeting 12 S rRNA gene) described by Isaksson et al. (2014). The technique has been well described. A slight modification of the technique has been realised and it has been indicated in the report.

Test sensitivity

The test sensitivity used for the estimation of the sample size was 0.78, as suggested by EFSA (EFSA, 2015). However, an overall system sensitivity of 0.87 (0.83–0.90) has been estimated based on internal validations performed by Evira/Finnish Food Authority. The additional positive (spiked) samples tested in 2020 help in narrowing the uncertainty around the sensitivity of the test in use (Table 10).

Table 10: Results of the internal validation round of tests performed by Finland over time

Year	Spiked samples (n, positive controls)	Samples testing positive (s)	Estimated sensitivity for each trial (exact binomial test)	Bayesian cumulative [a]
2014	131	102	0.78 (0.70–0.85)	0.78 (0.7–0.84)
2015	38	32	0.84 (0.69–0.94)	0.79 (0.73–0.85)
2016	32	31	0.97 (0.84–1)	0.82 (0.76–0.87)
2017	76	72	0.95 (0.87–0.99)	0.85 (0.81–0.89)
2018	31	31	1 (0.89–1)	0.87 (0.83–0.90)
2019	24	24	1 (0.86–1)	0.88 (0.84–0.91)
2020[b]	–	–	–	–
2021	47	45	0.96 (0.85–1)	**0.89 (0.86–0.92)**
Total	**379**	**337**	**0.89 (0.85–0.92)**	

(a): Estimated based on the distribution $Beta\left(\sum_{i=1}^{y} s_i + 1, \sum_{i=1}^{y} n_i - \sum_{i=1}^{y} s_i + 1\right) + 1$ where y is the number of years/rounds of test.

(b): In 2020, an internal validation exercise was performed, but the quality of the positive samples (i.e. the eggs in the sample) was not considered comparable to the ones used in other years.

An exact binomial test shows a 'probability of success' ('best guess' of the sensitivity) equal to 0.89, with a confidence interval going from 0.85 to 0.92 (bottom row of Table 8) and a Bayesian approach leads substantially to the same results.

4.1.2. Selection of the target population

Definition of susceptible host population target by the system

The selection of raccoon dogs and red fox species as target populations was based on their role as definitive hosts in the cycle. This is an assumption also confirmed by the EFSA Scientific opinion on *E. multilocularis* infection in animals (EFSA AHAW Panel, 2015). It is not possible to conclude on the role of the age and gender composition of the target population in the epidemiology and the lifecycle of EM, due to lack of appropriate data and studies (EFSA AHAW Panel, 2015).

Size of susceptible host population targeted by the system

Host population sizes were based on a scientific study performed in 2007. Although population data have not been updated since 2007, new information regarding annual hunting bags has been included in the report. The decision to accept the size of the population as published by Kauhala (2007) and adjusting for the change of the size of the hunting bag is scientifically sound, particularly considering that the sample size calculation is not heavily affected when the population size has these dimensions (~ infinite population) (see EFSA AHAW Panel, 2015). The fact of considering the sum of the red fox and raccoon dog populations as the target population size seems to be correct, as raccoon dogs can act as DHs in conjunction with the red fox (EFSA AHAW Panel, 2015).

4.1.3. Sampling strategy

Epidemiological unit

The epidemiological unit appears in the report and is defined as the individual animal. Individual rectal contents were collected by Finnish Food Authority from hunter-submitted carcasses.

Sample size calculation

The method used to calculate the sample size of Finland was the RIBESS tool. The sample size was calculated with an overall sensitivity of the diagnostic approach of 0.78 and a population size of 456,740 (sum of red fox and raccoon dog population). The sample size required in this case is 383. The sample size collected (N = 653) is sufficient to satisfy the legal requirements.

Implementation of the sampling activity

The geographical information shows that, in 2021, 16 (15 in 2020) of 20 NUTS3 regions were included in the sampling activity (see Figure 6). There was a higher intensity of the sampling in the south-east of the country. The date of hunting is not always communicated to the laboratory and for

this reason only the month of sampling is submitted to EFSA. The surveillance strategy as described in the Finnish report cannot be considered a simple random sample, but rather a 'convenience sample', biologically driven. Most of the samples were collected by hunters and efforts were concentrated in the north and south-east of the country. However, in the case of wildlife animals, 'convenience sampling' is the most frequently used method. To mitigate the potential bias caused by this sampling activity, more samples than required were collected. Samples were collected during a period of 12 months as established in the relevant Regulation. The reduction of the intensity of the sampling during the summer months (from May to August) is well justified and may not compromise the success of the detection of the parasite. A previous EFSA assessment suggested that a sampling distribution concentrated in the second half of the year – in a Freedom from Disease framework – could be more effective than a sampling distributed over the whole year; however, a quantitative evaluation was not performed (EFSA, 2013).

4.1.4. Methodology

Design prevalence

The DP was equal to 1% (0.01), as it is specified in Annex I to Commission Delegated Regulation (EU) 2018/772.

Epidemiological geographical unit

The geographical unit was specified to be the entire territory of Finland. The choice is sound as no risk factors were reported to justify the identification of subareas within the Finnish territory.

Methodology for calculation of the area sensitivity

The area sensitivity was estimated by FI using the RiBESS tool. The parameters included for the calculation were the following, all fully documented:

- DP of 1% (0.01),
- test sensitivity of 0.78,
- population size of 456.740 (raccoon dogs + red foxes) and
- sample size of 653.

