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Annual assessment of *Echinococcus multilocularis* surveillance reports submitted in 2019 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, the United Kingdom (UK) and Norway in their respective surveillance programmes. The surveillance programmes of these four countries were evaluated by checking the information submitted by each of them and verifying that the technical requirements were fulfilled as laid down in Commission Delegated Regulation (EU) 2018/772 of 21 November 2017 supplementing Regulation (EU) No 576/2013 of the European Parliament and of the Council with regard to preventive health measures for the control of *Echinococcus multilocularis* infection in dogs, and repealing Delegated Regulation (EU) No 1152/2011. The information was divided into four different categories for assessment: the type and sensitivity of the detection method, the selection of the target population, the sampling strategy and the methodology. For each category, the main aspects that need to be considered in order to accomplish the technical requirements of the legislation were checked against compliance of several criteria. All the countries participating in this surveillance (Finland, the UK, Ireland and Norway) succeeded in the fulfilment of the technical legal requirements foreseen in Commission Delegated Regulation (EU) 2018/772 concerning these four different categories. Within the UK, Northern Ireland fulfils those requirements only when assuming a diagnostic test sensitivity value of 0.99, provided by the national reference laboratory, which is higher than the sensitivity value suggested by EFSA (conservative value of 0.78). None of the four countries recorded positive samples in the 12-month reporting period. It should be noted that Malta did not have to report surveillance data in order to maintain its eligibility to continue applying preventive health measures for the control of *E. multilocularis* infection in dogs entering its territory, but to meet the conditions laid down in Article 4 (1) of Commission Delegated Regulation (EU) 2018/772 including the submission of evidence to the European Commission of the absence of the red fox from the territory.

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

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Summary

Following a request from the European Commission and, indirectly, from the European Free Trade Association (EFTA) Surveillance Authority, the 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, the United Kingdom (UK) and Ireland and Norway.

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

In accordance with this Article, Malta falls under the category described in paragraph 2, i.e. it is in the position of demonstrating that the infection with *E. 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 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 UK, 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, the UK and Norway as reported in 2019 were assessed by checking the reports for completeness against relevant elements that need to be addressed when performing an *E. multilocularis* surveillance in the context of Commission Delegated Regulation (EU) 2018/772 and analysing the raw data collected by these countries. In order to facilitate the assessment, the information given by the different countries was divided into four different categories corresponding to the critical points that are addressed in the legislation in the requirements for the pathogen-specific surveillance programme provided for in point c) of Article 4(2): (i) the type and sensitivity of the detection method, (ii) the selection of the target population, (iii) the sampling strategy and (iv) the methodology.

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

All the countries selected adequate wild definitive hosts in order to perform the surveillance. In the UK, Northern Ireland fulfils the requirements of Commission Delegated Regulation (EU) 2018/772 related to the desired confidence level of 95% only 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.

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1. Introduction

Overall, at any time, more than 1 million people are affected by one of the four forms of human echinococcosis: alveolar (caused by *Echinococcus multilocularis*), cystic (caused by *Echinococcus granulosus* senso lato), polycystic (caused by *Echinococcus vogeli*) or unicystic (caused by *Echinococcus oligarthra*). The WHO is working towards the validation of effective cystic echinococcosis (CE) control strategies by 2020.[1]

Human alveolar echinococcosis (AE), caused by the larval stage of the fox tapeworm *Echinococcus multilocularis* (EM), is a serious parasitic zoonosis (Torgerson et al., 2010; EFSA AHAW Panel, 2015; EFSA and ECDC, 2017). AE is confined to the northern hemisphere, in particular to regions of China, the Russian Federation and countries in continental Europe and North America.[1] *E. multilocularis* is an emerging parasite in Hungary. Because of its still low incidence, differential diagnosis and therapy of AE is a new challenge in clinical practice in Hungary[2] (Dezsényi et al., 2019).

