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Annual assessment of *Echinococcus multilocularis* surveillance reports submitted in 2021 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 in 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. Northern Ireland did not fulfil those requirements, not even 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.

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Summary

Following a request from the European Commission and, indirectly, from the European Free Trade Association (EFTA) Surveillance Authority, the Animal and Plant Health Unit (ALPHA) 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: 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 the 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 by Finland, Ireland, United Kingdom (Northern Ireland) and Norway as reported in 2021 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%, with a 95% confidence level.

All the countries selected adequate wild definitive hosts in order to perform the surveillance. In United Kingdom, Northern Ireland does not fulfil the requirements of Commission Delegated Regulation (EU) 2018/772 related to the desired confidence level of 95% not even when 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 Article 2 of Regulation EU 2018/878 of 18 June 2018 United Kingdom in respect of Northern Ireland is referenced as 'Member State'.

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 oligarthra*). 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%).² Table 1 reports the number of cases and notification rates in the EU/EFTA by country and year. *Echinococcus 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, 2019)

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			
				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 hosts (rodents) and the oncosphere migrates inside them until reaching target organs such as the liver (Peregrine et al., 2012; CDC, online). 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 2; 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 – updated by Adriano Casulli, 2021, personal communication)

Definitive hosts	
Red fox (<i>Vulpes vulpes</i>)	Considered the main DH
Arctic fox (<i>Vulpes lagopus</i>)	In Europe, only relevant in Svalbard (Norway)
Raccoon dog (<i>Nyctereutes procyonoides</i>), Wolf (<i>Canis lupus</i>), Golden jackal (<i>Canis aureus</i>)	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 (<i>Canis lupus familiaris</i>) and wild cat (<i>Felis s. silvestris</i>)	Overall, prevalence of dogs with the parasite is low. Even if they may play a secondary role in the maintenance of the life cycle of the parasite, because of the strict contact with humans, they pose a risk for human infection. It should be also considered that some behaviours of dogs [scent-rolling on fox (positive) faeces, or ingestion of fox (positive) faeces] even if dogs are not infected, may facilitate human infections (Conraths et al., 2017). On the contrary, cats hardly get infected experimentally, but their natural infection has been reported in numerous occasions. However, when worms are present, eggs seem not viable. For both species, further information is needed.
Intermediate hosts	
Common vole (<i>Microtus arvalis</i>), field vole (<i>Microtus agrestis</i>), common pine vole (<i>Microtus subterraneus</i>), sibling vole (<i>Microtus levis</i>), bank voles (<i>Myodes</i> spp.), water voles (<i>Arvicola</i> spp.), snow vole (<i>Chionomys nivalis</i>), lemming (<i>Lemmus lemmus</i>)	Various species of voles are confirmed as suitable hosts. However, factors such as their population densities and predation rates may influence their role in the cycle.
Muridae (<i>Apodemus</i> spp., <i>Mus</i> spp., <i>Rattus</i> spp.), brown hare (<i>Lepus europaeus</i>), shrew (<i>Sorex</i> spp.)	Although some murid rodents, hares and shrews are susceptible, natural infections occur only sporadically
Muskrat (<i>Ondatra zibethicus</i>), beaver (<i>Castor</i> spp.), nutria (<i>Myocastor coypu</i>), Alpine marmot (<i>Marmota marmota</i>)	Large rodents are susceptible hosts. Their role seems to be related to the dispersion of the parasite; e.g. through introduction/reintroduction (beaver)
Suids, horses and domestic dogs	Only accidental or refractory intermediate hosts. They are dead end hosts: vesicles are abortive and cannot transmit the parasite to DE.

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, 2019). See Table 3 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 southwestern part of Hungary, with a prevalence of 15.6% and mean intensity of 664 worms (Balog et al., 2021).

Table 3: Summary of human and animal echinococcosis caused by *Echinococcus multilocularis* and *Echinococcus granulosus sensu lato* in the EU, 2015–2019 (EFSA and ECDC, 2019)

	2019	2018	2017	2016	2015	Data source
Humans (cystic and alveolar)						
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

	2019	2018	2017	2016	2015	Data source
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 s.l. 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
Echinococcus granulosus s.l. 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 s.l. 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

ECDC: European Centre for Disease Prevention and Control; EFSA: European Food Safety Authority; MS: Member States.

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 4). The prevalence has been reported to range from 0% to more than 50% (EFSA AHAW Panel, 2015).

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%–< 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 *E. 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, considering 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 *E. 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 *E. 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 31 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 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 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 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 countries, in accordance with certain requirements regarding the sampling, the detection techniques and the reporting.

[omissis]

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 2021 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 5) 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 <i>E. multilocularis</i> 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 <i>E. multilocularis</i> 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 <i>E. multilocularis</i> parasite, if present in any part of the Member State, at the design prevalence of less than 1%.	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%.	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.

Points addressed in the Annex II	Element	Description of element
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.
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. Time frame 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% 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 (R core Team, 2019) was used to compute descriptive statistics. Table 7 lists and describes all the parameters that were extracted from the data submitted.

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.

	Parameter	Description
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).
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 12S 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 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 2015.

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 12S 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/12S rDNA real-time by Isaksson et al., 2014). (Figure 1 on results of spiked samples).

Table 2 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	0.68 [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]

Values of Sensitivity (SE), Specificity (SP), Positive predictive values (PPV) Negative predictive values (NPV) and Area under the curve (AUC) for the different DNA extractions and detection methods tested in the study. The table is divided into the whole dataset of all samples (n = 144), and separately for samples containing low numbers of *E. multilocularis* eggs (<15) (n = 84). Detection was performed in triplicate, however, due to little DNA available, *CO1rtPCR on DNA-fishing was only possible in duplicate. Egg numbers added to each sample are available in Additional file 1: Table S2.

Figure 1: Table extracted from Øines et al., 2014 on the results of the molecular analysis of the spiked fox faeces batches using four detection methods

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, which demonstrates an overall sensitivity of 0.70. The sensitivity is positively correlated with the amount of DNA in the samples. In samples with ≥ 10 eggs, the sensitivity is > 0.83. For samples with ≥ 5 eggs, the sensitivity is > 0.58 (see Figure 2).

Year	1 egg		5 eggs			10 eggs			50 eggs			one whole worm			
	Number of samples tested	Number of positive samples	Se*	Number of samples tested	Number of positive samples	Se*	Number of samples tested	Number of positive samples	Se*	Number of samples tested	Number of positive samples	Se*	Number of samples tested	Number of positive samples	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
overall	38	16	0,42	12	7	0,58	36	30	0,83	2	2	1,00	35	31	0,89

* sensitivity

Figure 2: Table reporting the results from testing spiked samples (2015–2020 data)

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 2020 both on identification of *Echinococcus* worms in the intestinal mucosa of the definitive host and identification to species level was positive.

3.2. Target population

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 on line: <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 2015–2019 (latest available data) was 168,100 raccoon dogs and 46,100 red foxes. Therefore, FI estimated the population sizes of the **raccoon dog and the red fox** to be $2 \times 168,100 = 336,200$ individuals and $2.5 \times 46,100 = 115,250$ individuals, respectively. The estimated size of the susceptible population is therefore **451,450**.

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.