The value of the area sensitivity (0.994) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements of Commission Delegated Regulation (EU) 2018/772.

In summary, the set of data relative to the surveillance activity in 2021 ensures the fulfilment of all the technical legal requirements included in the Annex I of Commission Delegated Regulation (EU)2018/772.

4.2. Ireland

4.2.1. Type and sensitivity of the detection method

Type of the detection method: The diagnostic test chosen by Ireland is well described (PCR Cest1-Cest2 NAD1) and is based on a peer-reviewed method with a correct reference included in the report.

Test sensitivity: Ireland followed EFSAs advice regarding the setting of the conservative, lowest value of the sensitivity (0.78) (EFSA AHAW Panel, 2015).

4.2.2. Selection of the target population

Definition of susceptible host population target by the system: The red fox has been recognised as the main wildlife definitive host species for this parasite (EFSA AHAW Panel, 2015). The selection of this species to perform the pathogen surveillance is well explained and referenced. The absence of other important definitive wild hosts (raccoon dogs and wolves) is also supported by scientific literature. Regarding the age or gender of the target population, their role in the epidemiology and in the lifecycle of *E. multilocularis* is not known due to the lack of appropriate data and studies (EFSA AHAW Panel, 2015).

Size of susceptible host population targeted by the system: Although the original information regarding the red fox population size was published in 2000 and 2009 (Hayden and Harrington, 2000; Marnell et al., 2009), Dr Tomás Murray, of the National Biodiversity Data Centre, Ireland, specifically provided additional information regarding the Irish fox population in 2015, including more recent data on the relative population density distribution based on ongoing observation records. Nevertheless, at

a population size greater than 10,000, moderate fluctuations in the population size would not significantly change the sample size required to achieve the same statistical confidence of less than 1% (0.01) prevalence at a specific test sensitivity (EFSA, 2014). Therefore, fluctuations in the previous population size of 150,000 do not significantly alter the sample size required (EFSA, 2014).

4.2.3. Sampling strategy

Epidemiological unit: The epidemiological unit is defined in the report as the individual animal. Faeces samples were obtained post-mortem from culled (control programmes) or animals trapped inadvertently.

Sample size calculation: The method used to calculate the sample size for Ireland was the RIBESS tool. The sample size was calculated with: (a) an overall sensitivity of 0.78 (as recommended by EFSA AHAW Panel, 2015) and (b) a population size of 150,000 (red fox population). With these conditions, the minimum number of samples to collect in order to obtain a minimum of 0.95 of area sensitivity is 383. The total number of samples collected by Ireland was 398, which ensures the fulfilment of the technical legal requirements in Commission Delegated Regulation (EU) 2018/772 concerning a confidence level of at least 0.95 against a design prevalence of 1%. Although EFSA would recommend considering the population size as the maximum value of the range instead of the minimum number (200,000 instead of 150,000), the minimum sample size thus calculated to achieve the same confidence would not differ significantly.

Implementation of the sampling activity: The geographical information shows that all regions were included in the sampling activity. The sampling activity per 1,000 km^2 shows a slightly higher intensity (max = 8, min = 2) in the north and south-east regions of the country, i.e. the target sample size is distributed across the territory almost as a function of the area size, adjusted for the density of the population. Such a sampling strategy, leading to a so-called proportional sample, is more likely to be representative compared to other strategies. Samples were obtained during the whole year excluding April, June, July and August. The reduction of collection of samples during spring and summer is justified to avoid culling adult female foxes during the nursing period. This fact might not influence the representativeness of the sample, as suggested in a previous EFSA assessment (EFSA, 2013). A sampling distribution concentrated in the second half of the year – in a Freedom from Disease framework – could be more effective than a sampling distributed across the whole year (EFSA, 2013).

4.2.4. Methodology

Design prevalence: The DP was equal to 1% (0.01), as it is specified in Annex I Commission Delegated Regulation (EU) 2018/772.

Epidemiological geographical unit: The geographical unit was specified to be the entire territory of Ireland. The choice is sound as no risk factors were reported to justify the identification of subareas within the Irish territory.

Methodology for calculation of the area sensitivity: The area sensitivity was estimated by Ireland using the RiBESS tool. The parameters included for the calculation were the following:

- design prevalence of 1%,
- test sensitivity of 0.78,
- population size of 150,000 and
- sample size of 398.

The value of the area sensitivity 0.956 (> 0.95) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements described in Commission Delegated Regulation (EU) 2018/772. With a population size of 200,000, the value of the area sensitivity would also reach this CL, 0.956 (> 0.95).

In summary, the set of data relative to the surveillance activity in 2021 ensures the fulfilment of the technical legal requirements included in all the paragraphs in Annex I of Commission Delegated Regulation (EU) 2018/772.

4.3. United Kingdom (Northern Ireland)

4.3.1. Type and sensitivity of the detection method

Type of test: The Sedimentation and Counting Technique (SCT) test (Eckert, 2003), considered as the reference standard for detection of *E. multilocularis* from individual intestinal content, was used.