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. 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 stage (strobilar stage) of the cestode residing in the small intestine of the definitive hosts release the eggs into the environment via faeces (Peregrine et al., 2012; EFSA AHAW Panel, 2015). The infective eggs are ingested by the intermediate hosts and the oncosphere migrates inside them until reaching organs, especially the liver (CDC, online; Peregrine et al., 2012). In the liver, the oncosphere develops into an encysted larval (metacestode stage) which resembles a malignancy in appearance and behaviour, because it proliferates indefinitely by exogenous budding and invades the surrounding tissues. In rodents, hydatid cysts contain numerous small vesicles with multiple 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 directly through close contact with the definitive host or indirectly through ingestion of food or water contaminated with eggs of the parasite (Torgerson et al., 2010).

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

Table 1: Potential definitive and intermediate hosts of *E. multilocularis* in Europe (EFSA AHAW Panel, 2015)

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

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

[2] Emerging human alveolar echinococcosis in Hungary. Early experiences in clinical management in a single center study from 2005-2018 Dezsényi, B. et al.; International Journal of Infectious Diseases, 79, 118–119.

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

DH: definitive host.

The distribution of the parasite seems[3] to expand over time. 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). The latest available information indicates that at least 24 European countries have found the presence of *E. multilocularis* in the main definitive host, the red fox (EFSA and ECDC, 2018[4]). In addition, human cases of AE are notified every year in some of these countries. Although human AE is a notifiable disease in some Member State, in practice, this parasitic disease (together with the CE) is largely underreported in Europe. In 2017, 827 confirmed human echinococcosis cases were reported in the European Union (EU). The EU notification rate was 0.19 cases per 100,000 population, a decrease by 13.6% compared with 2016. Species information was provided for the majority (71.4%) of cases and *E. multilocularis* accounted for 146 cases (26.3%). A high proportion (> 75%) of the human echinococcosis cases were reported where no information on importation and travel destination were available. The proportion of cases who were hospitalised continues to decrease, compared to 2016, with higher hospitalisation rates for AE than for CE. One fatal case (species not specified) was reported in 2017 (EFSA and ECDC, 2018[4]).

The prevalence of the parasite is not homogeneous and may vary depending on multiple elements such as for example microclimatic conditions, geographical location, host population dynamics and amount of IHs (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 EU and adjacent countries found differences between countries (Oksanen et al., 2016; **Table 2**). The prevalence has been reported to range from 0 to more than 50% (EFSA AHAW Panel, 2015).

[3] The uncertainty is linked to the fact that no baseline study has ever been performed at European level. The data relate to scientific literature.

[4] EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal 2018;16(12):5500, 262 pp. https://doi.org/10.2903/j.efsa.2018.5500

Table 2: 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	Human AE cases[1]
Finland, Ireland, Malta, United Kingdom, Norway*	0	Austria, Belarus, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, France, FYR Macedonia, Germany, Greece, Hungary, Latvia, Lithuania, Moldova, Poland, Romania, Slovakia, Slovenia, Switzerland, Netherlands, Turkey and Ukraine
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%	

1: Only included the confirmed *E. multilocularis* species.
*: Excluding Svalbard.

The EU 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, taking into account the relative risk of infection with the *E. multilocularis* parasite in different species and subpopulation of the target wild definitive host population;
3) The pathogen-specific surveillance programme shall consist in the ongoing collection, during the 12-month surveillance period, of samples from wild definitive hosts, to be analysed using:
    a) the sedimentation and counting technique (SCT), or a technique of equivalent sensitivity and specificity, by examination of intestinal contents for the detection of the *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 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 the 31 of May.

At the moment, only four Member States (Finland, Ireland, Malta and the United Kingdom (UK)) 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; EFSA and ECDC, 2018) 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[5] to the list of countries complying with the conditions of Article 3 of the Regulation.