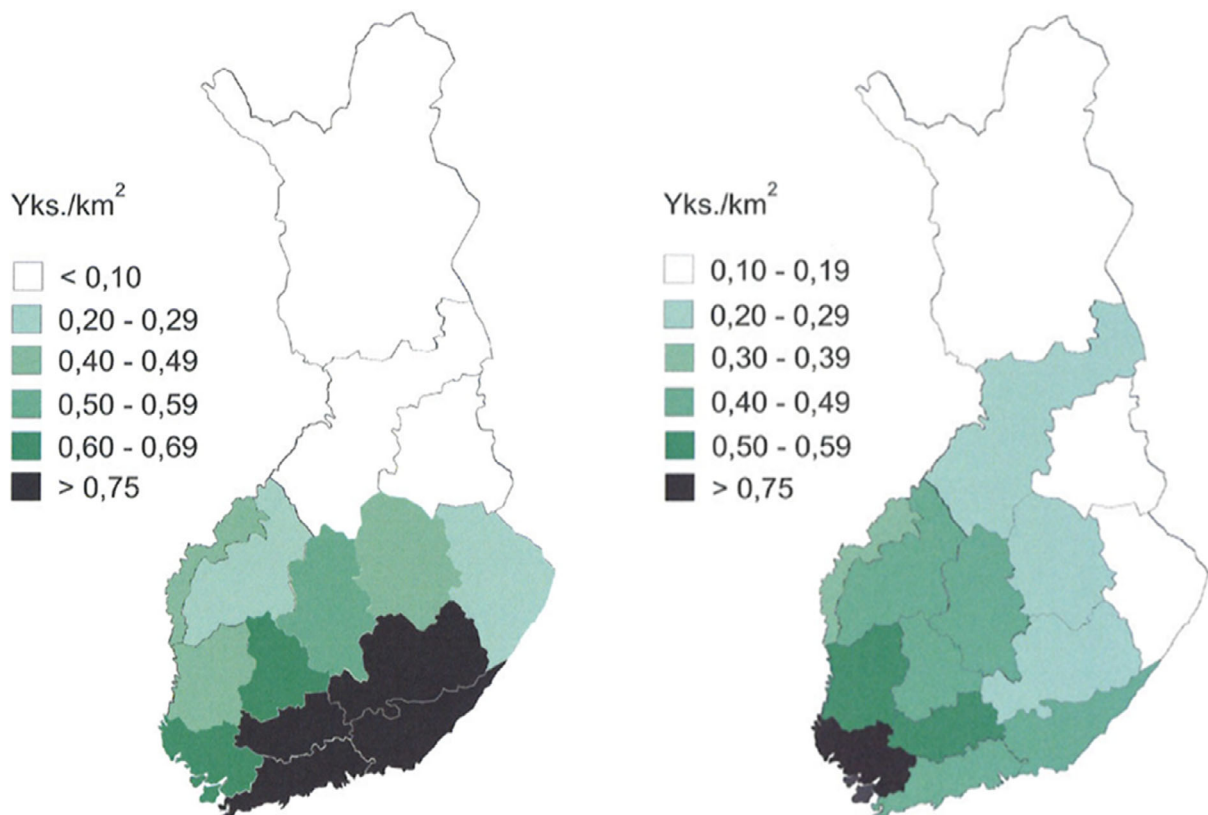


Figure 3: Finland – Raccoon dog densities (left) and red fox densities (right) according to Kauhala (2007) (Yks./km² = individuals/km²)

The hunting bag of the raccoon dog has been biggest in the four south-eastern regions in 2017–2019. As for the red fox, the largest regional hunting bag in recent years has been achieved in Lapland where hunting effort has been high. 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 southwestern 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. 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. The red fox inhabits the whole country including the northernmost 'fjeld' regions where densities can be locally high. The raccoon dog is continuously spreading towards north and nowadays a few hundred individuals are hunted yearly even in southern Lapland.

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 (that is, 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.

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.

³ <https://www.riistakolmiot.fi/raportit/kettu-2021/>

⁴ <https://www.npws.ie/sites/default/files/publications/pdf/RL3.pdf> see p.35.

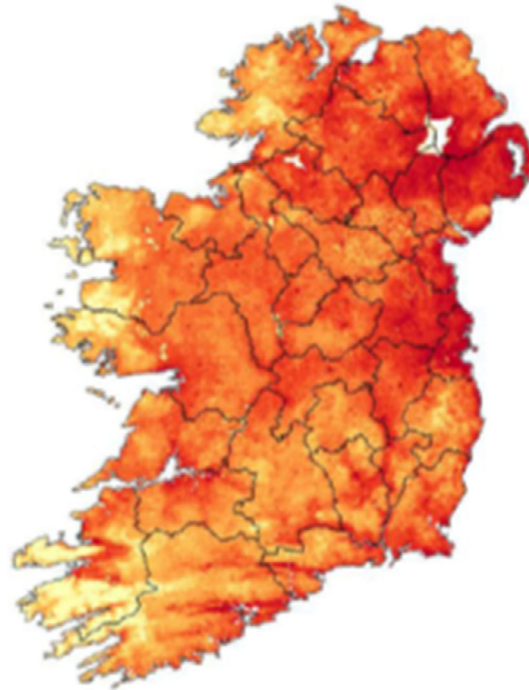


Figure 4: Ireland – Probability of presence per 1 km² from the final Maxent species distribution model (Phillips et al., 2006) for red fox. Source: data up to 2015 provided by Dr. Tomás Murray, from National Biodiversity Data Centre (Ireland)

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, an estimate of 14,000 is given, which is equivalent of 1 fox per km² and accounts for the large area of rural land in contrast to the urban land use (Conserve Ireland, 2009). This probability of presence per 1 km² 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. Tom as Murray, from National Biodiversity Data Centre (Ireland). 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.

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. It has been estimated that for the period 2018–2020, a population of 273–338 adult animals.⁵ In winter 2020–2021, there were 57–58 wolves registered in Norwegian territories and 52–56 wolves living in territories that are located partly in Norway and partly in Sweden.⁶ In addition to the 532 red foxes tested in 2020 as part of our official surveillance programme, samples from small number (i.e. 20)

⁵ <https://brage.nina.no/nina-xmlui/handle/11250/2719248>

⁶ <https://rovdata.no/Ulv/Bestandsstatus.aspx>

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 km² (mountain areas), 3 red foxes/10 km² (forest/marsh) and 10 red foxes/10 km² (urban/agricultural areas; e.g. Akershus, Vestfold, Østfold) (personal communication Prof. Olav Hjeljord, 29/6/2020). See also Figure 5.

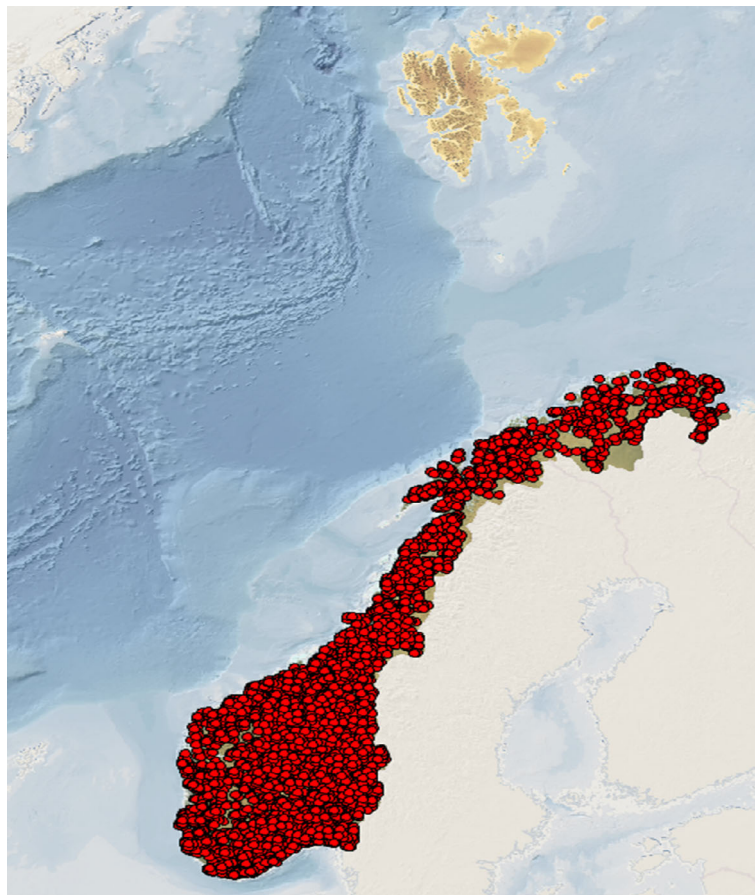


Figure 5: Map showing observations of red fox in Norway. Online service where citizens can logon and register their observations of fauna and flora in Norway. Source: Norwegian Biodiversity Information Centre. <https://artsdatabanken.no/Pages/180936>

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 451,450 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 website⁷ 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. A total of 310 and 216 samples were collected from raccoon dogs and foxes, respectively (N = 526).

The majority of the samples originated from Southeast Finland as this is the region where active monitoring of rabies control programme has taken place since 1990 (Pohjois-Karjala, Etelä-Karjala, Etelä-Savo, Kymenlaakso, 59% of the samples). 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. The red fox inhabits the whole country including the northernmost 'fjeld' regions where densities can be locally high. 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 1:1.45) but not in raccoon dogs (1:0.99). Of the animals that could be classified by age (N-age = 477), 61% were juveniles. The proportion of juveniles was 67% in raccoon dogs and 47% 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, 59% of the samples). A large sample of foxes (28% of all animals) was received from Lappi (Lapland) where active red fox population reduction to protect the arctic fox was ongoing (see Figures 6 and 8).

Samples were collected throughout 2020 (see Figure 7). 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 and female raccoon dogs with pups are protected, and consequently, hunting is only focused on diseased or injured individuals. However, the raccoon dog was recently (1 June 2019) classified in the Finnish law as an alien invasive species with no protection seasons.

All 526 samples were negative by PCR. Thus, no sample was found positive for *E. multilocularis*.