Test sensitivity: According to Casulli et al. (2015) and Conraths et al. (2015), the SCT method selected by Northern Ireland (NI) has a sensitivity of 98% and 83.8%, respectively. The analyses performed at the Agri-Food and Biosciences Institute (AFBI) considered an Se of 99% (Eckert, 2003). The considerations about the appropriateness of the TSe value chosen are the same as the previous years: The evidence provided to support the test sensitivity value for the SCT (Eckert, 2003) actually refers to a previous work (Hofer et al., 2000). However, the aim of the latter study was not to estimate the sensitivity of the SCT test, but rather to estimate the prevalence in the target population. In the paper of Hofer, it is reported that 'no sample classified as negative by the SCT was detected positive by the intestinal scraping technique (IST)'. This observation could falsely lead to the conclusion that the SCT has a sensitivity close to 100%, but in reality, the only possible conclusion is that the IST sensitivity is not higher than the one of the SCT, but both of them are unknown. To estimate the diagnostic sensitivity of a test, it is essential to know the real conditions of the samples that are examined, i.e. if they are truly infected or if they are negative controls. In the absence of this information, it is impossible to estimate the probability of the test to detect a positive sample given that the sample is truly infected (as the latter condition is not known). Note that this procedure was not followed to estimate the diagnostic sensitivity of the IST technique neither. As a conclusion, the almost perfect sensitivity of the SCT is, in reality, an assumption not supported by adequate scientific evidence. EFSA recommends using a test sensitivity of 0.78 as a more conservative option: An overestimation of the performance of the test can lead to wrong conclusions on the area sensitivity achieved.

4.3.2. Selection of the target population

Definition of susceptible host population target by the system: The selection of red fox to perform the pathogen surveillance seems appropriate, as this species has been recognised as the main wildlife definitive host species for this parasite (EFSA AHAW Panel, 2015). Regarding the absence of other potential wild definitive hosts (raccoon dogs, wolves), the information is consistent with the report of Ireland. However, no reference has been provided. Size of susceptible host population targeted by the system: Data of fox population size are well documented (14,000) and it can be assumed to be almost stable.

4.3.3. Sampling strategy

Epidemiological unit

For United Kingdom (Northern Ireland), intestinal contents from hunted or roadkill individual animals were sampled.

Sample size calculation

The method used to calculate the sample size of NI was the RIBESS tool. The sample size was calculated with an overall sensitivity of the diagnostic approach of 0.99 and a population size of 14,000 (red fox population). With these conditions, the minimum number of samples to collect in order to obtain a minimum of 0.95 of area sensitivity is 298. The total number of samples collected by NI was 346. However, if a sensitivity of 0.78 is considered, as suggested by EFSA as a worse-case scenario (EFSA, 2015), the required samples to fulfil the technical legal requirements regarding a confidence level of at least 0.95 against a design prevalence of 1% increase to 379 (with 33 additional samples required). As an internal validation of the test sensitivity has not been made (ideally it should be determined by each lab for the protocol used in-house), a value of 0.78 would be the most suitable value in order to calculate the sample size. The total number of samples collected by NI, assuming the theoretical value of 0.78 as test sensitivity, returns a confidence level equal to 0.935, slightly lower than the value indicated among the technical legal requirements of Commission Delegated Regulation (EU) 2018/772 regarding a confidence level of at least 0.95 against a design prevalence of 1%. On the other hand, the effort to increase the required sample size must be acknowledged: compared to 2020, 182 additional samples were tested. In addition, the sampling carried out in the Republic of Ireland,

given the lack of geographical barrier between the two regions, would provide additional guarantees that Northern Ireland remains disease free this year, even if a lower test sensitivity were used for the sample calculation.

Implementation of the sampling activity

The sampling process has more of the characteristics of a convenience sampling, rather than a simple random sample. The difficulties in performing a simple random sampling technique, however, are well known and are broadly discussed in previous reports. Although since 2021 there are no more hunting restriction for the fox, the reduction of the intensity of the sampling during the summer months (from April to August) could be due to a habit. This should not compromise the success of the detection of the parasite. A previous EFSA assessment suggested that a sampling distribution concentrated in the second half of the year – in a Freedom from Disease framework – could be more effective than a sampling distributed over the whole year; however, a quantitative evaluation was not performed (EFSA, 2013). For the first time since the annual scientific assessment was performed, United Kingdom (Northern Ireland) tested samples collected in the previous calendar year (2021), aligning with the other involved countries.

4.3.4. Methodology

Design prevalence

The DP used was equal to 1%, as it is specified in Annex I to Commission Delegated Regulation (EU) 2018/772. Epidemiological geographical unit: The geographical unit was specified to be the entire territory of Northern Ireland.

Methodology for calculation of the area sensitivity

The area sensitivity was estimated by Northern Ireland using the RiBESS tool. The parameters included for the calculation were the following:

- design prevalence of 1%,
- test sensitivity of 0.99,
- population size of 14,000 and
- sample size of 346.

With these conditions, area sensitivity was higher than 0.95 (0.968). However, if a test sensitivity of 0.78 is assumed, as suggested by EFSA (EFSA, 2015), the area sensitivity (0.935) is not sufficient to comply with the EU regulation in force (34 additional tests would be required).

In summary, the set of data relative to the surveillance activity in 2020 does not ensure the fulfilment of the technical legal requirements of Annex I of Commission Delegated Regulation (EU) 2018/772.

From a purely epidemiological point of view, to consider the whole island of Ireland as one epidemiological unit would be a scientifically sound approach. The fox population is widely distributed in the island of Ireland and individual animals move freely throughout the territory without physical barriers. EFSA performed a theoretical analysis considering the population of foxes of the whole territory of Ireland by means of combining the results of NI and Ireland. The global area sensitivity achieved would be 0.997, i.e. above the confidence required by the legislation.

	Component Sensitivity	Overall Area Sensitivity
IE	0.956	0.997
NI	0.935 (TSe 0.78)	

4.4. Norway

4.4.1. Type and sensitivity of the detection method

Type of the detection method: Norway used a DNA-fishing technique, the PCR 12 S rRNA (Isaksson et al., 2014), which is well described and appropriately referenced in the report.