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

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

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

1.2. Interpretation of the Terms of Reference

This report addresses ToR 2 of the mandates M-2012-0200 and M-2014-0287 submitted to EFSA by the European Commission and the EFTA Surveillance Authority, respectively, and applies the principles

[5] 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.

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.

2. Data and methodologies

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

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

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

Table 3: Assessment categories and their equivalence in the Commission Delegated Regulation (EU) 2018/772 (Annex I)

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

For each of the four evaluation parts, the most relevant elements were extracted from the reports submitted by the Member States and checked against the criteria described below (Table 4).

Table 4: Relevant elements checked for compliance of the technical requirements of Annex I of Commission Delegated Regulation (EU) 2018/772

Points addressed in the Annex II	Element	Description of Element
Type and sensitivity of the detection method	**Type of test**	The diagnostic test used for the detection of EM must be defined. Modifications of the original method should be indicated
	Test sensitivity	The sensitivity and specificity of the test used in the surveillance system must be reported. This would ideally be estimates from each participating laboratory reported as a point estimate (average) of the values across the country with minimum and maximum values or a probability distribution. Alternatively, a value of 0.78, as recommended by EFSA (2015), shall be used
Selection of the target population	**Definition of susceptible host population targeted by the system**	The susceptible wild definitive host population(s) (red foxes, raccoon dogs) targeted by the surveillance system should be described and the choice justified. If domestic host species (dogs or cats) are sampled, evidence for the absence of wild definitive hosts and for these domestic animals having had access to outdoors should be provided
	Size of susceptible host population targeted by the system	The size of the targeted (wildlife) population should be reported, together with the evidence for this. Historical population data should be updated since these may not reflect current populations
Sampling strategy	**Epidemiological unit**	It should be clearly defined if individual animals or individual faecal samples collected from the environment constitute the epidemiological unit. If individual faecal samples are collected from the environment, the method applied to establish the species from which the faeces originated has to be reported
	Sample size calculation	The applied survey design should be fully documented, including considerations regarding potential biases inherent in the survey design. The method and the formula used to calculate the sample size should be fully documented
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must to be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be representative
Methodology	**Design prevalence (DP)**	DP is specified in Annex I to Regulation (EU) No 2018/772 and must be 1% (0.01) or lower
	Geographic epidemiological unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification
	Methodology for calculation of area sensitivity	For the calculation of the area sensitivity, the diagnostic sensitivity should be set conservatively to the lowest value, excluding the lowest 20th percentile, from the ones reported in the scientific literature and related to the diagnostic tests implemented by the countries listed in Annex I of the Commission Delegated Regulation (EU) No 2018/772. In this case, is 78% (EFSA AHAW Panel, 2015)

A summary of the assessment of the relative elements of the different countries is given at the end of the document (Appendices 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)[6] was used to compute descriptive statistics. Table 5 lists and describes all the parameters that were extracted from the data submitted.

Table 5: List of the parameters extracted from the raw data submitted by the Member States via the Data Collection Framework

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

3. Assessment

3.1. Finland

3.1.1. Information as submitted in the report by the Member State

The Finnish Food Safety Authority – Evira – (which merged in 2019 into the Finnish Food Authority) 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 in Evira in 2014, 2015 and 2016 (EFSA, 2017). In 2017 and 2018, additional positive (spiked) samples (76 and 31, respectively) were blindly tested.

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 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). The raccoon dog is more numerous (342,000) in Finland than the red fox (120,000), based on hunting bag statistics provided by the Natural Resources Institute (http://statdb.luke.fi/PXWeb/pxweb/en/, see also Figure 2), and Kauhala (2007). The population densities for both species are highest in the southern part of the country. See maps in Figure 1.