⁷ <https://www.ruokavirasto.fi/>

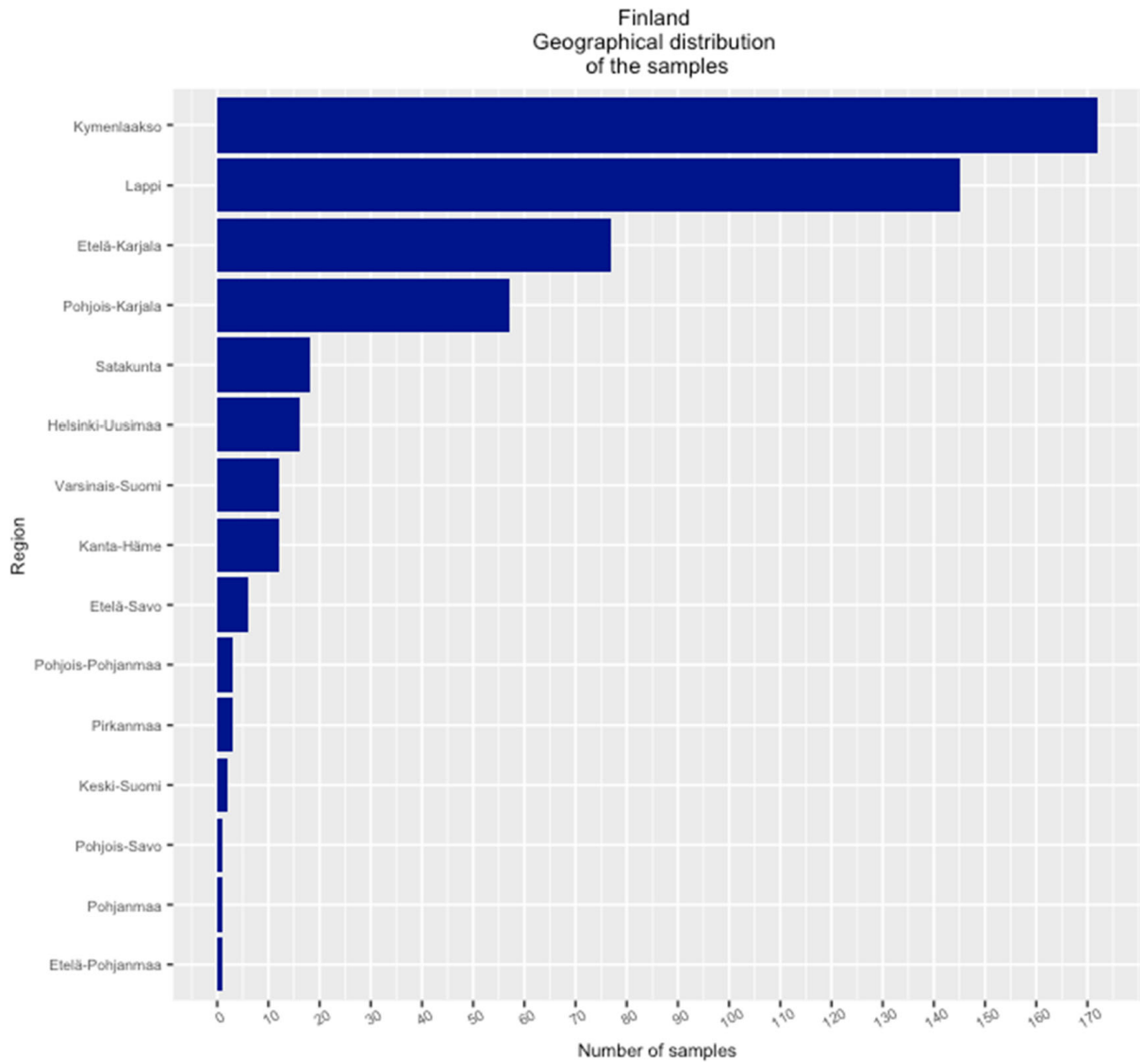


Figure 6: Finland – Geographical distribution of samples

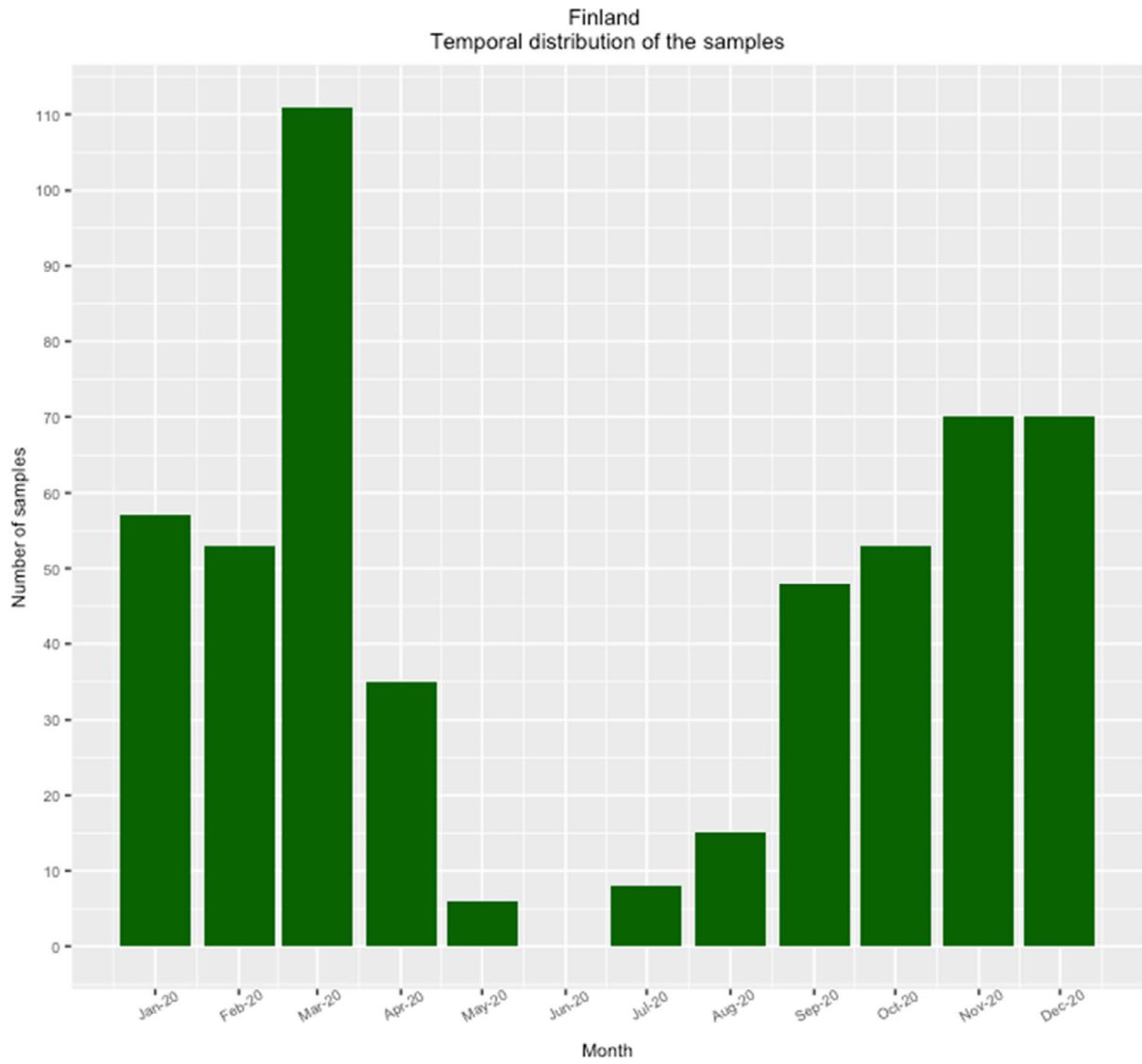


Figure 7: Finland – Temporal distribution of samples

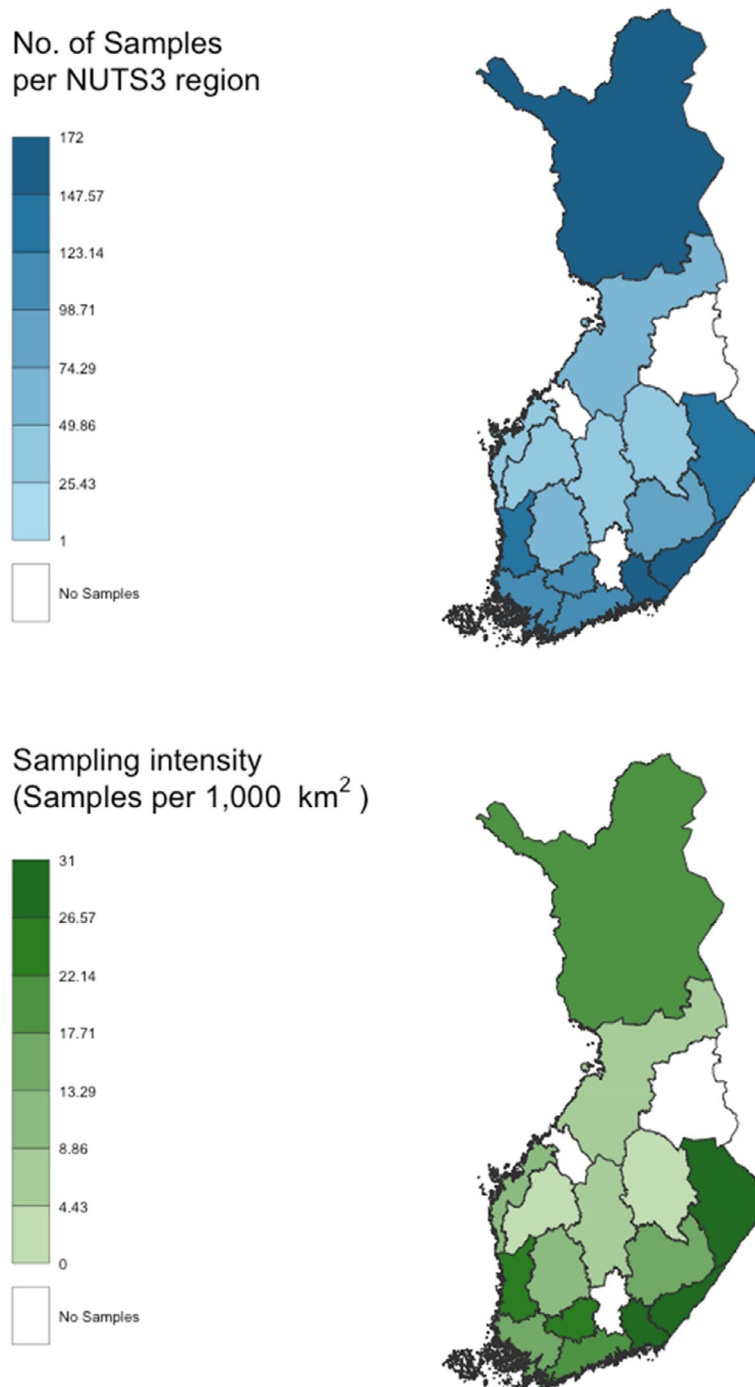


Figure 8: Finland – Sampling activity and intensity by NUTS 3 region

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 were 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 404 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 8 and 11). Samples were obtained during 9 months of the year (see Figure 9). 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).