Test sensitivity: For precautionary reasons, the diagnostic sensitivity was set to the sensitivity obtained by Øines et al., 2014 (0.63), a lower value than the minimum recommended by EFSA (0.78).

Such a low test sensitivity implies a much higher effort to reach the 95% of confidence stated in the legislation, as a large sample size is required. Table 9 summarises the results of the set of trials performed in Norway on samples spiked with different concentrations of eggs and worms (Inger Sofie Hamnes, 2022, personal communication) (Table 11).

Table 11: Summary of the number of tested spiked samples (n) and number of samples testing positive (s) for each concentration of egg/worm. The last column reports the outcome of an exact binomial test (R Core Team, 2022)[(a)]

Spike	s	n	Test Se 50th perc (95% CI)
1 egg	30	54	0.56 (0.41–0.69)
5 eggs	21	28	0.75 (0.55–0.89)
10 eggs	44	52	0.85 (0.72–0.93)
50 eggs	2	2	1 (0.16–1)
1 worm	47	51	0.92 (0.81–0.98)
Overall	**144**	**187**	**0.77 (0.70–0.83)**

(a): R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: https://www.R-project.org/

Taken individually and looking at the 50th percentile, there is a positive correlation between the concentration of the parasite in the sample and the sensitivity. The small number of samples used to test high concentrations (50 eggs) brings a huge uncertainty around the estimate associated with the results (95% CI: 0.16–0.98). This uncertainty also affects the estimation of the overall performance of the test: Pooling all the results together allows to estimate the performance of the test in a condition that may reflect the situation in the field, i.e. where the amount of the parasite or its eggs is unknown. The bottom line in Table 9 shows the result of this estimation: **based on the available data**, the test appears to have a sensitivity greater than 0.77 in 50% of the cases; however, the lower bound of the confidence interval suggests that a more conservative value would be 0.70. This low value, as said, is data driven and affected by the sample size: Additional test will contribute to narrow the uncertainty around the 50th percentile. On the other hand, the likelihood of analysing samples with 50 eggs appears to be quite low, based on expert opinion. More studies on this topic should be performed in order to assign a weight to each spiked sample based on the egg content. To check whether the number of eggs in a sample has an impact on the performance of the test (i.e. the test sensitivity), two models were fit to the data shown in Table 9. Both models have as dependent variable the test sensitivity, i.e. the ratio between the number of spiked samples that were correctly detected as positive and the total number of spiked samples. The first model, a log-logistic model, was fit to the data with the predictor containing the number of eggs in a sample. The second one, a logistic model, with no information about the number of eggs, was also fit to the data. By comparing the two models by means of a likelihood ratio test, the log-logistic model fits better the data compared to the logistic model with no predictors. This modelling exercise confirms that the number of eggs in the samples has an impact on the ability of the test to detect truly positive samples, i.e. the test sensitivity: the higher the number of eggs, the higher the test sensitivity. Further analysis should be performed to better estimate what value of the test sensitivity could better fit a field situation.

4.4.2. Selection of the target population

Definition of susceptible host population target by the system: Red fox was considered the target species for Norway, and only few numbers of wolves were also included in the surveillance, but not reported. The reasons put forward by Norway to justify its decision of not including other wild definitive hosts (arctic foxes and raccoon dogs) are valid.

Size of susceptible host population targeted by the system: In the absence of data on fox populations in Norway, the size was estimated considering the annual hunted foxes.

4.4.3. Sampling strategy

Epidemiological unit: The epidemiological unit appears in the report and is defined as the red fox (*Vulpes vulpes*). Individual rectal contents were collected directly by hunters.

Sample size calculation: The EFSA RiBESS tool was used to verify that the sample size was sufficient to claim a prevalence of not more than 1% at a confidence level of at least 95%.

Considering design prevalence of 1%, a test sensitivity of 0.63 and a population size of 151,000, the sample size required is 474. The number of samples collected by Norway in 2021 (511 samples) is more than required.

Implementation of the sampling activity: Samples were collected from all the Norwegian NUTS3 regions with an increase of the sampling in the southeast and northwest of the country. The differences of sampling intensities among the different areas have also been justified in the report.

4.4.4. Methodology

Design prevalence

The DP was equal to 1% (0.01), as it is specified in Annex I to Commission Delegated Regulation (EU) 2018/772.

Epidemiological geographical unit

The geographical unit is deduced to be the entire territory of Norway. The choice is sound as no risk factors were reported to justify the identification of subareas within the Norwegian territory.

Methodology for calculation of the area sensitivity

The area sensitivity was estimated for Norway using the RiBESS tool and considering the following parameters:

- design prevalence of 1%,
- test sensitivity of 0.63,
- population size of 151,000 and
- sample size of 511.

The area sensitivity value is 0.961 (> 0.95), which exceeds the established minimum value of 0.95 needed to fulfil the technical legal requirements of Commission Delegated Regulation (EU) 2018/772.

In summary, the set of data relative to the surveillance activity in 2020 ensures the fulfilment of the technical legal requirements of all the paragraphs included in the Annex I of Commission Delegated Regulation (EU) 2018/772.