[6] R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: https://www.R-project.org/

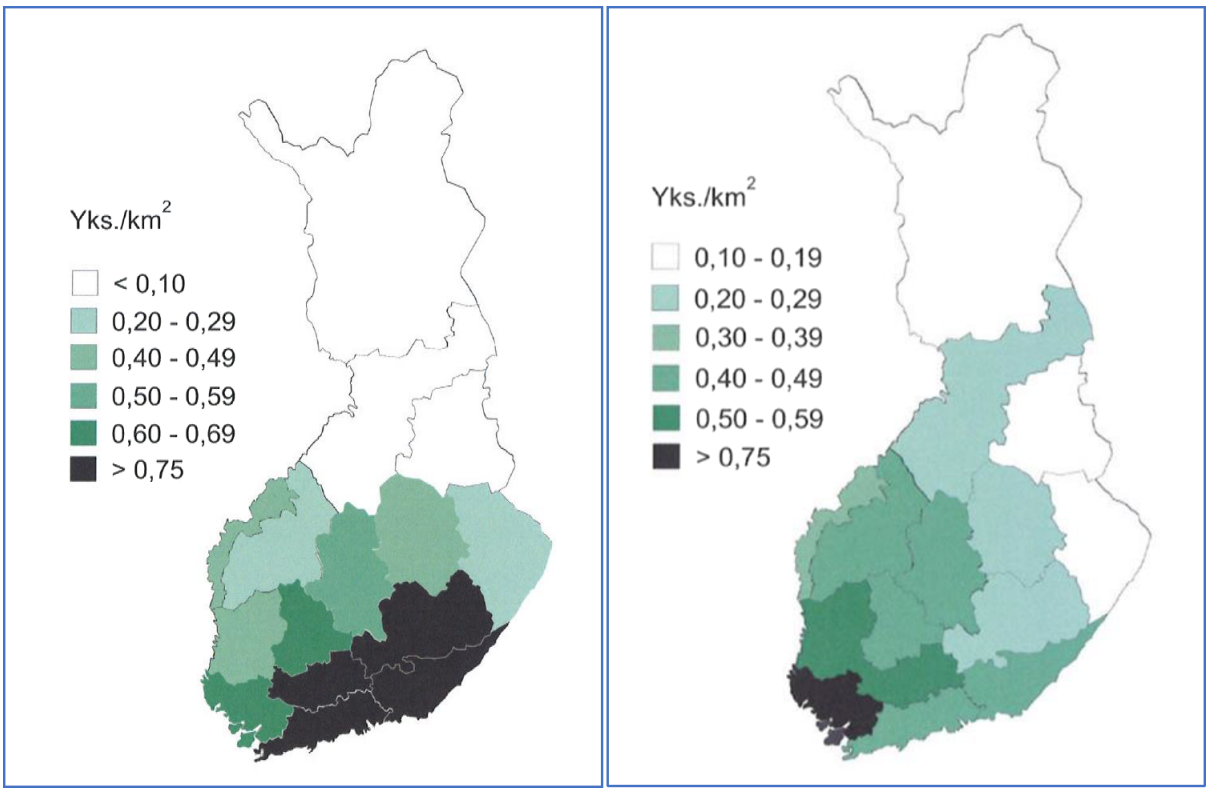
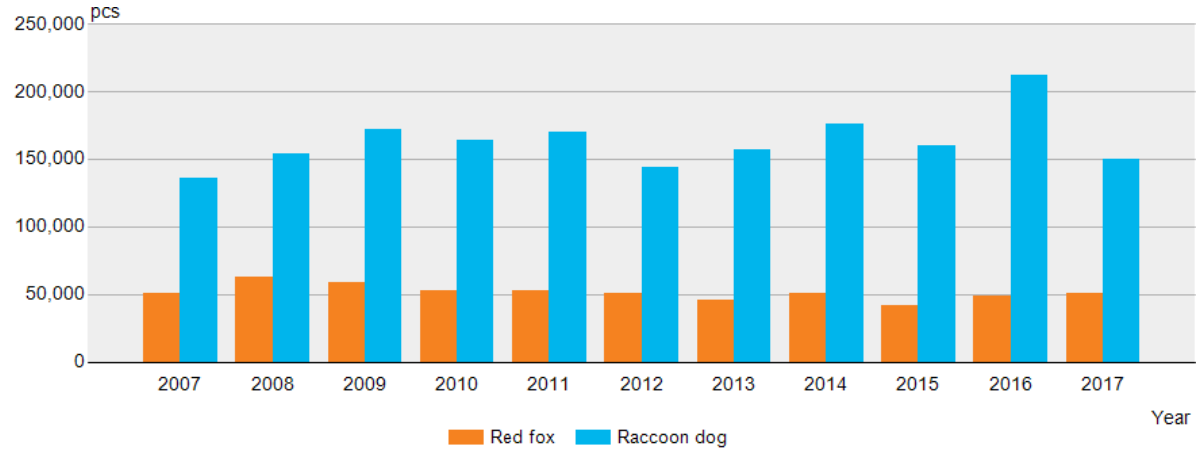