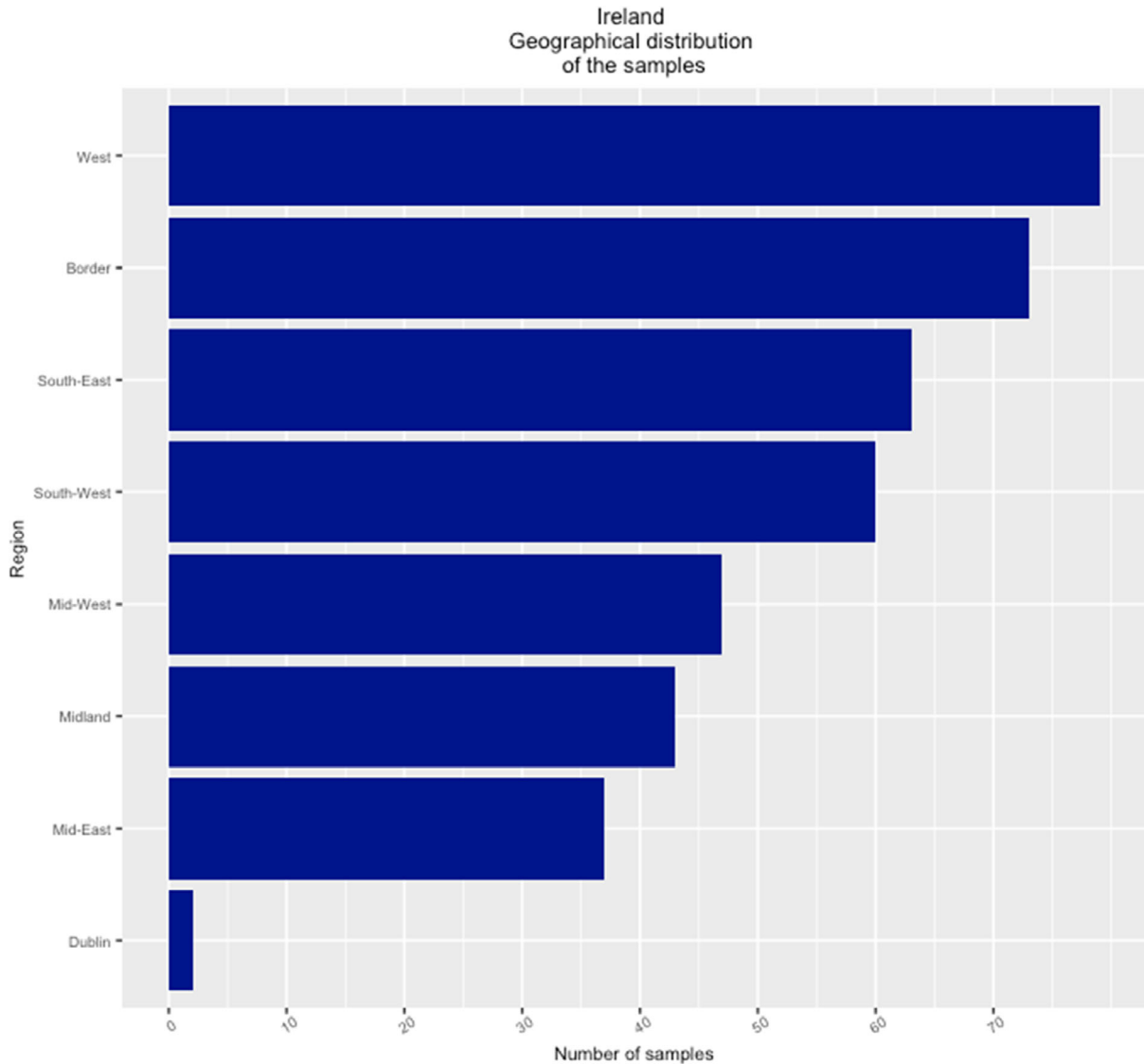


Figure 9: Ireland – Geographical distribution of samples

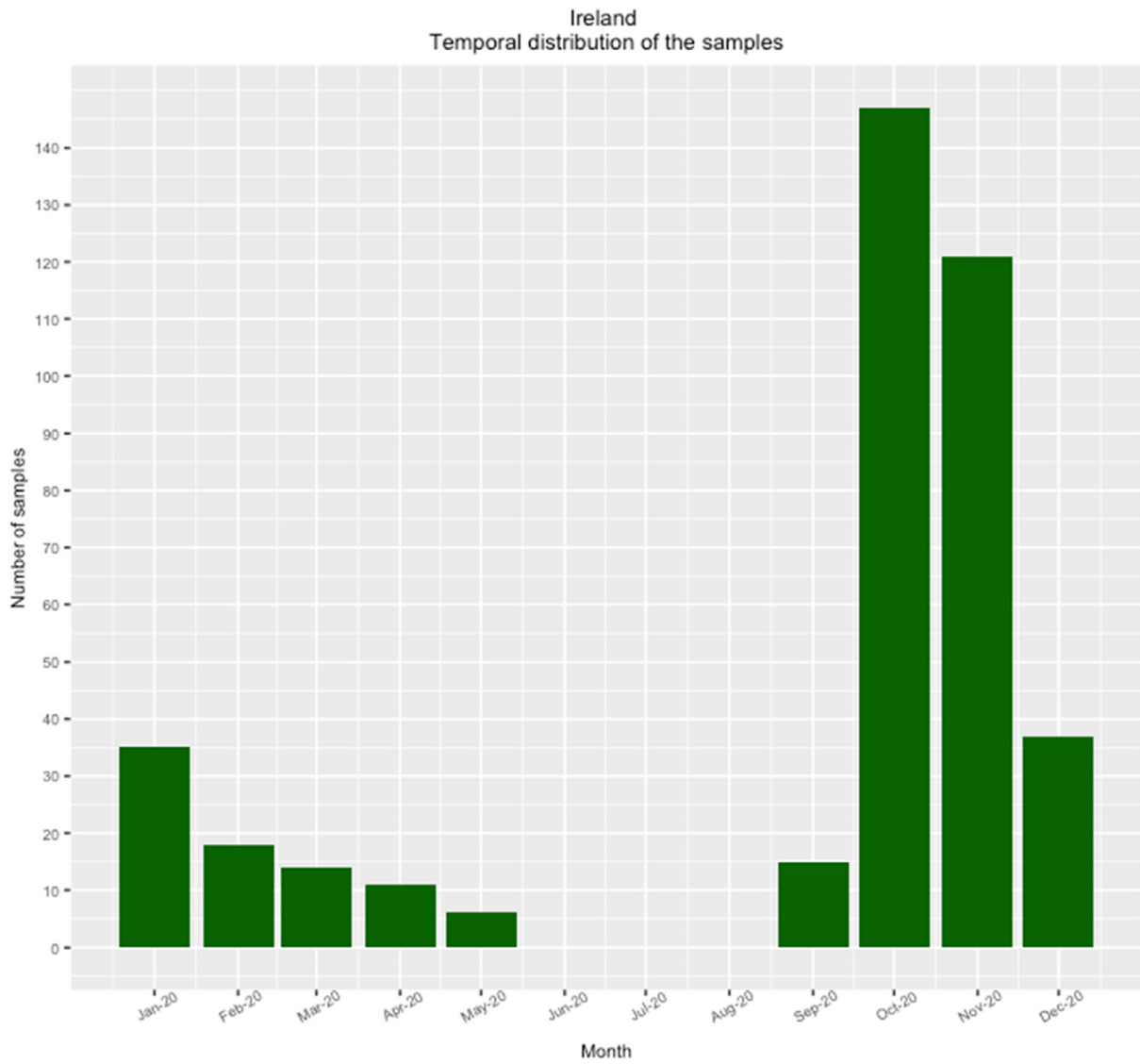


Figure 10: Ireland – Temporal distribution of samples

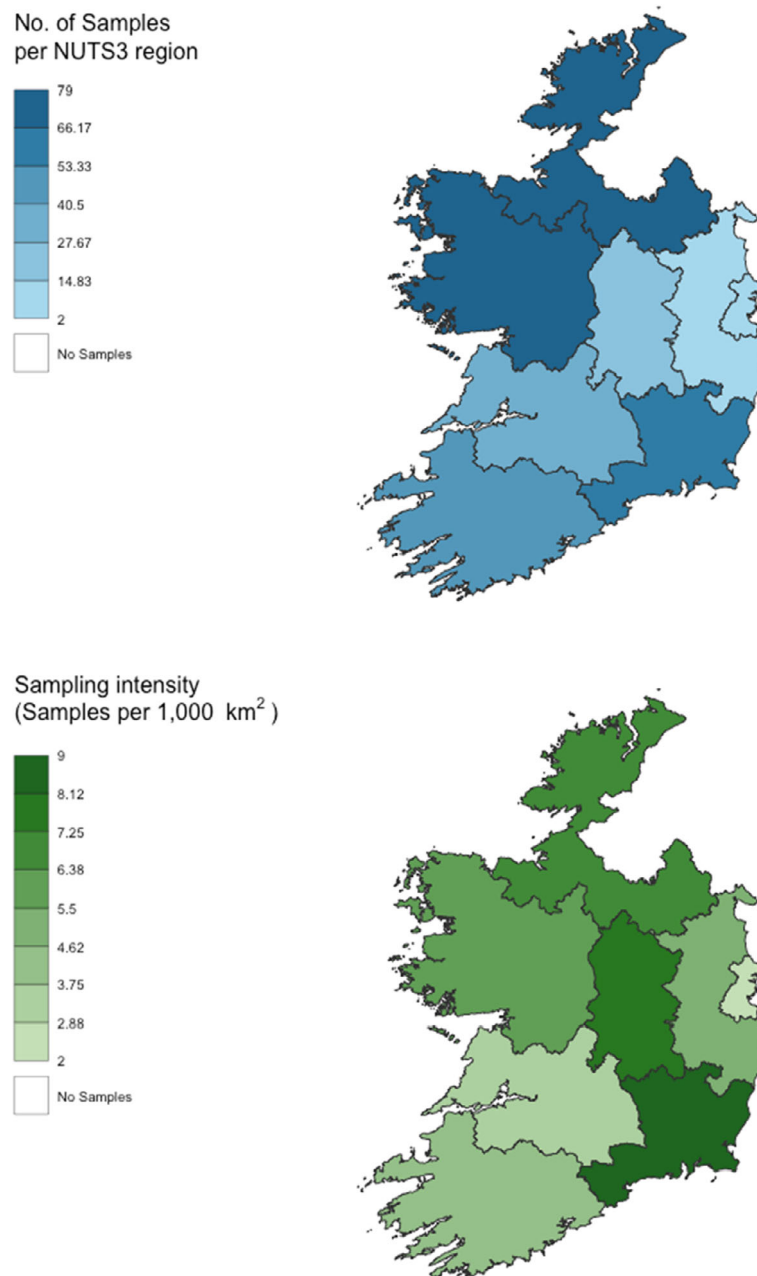


Figure 11: Ireland – Sampling activity and intensity by NUTS 3 region

3.3.3. United Kingdom (Northern Ireland)

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

The sample size was calculated using the EFSA RiBESS tool. 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, road kills or research stations; therefore, only an approximate location of the animal can be used. Hunters and gamekeepers who shoot foxes as part of pest population control were contracted to collect carcasses. Carcasses were delivered to field stations and frozen until sampling was undertaken. Road kills were only occasionally suitable for testing; therefore, the number was low. There have been significant issues this year with the sampling plan resulting in a lower than expected number of samples from Northern Ireland. Firstly, the COVID-19 restrictions meant there were fewer people on the roads and therefore far fewer road kills. Pest control agents were not considered a key worker role, and therefore, the number of foxes killed was

fewer. The change to the reporting dates also means many foxes all of which tested negative were not counted in this year report. However, COVID restrictions also meant no people were travelling with their pets to countries where *E. multilocularis* is considered endemic and which could have resulted in the pets bringing infection to the area. The only land border is with the Republic of Ireland which had a similar approach to travel and working restrictions. Therefore, despite the lower number of definitive hosts being tested this year for the area of Northern Ireland, there is every reason to believe the likelihood of disease incursion was greatly reduced. Reports were made at NUTS 3 level (the lowest level of NUTS: districts in Northern Ireland) (See Figure 14). The NUTS boundaries are only rarely amended, and therefore, comparisons could be made from one year to the next in terms of distribution.

In NI, 164 samples were collected and tested. The sampling activity targeted the regions with higher fox density (See Figure 4).

Sampling was carried out at certain times of the year; the target was the wild population and hunting was not permitted during the breeding season (See Figures 12 and 13).