5. Conclusions

- *Echinococcus multilocularis* was not detected in any of the samples from the four countries (Finland, United Kingdom (Northern Ireland), Ireland and Norway) collected in the reporting period (2021).
- All the countries participating in this surveillance (Finland, United Kingdom (Northern Ireland), Ireland and Norway) fulfil the technical legal requirements regarding the use of appropriate techniques for the detection of *E. multilocularis* in intestinal contents or faeces. All these countries use different methods for detection of the parasite as described in the report. Sensitivity (and specificity) values of the techniques have been reported for a proper assessment of the surveillance performance.
- All the countries participating in this surveillance (Finland, United Kingdom (Northern Ireland), Ireland and Norway) fulfil the technical legal requirements regarding the collection of samples from wild definitive hosts. The four countries selected adequate wild definitive hosts in order to perform the surveillance.
- The sampling strategies performed by Finland, United Kingdom (Northern Ireland), Ireland and Norway cannot be considered based on a simple random sampling. For contingent, technical reasons, the sampling strategy in live wild animals cannot be random sampling but rather convenience sampling. Also, obtaining representative samples from wildlife populations is often hampered by the lack of precise knowledge on the distribution of wild host populations (EFSA, 2015), although some countries demonstrated that they had such information, based on combining sampling activity results and modelling.
- All the countries participating in this surveillance (Finland, United Kingdom (Northern Ireland), Ireland and Norway) fulfil the technical legal requirements regarding the 12-month surveillance period collection. In general, the lower number of wild animal samples during spring and summer was well justified and historical data show that this lower number does not compromise the success of the detection of the parasite.

- All the countries participating in this surveillance (Finland, United Kingdom (Northern Ireland), Ireland and Norway) fulfil the technical legal requirements regarding the confidence level of at least 0.95 against a design prevalence of 1%.
- The United Kingdom (Northern Ireland) did not provide sufficient scientific evidence to support the test sensitivity (0.99) claimed for the diagnostic test used. Reasons are illustrated in the assessment. Assuming a more conservative test sensitivity value of 0.78, as per EFSA recommendation in the absence of scientific evidence supporting other values, and only in this case, the number of samples collected by the United Kingdom (Northern Ireland) is not sufficient to achieve the confidence level of at least 0.95 against a design prevalence of 1% as required in the relevant legislation. Nonetheless, the confidence achieved in the isle of Ireland, considering the combined data from Ireland and Northern Ireland, is 99.7%.

6. Recommendation

- Norway and Finland are recommended to publish the results of their internal trials performed in order to estimate the sensitivity of the diagnostic assays used. The scientific publication(s) may serve as a basis for an overall project that enable a sound scientific approach in order to validate and estimate the diagnostic sensitivity (and specificity) of the diagnostic assays used for *E. multilocularis* at EU level. This project could be set up in collaboration with EFSA and the EURLP.
- United Kingdom (Northern Ireland) shall consider the critical appraisal provided in this report on the work published by Eckert (2003) related to the TSe (Test Sensitivity) of the SCT and reconsider the value used to substantiate their freedom of *E. multilocularis*. An internal study to estimate the performance of the SCT in United Kingdom (Northern Ireland) is recommended.

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Glossary

Alveolar echinococcosis	The human disease caused by infection with the larval stage (metacestode) of ***E. multilocularis***. It is characterised by infiltrative, tumour-like growth, initially in the liver, potentially causing high fatality rates.
EFSA Data Collection Framework (DCF)	The EFSA web interface accessible by most common web browsers through which data providers can submit their files. The system provides automatic feedback on errors in structure and content, and confirmation of successful submissions.
Enzyme-linked Immunosorbent Assay (ELISA)	The test that applies the immunological concept of an antigen binding to its specific antibody, which allows detection of very small quantities of antigens such as proteins, peptides, hormones or antibody in a fluid sample, utilising enzyme-labelled antibodies or antigens and a chromogenic substrate for the enzyme to detect the target molecules.
GB	Great Britain
Geographical epidemiological unit	The portion of territory within a given Member State characterised by a specific risk of presence which differs from other portions, if any. An example could be the portion of territory within a defined distance from the border. In this assessment, all countries have assumed the entire territory as a unique geographical epidemiological unit.
NI	Northern Ireland
NUTS	The Nomenclature of Territorial Units for Statistics (NUTS), or in French Nomenclature Unités Territoriales Statistiques, is a geocode standard for referencing the administrative divisions of countries for statistical purposes. The standard was developed by the European Union and subdivides the territory of the European Union into regions at three different levels (NUTS 1, 2 and 3), moving from larger to smaller territorial units (see also http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Glossary:NUTS).
Odds Ratio (OR)	The ratio of the odds of an event occurring in one group to the odds of it occurring in another group. It estimates the probability of the event given exposure to a specific factor by measuring the probability of exposure given the presence of the event.

Risk-based Estimate of System sensitivity and Sample size (RiBESS) tool	The Microsoft Excel-based tool developed by EFSA for the calculation of the sample size needed to substantiate absence of a given disease and/or to calculate the survey sensitivity (confidence) once the samples have been collected.
Sedimentation and Counting Technique (SCT)	The technique for the quantitative assessment of the ***E. multilocularis*** burden of foxes or other definitive hosts, where intestinal material is washed and sedimented several times and the resulting sediment is examined under a stereomicroscope for the presence of the parasite.