Figure 1: Finland – Raccoon dog densities (left) and red fox densities (right) according to Kauhala (2007) (Yks./km^2 = individuals/km^2)



Source: OSF: Natural Resources Institute Finland, Hunting

Figure 2: Finland – Annual hunting bag of foxes and racoon dogs (2007–2017) (Source: OSF Natural Resources Institute Finland). Pcs: number of animals

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

The epidemiological unit was defined as the individual animal (red fox or raccoon dog).

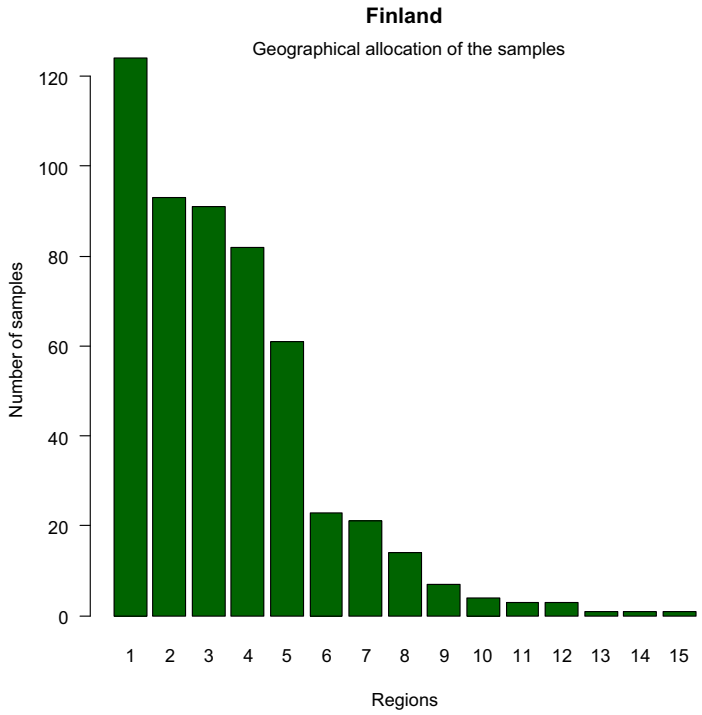
For the whole country of Finland, the entire wild small canid population(s) of the country was defined as the geographical epidemiological unit (even though the population is a continuum of the northwestern taiga population).

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.

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

A total of 326 and 203 samples were collected from raccoon dogs and foxes respectively (N = 529). Gender ratio was male-biased in foxes (1:1.24) while it was close to one in raccoon dogs (1:1.06). Of the animals that could be classified by age (N = 419), 50% were juveniles. The proportion of juveniles was 60% in raccoon dogs and 33% in foxes.