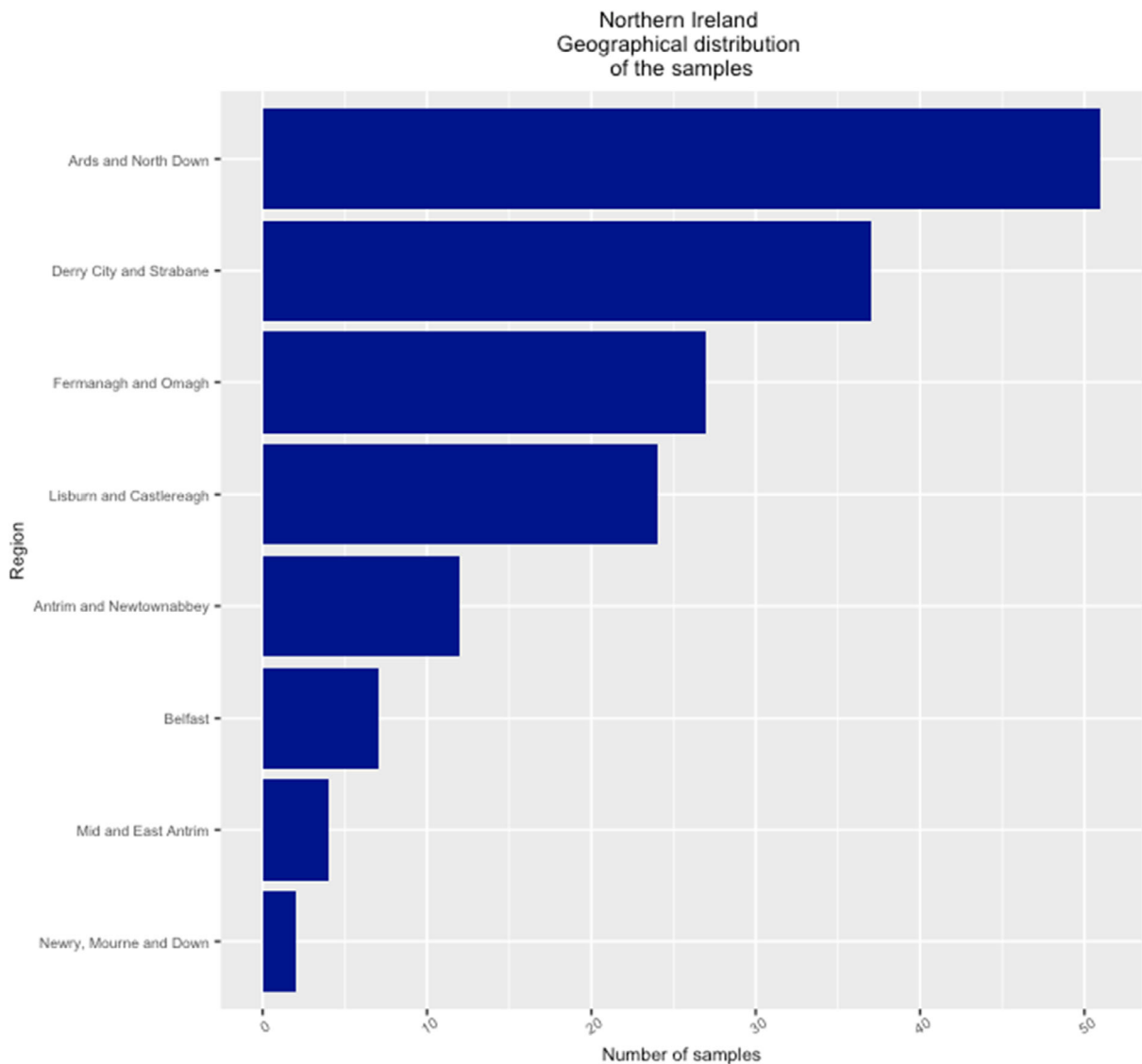


Figure 12: Northern Ireland – Geographical distribution of samples

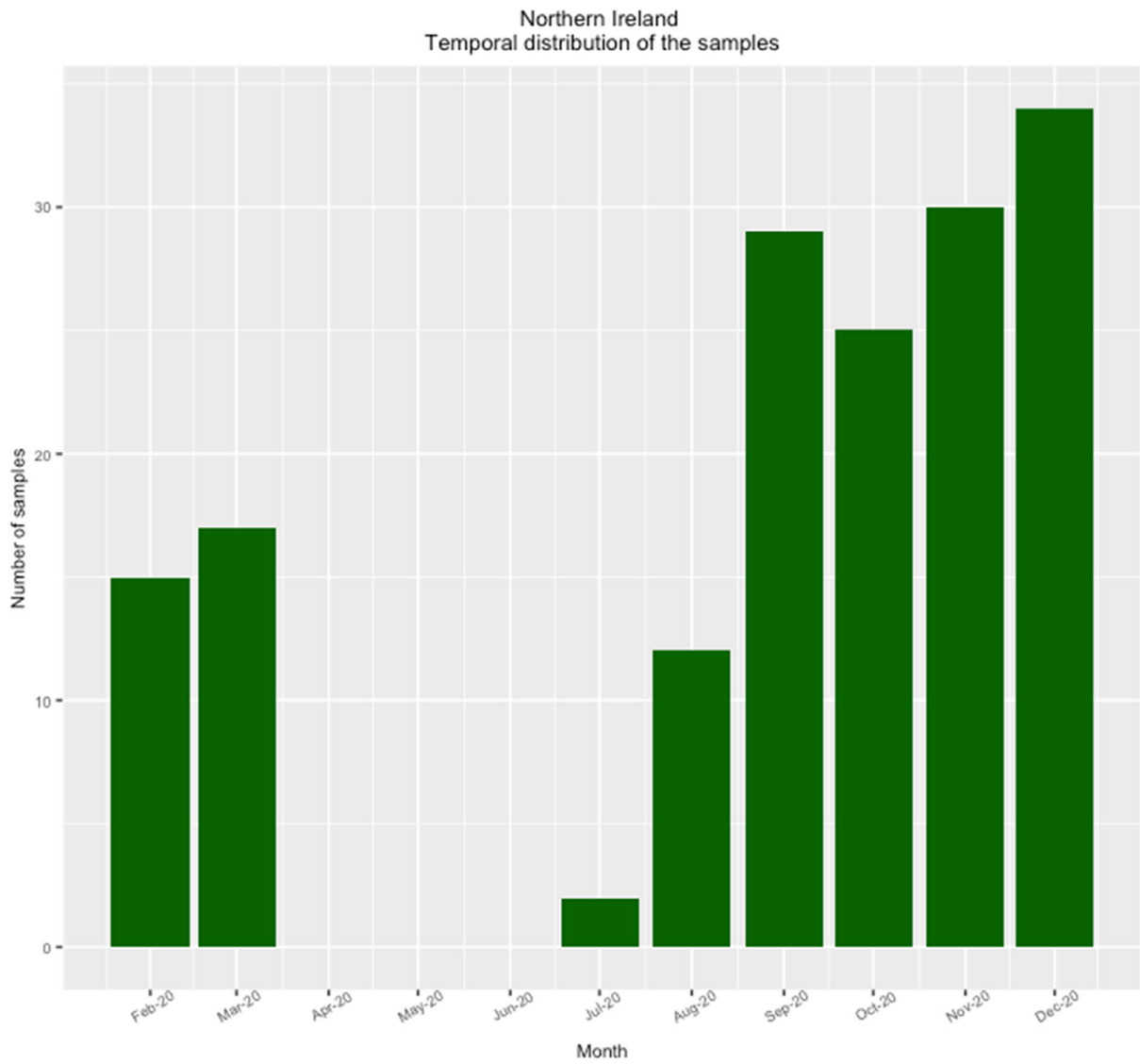


Figure 13: Northern Ireland – Temporal distribution of samples

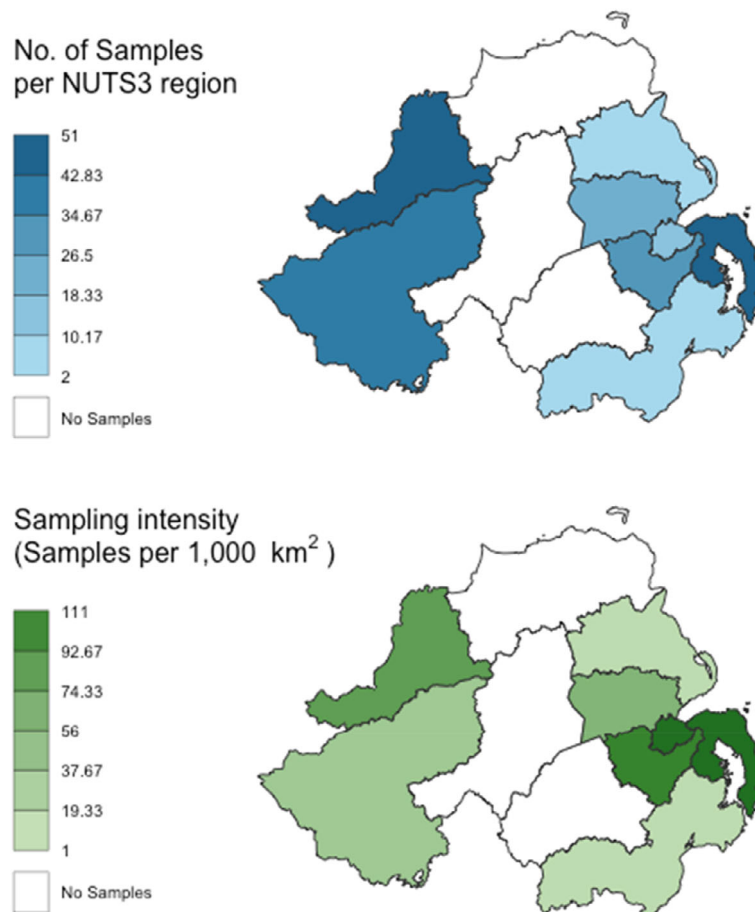


Figure 14: Northern Ireland – Sampling activity and intensity by NUTS 3 region

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 2020, 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, and for calculation of the sample size needed to achieve the desired target confidence of freedom, we used a probability of introduction of 0.5.

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 4–5 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 program 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 2020 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 thirty-two samples were collected from red foxes in 2020 and all were negative in PCR.

Samples were collected throughout 2020. 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 15 and 17). The temporal distribution of samples is also somewhat uneven 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 (See Figure 16).