Abbreviations

ASe	Area sensitivity
CL	Confidence Level
DCF	EFSA Data Collection Framework
DH	Definitive host
DNA	Deoxyribonucleic acid
EFTA	European Free Trade Association
EM	*Echinococcus multilocularis*
GB	Great Britain (including England, Wales and Scotland)
N	Target population size
NI	Northern Ireland
OR	Odds ratio
PCR	Polymerase Chain Reaction
RR	Relative risk
SCT	Sedimentation and Counting Technique
Se	Sensitivity
Sp	Specificity
SSe	System sensitivity
TSe	Test sensitivity
UK(NI)	United Kingdom (Northern Ireland)

Appendix A – Finland. Assessment tables of the surveillance report

A.1. Finland – Part I of surveillance report: checklist on the surveillance system for a representative sample survey and comments

Points addressed in Annex II	Element	Description of Element	Information provided in surveillance report	Requirement fulfilled	Comments
Type and sensitivity of the detection method	Type of test	The diagnostic test used for the detection of EM must be defined. Modifications of the original method should be indicated.	Yes	Yes	Technique well described. A slight modification has been realised and it is indicated in the report.
	Test sensitivity	The sensitivity and specificity of the test used in the surveillance system must be reported. This would ideally be estimates from each participating laboratory reported as a point estimate (average) of the values across the country with minimum and maximum values or a probability distribution. Alternatively, a value of 0.78, as recommended by EFSA, 2015, shall be used.	Yes	Yes	An exact binomial test indicates that the actual value may lie between 0.85 and 0.92 (95% CL). A Bayesian approach gives similar results. Therefore, the lowest value (0.85) may be the most conservative choice for estimating the overall system sensitivity considering a worst-case scenario. However, these results should be published on a scientific journal. Finland used a more conservative approach assuming a test sensitivity of 0.78
Selection of the target population	Definition of susceptible host population targeted by the system	The susceptible wild definitive host population(s) targeted by the surveillance system should be described and the choice justified. If domestic host species are sampled, evidence for the absence of wild definitive hosts and for these domestic animals having had access to outdoors should be provided.	Yes	Yes	NA

Points addressed in Annex II	Element	Description of Element	Information provided in surveillance report	Requirement fulfilled	Comments
	Size of susceptible host population targeted by the system	The size of the targeted (wildlife) population should be reported, together with the evidence for this. Historical population data should be updated since these may not reflect current populations.	Yes	Yes	Although population data have not been updated since 2007, new information regarding annual hunting bags has been included in the report. The decision to use the size of the population as published by Kauhala in the estimations is scientifically sound, considering that the sample size calculation is not heavily affected when the population size has large dimensions (see EFSA AHAW Panel, 2015). The fact of considering the sum of the red fox and raccoon dog populations as the target population size seems to be correct, as raccoon dogs can act as DHs in conjunction with the red fox (EFSA AHAW Panel, 2015).
Sampling strategy	Epidemiological unit	It should be clearly defined if individual animals or individual faeces samples collected from the environment constitute the epidemiological unit. If individual faeces samples are collected from the environment, the method applied to establish the species from which the faeces originated has to be reported.	Yes	Yes	NA
	Sample size calculation	The applied survey design should be fully documented, including considerations regarding potential biases inherent in the survey design. The method and the formula used to calculate the sample size should be fully documented.	Yes	Yes	NA
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must to be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be homogeneous.	Yes	Yes	NA

Points addressed in Annex II	Element	Description of Element	Information provided in surveillance report	Requirement fulfilled	Comments
Methodology	Design Prevalence (DP)	DP is specified in Annex II to Commission Delegated Regulation (EU) 2018/772 and must be 1% or lower.	Yes	Yes	NA
	Geographical epidemiologic unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification.	Yes	Yes	NA
	Methodology for calculation of area sensitivity	For the calculation of the area sensitivity, the diagnostic sensitivity should be set conservatively to the lowest value, excluding the lowest 20th percentile, from the ones reported in the scientific literature and related to the diagnostic tests implemented by the countries listed in Annex I of the Commission Delegated Regulation (EU) 2018/772. In this case, is 78% (EFSA AHAW Panel, 2015).	Yes	Yes	NA

A.2. Finland – Part II of surveillance report: descriptive statistics for a representative survey

Parameter	Evidence	Requirement fulfilled	Action/Comment
Theoretical Sampling period	From 1 January 2021 to 31 December 2021	Yes	NA
Actual Sampling Period	NA	NA	NA
Number of samples	653	Yes	NA
Number of test results	653	Yes	The sample size achieves an area sensitivity of 0.994 (> 0.95)
Sensitivity	0.78	Yes	NA
Host	Raccoon dog and Red fox	Yes	NA
Animal sample	Yes	Yes	NA
Sampling Strategy and Design Objective sampling	Objective sampling and Simple random sample	Yes	The sampling strategy is actually a convenience sampling, biologically driven. The latter, in wildlife, is considered adequate
Sampling point	Wild (Hunting)	Yes	NA

Appendix B – Ireland. Assessment tables of the surveillance report

B.1. Ireland – Part I of surveillance report: checklist of the description of the surveillance system for a representative sample survey