Sampling was targeted in the southern part of the country where populations are denser. The majority of the samples originated from south-east Finland, as this is the region where active monitoring of rabies control programme has taken place since 1990. The same area can be considered having an elevated risk of introduction of EM due to geographical closeness of infected areas in the south. Also, south-east Finland has the highest density of raccoon dogs in Finland (Kauhala, 2007). A large sample of foxes (17% of all animals) was received from Lappi where active red fox population reduction to protect the arctic fox was on-going (see **Figure 3**).



	Region	# samples
1	Etelä-Karjala	124
2	Kymenlaakso	93
3	Lappi	91
4	Kanta-Häme	82
5	Pohjois-Karjala	61
6	Varsinais-Suomi	23
7	Satakunta	21
8	Uusimaa	14
9	Etelä-Savo	7
10	Keski-Suomi	4
11	Itä-Uusimaa	3
12	Pirkanmaa	3
13	Kainuu	1
14	Päijät-Häme	1
15	Pohjois-Pohjanmaa	1

Figure 3: Finland – Geographical distribution of samples

Samples were collected throughout 2018 (see Figure 4). 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.

[7] https://www.ruokavirasto.fi/

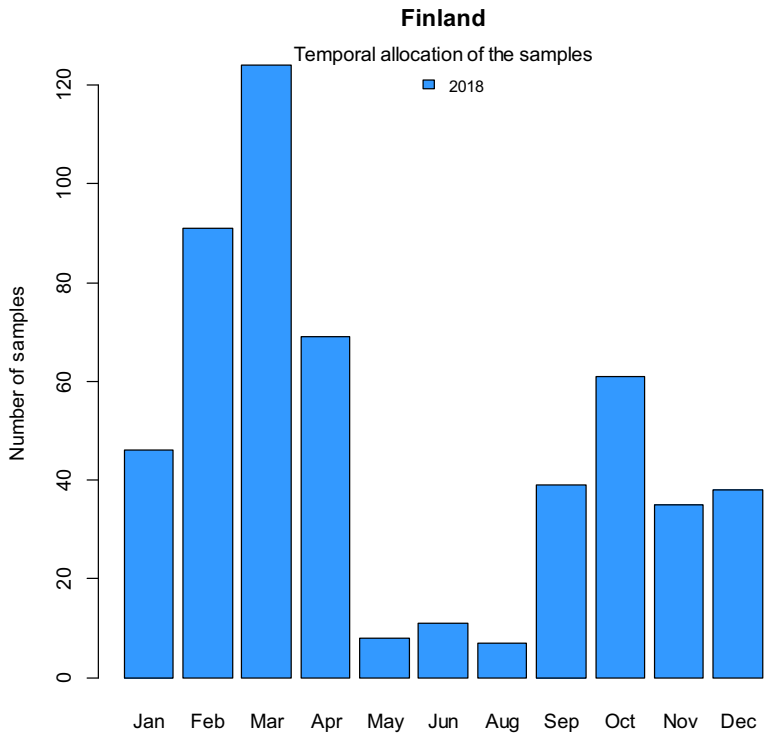


Figure 4: Finland – Temporal distribution of samples

All 529 samples were negative in PCR. Thus, no sample was found positive for *E. multilocularis*.

3.1.2. EFSA comments and considerations

3.1.2.1. 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: An overall system sensitivity of 0.76 has been estimated based on internal validations performed by Evira (EFSA, 2017). The additional positive (spiked) samples tested in 2018 help in narrowing the uncertainty around the sensitivity of the test in use.

Table 6: 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 sensitivity[(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)
Total	**232**	**196**	**0.87 (0.82–0.91)**	

(a): Estimated as Beta $(\sum_{i=1}^{y} s_i + 1, \sum_{i=1}^{y} n_i - \sum_{i=1}^{y} s_i + 1)$ 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 0.82 to 0.91 (bottom row of Table 6) and a Bayesian approach leads substantially to the same results.