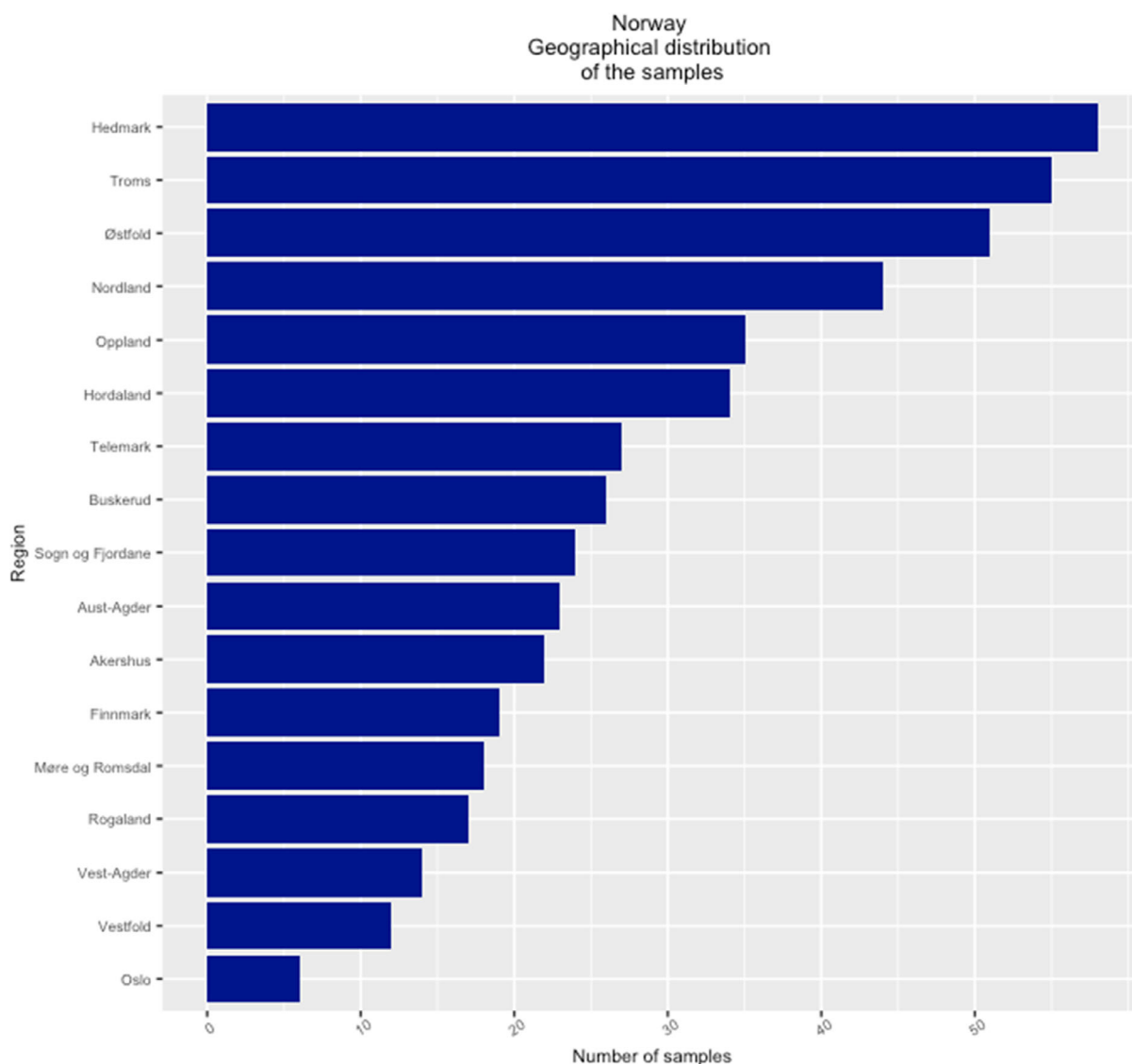


Figure 15: Norway – Geographical distribution of samples

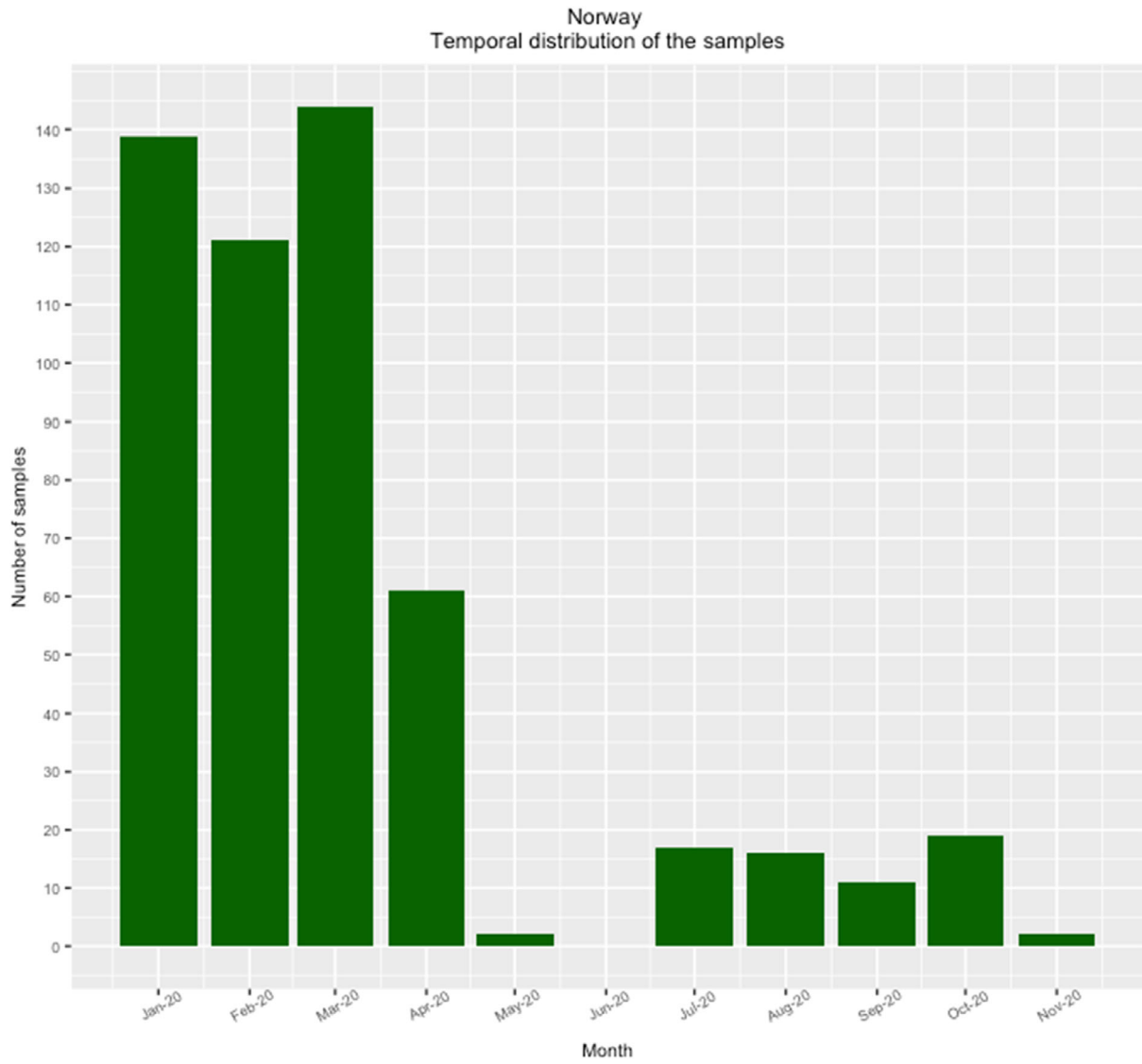


Figure 16: Norway – Temporal distribution of samples

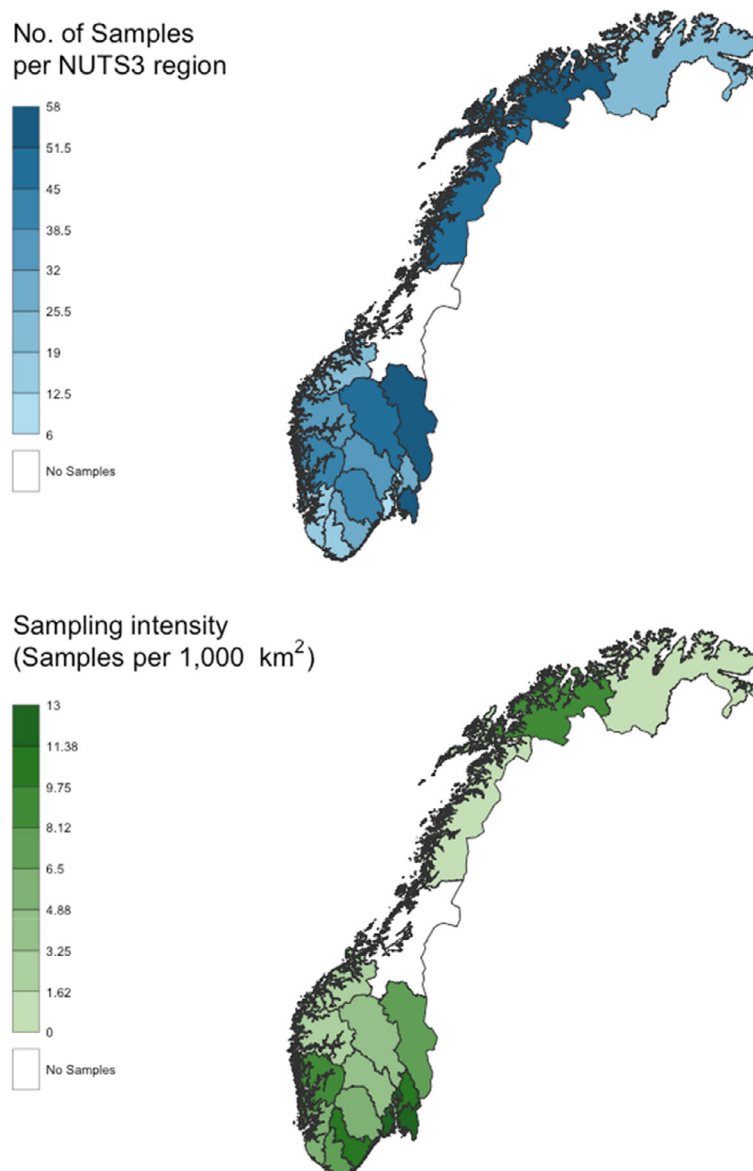


Figure 17: Norway – Sampling activity and intensity by NUTS 3 region

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 12S 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 (EFSA, 2019). The additional positive (spiked) samples tested in 2020 help in narrowing the uncertainty around the sensitivity of the test in use.