Points addressed in Annex II	Element	Description of Element	Information provided in surveillance report	Requirement fulfilled	Comments
Type and sensitivity of the detection method	Type of test	The diagnostic test used for the detection of EM must be defined. Modifications of the original method should be indicated.	Yes	Yes	The diagnostic test chosen by Ireland is well described (PCR Cest1-Cest2NAD1) and a reference for this peer-reviewed published method is provided
	Test sensitivity	The sensitivity and specificity of the test used in the surveillance system must be reported. This would ideally be estimates from each participating laboratory reported as a point estimate (average) of the values across the country with minimum and maximum values or a probability distribution. Alternatively, a value of 0.78, as recommended by EFSA, 2015, shall be used.	Yes	Yes	NA
Selection of the target population	Definition of susceptible host population targeted by the system	The susceptible wild definitive host population(s) targeted by the surveillance system should be described and the choice justified. If domestic host species are sampled, evidence for the absence of wild definitive hosts and for these domestic animals having had access to outdoors should be provided.	Yes	Yes	The absence of other important definitive wild hosts is also supported by scientific literature
	Size of susceptible host population targeted by the system	The size of the targeted (wildlife) population should be reported, together with the evidence for this. Historical population data should be updated since these may not reflect current populations.	Yes	Yes	The last update on the population size is from 2015. However, with a population size greater than 10,000,moderate fluctuations in the population size would not significantly change the sample size required

Points addressed in Annex II	Element	Description of Element	Information provided in surveillance report	Requirement fulfilled	Comments
Sampling strategy	Epidemiological unit	It should be clearly defined if individual animals or individual faeces samples collected from the environment constitute the epidemiological unit. If individual faeces samples are collected from the environment, the method applied to establish the species from which the faeces originated has to be reported.	Yes	Yes	NA
	Sample size calculation	The applied survey design should be fully documented, including considerations regarding potential biases inherent in the survey design. The method and the formula used to calculate the sample size should be fully documented.	Yes	Yes	NA
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must to be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be homogeneous.	Yes	Yes	NA
Methodology	Design Prevalence (DP)	DP is specified in Annex II to Regulation (EU) No 1152/2011 and must be 1% or lower.	Yes	Yes	NA
	Geographical epidemiologic unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification.	Yes	Yes	NA
	Methodology for calculation of area sensitivity	For the calculation of the area sensitivity, the diagnostic sensitivity should be set conservatively to the lowest value, excluding the lowest 20th percentile, from the ones reported in the scientific literature and related to the diagnostic tests implemented by the countries listed in Annex I of the Commission Delegated Regulation (EU) No 1152/2011. In this case, is 78% (EFSA AHAW Panel, 2015).	Yes	Yes	NA

B.2. Ireland – Part II of surveillance report: descriptive statistics for a representative survey

Parameter	Evidence	Requirement fulfilled	Action/Comments
Theoretical Sampling period	From 1 January 2021 to 31 December 2021	NA	NA
Actual Sampling Period	6 January 2021 to 31 December 2021	Yes	NA
Number of samples	398	Yes	The sample size achieves an area sensitivity of 0.956 (> 0.95)
Number of test results	398	Yes	NA
Sensitivity	0.78	Yes	NA
Host	Red fox	Yes	NA
Animal sample	Yes	Yes	NA
Sampling Strategy and Design Objective sampling	Objective sampling and Simple random sample	Yes	The sampling strategy is actually a convenience sampling, biologically driven. The latter, in wildlife, is considered adequate
Sampling point	Hunting and wildlife research stations	Yes	NA

Appendix C – United Kingdom (Northern Ireland). Assessment tables of the surveillance report

C.1. Northern Ireland – Part I of surveillance report: checklist of the description of the surveillance system for a representative sample survey

Points addressed in Annex II	Element	Description of Element	Information provided in surveillance report	Requirement fulfilled	Comments
Type and sensitivity of the detection method	Type of test	The diagnostic test used for the detection of EM must be defined. Modifications of the original method should be indicated.	Yes	Yes	The diagnostic test chosen is properly described
	Test sensitivity	The sensitivity and specificity of the test used in the surveillance system must be reported. This would ideally be estimates from each participating laboratory reported as a point estimate (average) of the values across the country with minimum and maximum values or a probability distribution. Alternatively, a value of 0.78, as recommended by EFSA, 2015, shall be used.	Yes	Yes	The evidence provided to support the test sensitivity value for the SCT (Eckert, 2003) actually refers to a previous work (Hofer et al., 2000) which focusses on the prevalence in the target population and not in the sensitivity of the SCT. **The almost perfect sensitivity of the SCT (0.99) is actually an assumption.** A safer option would be to follow the EFSA recommendation (Test Se = 0.78). As an alternative, Should provide evidence to support the suggested test sensitivity value of 0.99
Selection of the target population	Definition of susceptible host population targeted by the system	The susceptible wild definitive host population(s) targeted by the surveillance system should be described and the choice justified. If domestic host species are sampled, evidence for the absence of wild definitive hosts and for these domestic animals having had access to outdoors should be provided.	Yes	Yes	NA
	Size of susceptible host population targeted by the system	The size of the targeted (wildlife) population should be reported, together with the evidence for this. Historical population data should be updated since these may not reflect current populations.	Yes	Yes	NA

Points addressed in Annex II	Element	Description of Element	Information provided in surveillance report	Requirement fulfilled	Comments
Sampling strategy	Epidemiological unit	It should be clearly defined if individual animals or individual faeces samples collected from the environment constitute the epidemiological unit. If individual faeces samples are collected from the environment, the method applied to establish the species from which the faeces originated has to be reported.	Yes	Yes	NA
	Sample size calculation	The applied survey design should be fully documented, including considerations regarding potential biases inherent in the survey design. The method and the formula used to calculate the sample size should be fully documented.	Yes	Yes	The required sample size was estimated using the RiBESS tool. The method used is therefore scientifically correct. Some issues may originate from the assumption on the test sensitivity used in the formula.
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must to be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be homogeneous.	Yes	Yes	NA
Methodology	Design Prevalence (DP)	DP is specified in Annex II to Regulation (EU) No 1152/2011 and must be 1% or lower.	Yes	Yes	NA
	Geographical epidemiologic unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification.	Yes	Yes	NI was correctly considered as one independent epidemiological unit in the analysis.
	Methodology for calculation of area sensitivity	For the calculation of the area sensitivity, the diagnostic sensitivity should be set conservatively to the lowest value, excluding the lowest 20th percentile, from the ones reported in the scientific literature and related to the diagnostic tests implemented by the countries listed in Annex I of the Commission Delegated Regulation (EU) No 1152/2011. In this case, is 78% (EFSA AHAW Panel, 2015).	Yes	Yes	Only assuming the test sensitivity value proposed by NI (0.99) the area sensitivity achieved satisfies the legal requirements (0.968). Note: the absence of scientific evidence does not imply that the proposed test sensitivity (0.99) is wrong. EFSA, however, in the absence of adequate scientific evidence cannot assess the performance of the surveillance activity.

C.2. Northern Ireland – Part II of surveillance report: descriptive statistics for a representative survey

Parameter	Evidence	Requirement fulfilled	Action
Theoretical Sampling period	From 1 January to 31 December	NA	NA
Actual Sampling Period	4 January 2021 to 31 December 2021	Yes	NA
Number of samples	346	Yes	NA
Number of test results	346	Yes (IF TSe = 0.99)	The sample size is adequate to fulfil the legal requirements only assuming the assumed test sensitivity of 0.99
Sensitivity	0.99	Impossible to evaluate	The value is not supported by adequate scientific evidence
Host	Red fox	Yes	NA
Animal sample	Yes	Yes	NA
Sampling Strategy and Design Objective sampling	Objective sampling and Simple random sample	Yes	The sampling strategy is actually a convenient sampling based on biological considerations. Considered adequate in wildlife
Sampling point	Hunting and road transport	Yes	NA

Appendix D – Norway. Assessment tables of the surveillance report

D.1. Norway – Part I of surveillance report: checklist of the description of the surveillance system for a representative sample survey

Points addressed in Annex II	Element	Description of Element	Information provided in surveillance report	Requirement fulfilled	Comments
Type and sensitivity of the detection method	Type of test	The diagnostic test used for the detection of EM must be defined. Modifications of the original method should be indicated.	Yes	Yes	The diagnostic test chosen is properly described
	Test sensitivity	The sensitivity and specificity of the test used in the surveillance system must be reported. This would ideally be estimates from each participating laboratory reported as a point estimate (average) of the values across the country with minimum and maximum values or a probability distribution. Alternatively, a value of 0.78, as recommended by EFSA, 2015, shall be used.	Yes	Yes	Despite internal trials seem to indicate a better performance of the test (Test Se = 0.77, with a95% CI = 0.70–0.83), a more conservative value was set (0.63, Øines et al., 2014). This value is lower than the minimum recommended by EFSA (0.78). Such a low test sensitivity implies a much higher effort to reach the 95%of confidence stated in the legislation, as a large sample size is required.
Selection of the target population	Definition of susceptible host population targeted by the system	The susceptible wild definitive host population(s) targeted by the surveillance system should be described and the choice justified. If domestic host species are sampled, evidence for the absence of wild definitive hosts and for these domestic animals having had access to outdoors should be provided.	Yes	Yes	NA
	Size of susceptible host population targeted by the system	The size of the targeted (wildlife) population should be reported, together with the evidence for this. Historical population data should be updated since these may not reflect current populations.	Yes	Yes	NA

Points addressed in Annex II	Element	Description of Element	Information provided in surveillance report	Requirement fulfilled	Comments
Sampling strategy	Epidemiological unit	It should be clearly defined if individual animals or individual faeces samples collected from the environment constitute the epidemiological unit. If individual faeces samples are collected from the environment, the method applied to establish the species from which the faeces originated has to be reported.	Yes	Yes	NA
	Sample size calculation	The applied survey design should be fully documented, including considerations regarding potential biases inherent in the survey design. The method and the formula used to calculate the sample size should be fully documented.	Yes	Yes	NA
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must to be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be homogeneous.	Yes	Yes	NA
Methodology	Design Prevalence (DP)	DP is specified in Annex II to Regulation (EU) No 1152/2011 and must be 1% or lower.	Yes	Yes	NA
	Geographical epidemiologic unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification.	Yes	Yes	
	Methodology for calculation of area sensitivity	For the calculation of the area sensitivity, the diagnostic sensitivity should be set conservatively to the lowest value, excluding the lowest 20th percentile, from the ones reported in the scientific literature and related to the diagnostic tests implemented by the countries listed in Annex I of the Commission Delegated Regulation (EU) No 1152/2011. In this case, is 78% (EFSA AHAW Panel, 2015).	Yes	Yes	

D.2. Norway – Part II of surveillance report: descriptive statistics for a representative survey

Parameter	Evidence	Requirement fulfilled	Action
Theoretical Sampling period	From 1 January to 31 December	NA	NA
Actual Sampling Period	1 January 2021 to 20 December 2021	Yes	NA
Number of samples	511	Yes	NA
Number of test results	511	Yes	The sample size achieves an area sensitivity of 0.961 (> 0.95)
Sensitivity	0.63	Yes	NA
Host	Red fox	Yes	NA
Animal sample	Yes	Yes	NA
Sampling Strategy and Design Objective sampling	Objective sampling and Simple random sample	Yes	The sampling strategy is actually a convenient sampling based on biological considerations. Considered adequate in wildlife
Sampling point	Hunting	Yes	NA