Table 8: 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)
Total	332	292	0.87(0.84–0.91)	

(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.

An exact binomial test shows a 'probability of success' ('best guess' of the sensitivity) equal to 0.87, with a confidence interval going from 0.84 to 0.91 (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 targeted 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 (see Section 4.1.1) and a population size of 451,450 (sum of red fox and raccoon dog population). The sample size required in this case is 383. The sample size collected ($N = 526$) is sufficient to satisfy the legal requirements.

Implementation of the sampling activity: The geographical information shows that, in 2020, 15 (12 in 2019) of 20 NUTS3 regions were included in the sampling activity (see Figure 4). 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'. 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 (May, June and July) 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%, 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 Finland using the RiBESS tool. The parameters included for the calculation were the following, all fully documented:

- DP of 1%,
- test sensitivity of 0.78,
- population size of 451,450 (raccoon dogs + red foxes) and
- sample size of 526.

The value of the area sensitivity (0.984) 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 2020 ensures the fulfilment of the technical legal requirements of 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 EFSA's 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 targeted 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 EM 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 confidence. 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 404, 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 km² shows a homogenous intensity, i.e. the target sample size is distributed across the territory 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 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%, 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 404.

The value of the area sensitivity is 0.958, i.e. exceeding 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; 0.958 (> 0.95).

In summary, the set of data relative to the surveillance activity in 2020 ensures the fulfilment of the technical legal requirements of 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 a 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 an 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 targeted 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 is well documented (14,000) and has been recently updated.

4.3.3. Sampling strategy

Epidemiological unit: For NI, intestinal contents from hunted or road kill 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 164. 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 215 additional samples required). As an internal validation of the test sensitivity has not been made with a large number of samples year over year (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 0.725, 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 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. See also Figure 13. Regarding the sampling period, by submitting samples collected between February and December 2020, NI appears not to fulfil the requirements of the 12-month reporting period. However, it has to be noted that due to the United Kingdom exiting the EU, the EFSA policy on data collection changed according to the evolving situation, generating alterations in the regular data submission process of those countries that were submitting 12-month period data over 2 consecutive years.

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 164 (from February 2020 to December 2020).

With these conditions, area sensitivity was lower than 0.95 (0.806). Thus, NI would not be able to comply with the EU regulation in force when assuming a test sensitivity of 0.78.

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 0.99, i.e. above the confidence required by the legislation.

	Component sensitivity	Overall area sensitivity
IE	0.958	0.997
NI	0.725 (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 12S 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.

Table 9: 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, 2019)^(a)

Spike	s	n	Test Se 50th perc (95% CI)
1 egg	16	38	0.42 (0.26–0.59)
5 eggs	7	12	0.58 (0.28–0.85)
10 eggs	30	36	0.83 (0.67–0.94)
50 eggs	2	2	1 (0.16–1)
1 worm	31	35	0.89 (0.73–0.97)
Overall	86	123	0.70 (0.61–0.78)

(a): R Core Team (2019). 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: 0.15–0.98). 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 situation 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 the table shows the result of this estimation: **Based on the available data**, the test appears to have a sensitivity greater than 0.70 in 50% of the cases; however, the lower bound of the confidence interval suggests that a more conservative value would be 0.61. 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.

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 2020 (532 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%, 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 532.

The area sensitivity value is 0.965 (> 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 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 (2020).
- 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.
- All the countries participating in this surveillance (Finland, United Kingdom (Northern Ireland), Ireland and Norway) **fulfil the technical legal requirements concerning an 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%.
- The **sampling strategies** performed by Finland, United Kingdom (Northern Ireland), Ireland and Norway cannot be considered 'based on a simple random sample': The sampling strategy in wild live animals is not 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. Due to the impact of United Kingdom exiting the EU on the EFSA data collection policy, in this report the assessment of Northern Ireland sampling activity was performed on data covering 11 months. 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, Ireland and Norway) except for United Kingdom (Northern Ireland) fulfil the technical legal requirements regarding the **confidence level of at least 0.95 against a design prevalence of 1%**.
- Assuming a test sensitivity of 0.78 as per EFSA recommendation in the absence of scientific evidence supporting other values (as in the case of Northern Ireland), the number of samples collected by 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. The same is true also when considering a test sensitivity of 0.99, i.e. the one presented by Northern Ireland. Reasons are illustrated in the assessment. The confidence achieved in the isle of Ireland, considering the 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.
- 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 Northern Ireland is recommended.

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Glossary, abbreviations and acronyms

Alveolar echinococcosis	The human disease caused by infection with the larval stage (metacestode) of <i>E. multilocularis</i> . 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.
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 <i>E. multilocularis</i> 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.
ASe	Area sensitivity
CL	Confidence Level
DCF	EFSA Data Collection Framework
DH	Definitive host
DNA	Deoxyribonucleic acid
EM	Echinococcus multilocularis
N	Target population size
NI	Northern Ireland
OR	Odds ratio
PCR	Polymerase Chain Reaction
RR	Relative risk
SCT	Sedimentation and Counting Technique
Se	Sensitivity
TSe	Test sensitivity
EFTA	European Free Trade Association

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. The modification to the original technique is well 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	Based on the results of internal trials, an exact binomial test indicates that the actual value of the test used in Finland may lie between 0.84 and 0.91 (95% CL). A Bayesian approach gives similar results. Therefore, the lowest value (0.84) 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

Points addressed in Annex II	Element	Description of Element	Information provided in surveillance report	Requirement fulfilled	Comments
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	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).

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. Time frame 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 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

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

Parameter	Evidence	Requirement fulfilled	Action/Comments
Theoretical Sampling period	From 1 January 2020 to 31 December 2020	Yes	NA
Actual Sampling Period	January 2020 to December 2020	Yes	
Number of samples	526	Yes	The sample size achieves an area sensitivity of 0.984 (> 0.95)
Number of test results	526	Yes	NA
Sensitivity	0.78	Yes	NA
Host	Raccoon dog and Red fox	Yes	N A

Parameter	Evidence	Requirement fulfilled	Action/Comments
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-Cest2 NAD1) 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. Time frame 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 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 2020 to 31 December 2020	Yes	NA
Actual Sampling Period	7 January 2020 to 23 December 2020	Yes	NA
Number of samples	404	Yes	The sample size achieves an area sensitivity of 0.958 (> 0.95)
Number of test results	404	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	NA
	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	Impossible to evaluate	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, NI 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	Impossible to evaluate	The use of a test sensitivity (0.99) not supported by adequate scientific evidence makes impossible to evaluate the adequacy of the output from the RIBESS tool
	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. Time frame 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 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 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	Impossible to evaluate	Not even assuming the test sensitivity value proposed by NI (0.99) the area sensitivity achieved satisfies the legal requirements (0.806). 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/Comments
Theoretical Sampling period	From 1 January 2020 to 31 December 2020	NA	NA
Actual Sampling Period	1 February 2020 to 1 December 2020	Yes	The samples collected in January 2021 could not be reported to EFSA as an effect of the United Kingdom exiting the EU.
Number of samples	164	Impossible to evaluate	The sample size is not enough also if considering a test sensitivity value of 0.99.
Number of test results	164	Yes	NA
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

Parameter	Evidence	Requirement fulfilled	Action/Comments
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	NA
	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 = 0.70, with a 95% CI = 0.61–0.78), 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. Time frame 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 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

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

Parameter	Evidence	Requirement fulfilled	Action/Comments
Theoretical Sampling period	From 1 January 2020 to 31 December 2020	NA	NA
Actual Sampling Period	From 1 January 2020 to 1 November 2020	Yes	NA
Number of samples	532	Yes	The sample size achieves an area sensitivity of 0.965 (> 0.95)
Number of test results	532	Yes	NA
Sensitivity	0.63	Yes	NA
Host	Red fox	Yes	NA
Animal sample	Yes	Yes	NA
Sampling Strategy and Design	Objective sampling and Simple random sample	Yes	The sampling strategy is actually a convenient sampling based on biological considerations. Considered adequate in wildlife
Objective sampling			
Sampling point	Hunting	Yes	NA