3.1.2.2. Selection of the target population

Definition of susceptible host population target by the system: The selection of raccoon dogs and red fox species as target populations was based on their role as definitive hosts in the cycle. This is an assumption also confirmed by the EFSA Scientific opinion on *Echinococcus 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 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).

3.1.2.3. Sampling strategy

Epidemiological unit: The epidemiological unit appears in the report and is defined as the individual animal. Individual rectal contents were collected directly by hunters.

Sample size calculation: The method used to calculate the sample size of FI was the RIBESS tool. The sample size was calculated with an overall sensitivity of the diagnostic approach of 0.78 (see Section 3.1.2.1) and a population size of 462,000 (sum of red fox and raccoon dog population). The sample size required in this case is 383. For both sensitivity estimates, the sample size collected (N = 529) is sufficient to satisfy the legal requirements.

Implementation of the sampling activity: The geographical information shows that 15 (17 in 2017) out of 20 NUTS3 regions were included in the sampling activity (see Figure 5). There was a higher intensity of the sampling in the south-east of the country.

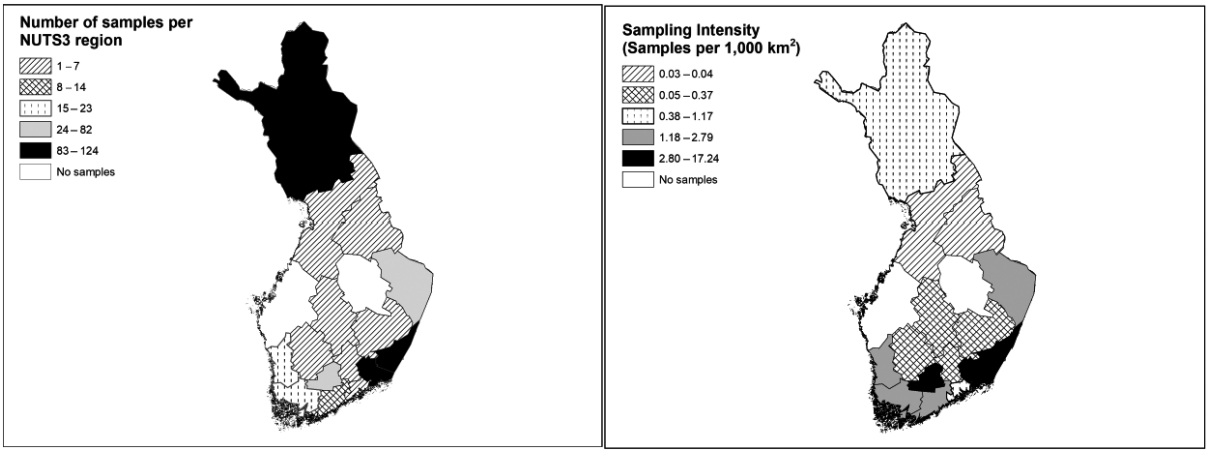


Figure 5: Finland – Sampling activity and intensity by NUTS 3 region

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).

3.1.2.4. Methodology

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

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

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

- DP of 1% (0.01),
- test sensitivity of 0.78,
- population size of 462,000 (raccoon dogs + red foxes) and
- sample size of 383.

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 2018 ensures the fulfilment of all the technical legal requirements included in the Annex I of Commission Delegated Regulation (EU) 2018/772.

3.2. Ireland

3.2.1. Information as submitted in the report by the Member State

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. 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 the scientific opinion by EFSA (EFSA AHAW Panel, 2015). In addition, the Irish National Reference Laboratory for Parasites is willing to participate in any test sensitivity assessment, if organised by the EU Reference Laboratory or other laboratory which could supply a large number of *E. multilocularis* positive samples.

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 (Hayden and Harrington, 2000; Marnell et al., 2009).

The red fox is a seasonal breeder; cubs are born in the spring and are almost fully grown by seven months of age (Hayden and Harrington, 2000). Therefore the age structure of the population between young and adult 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 6.

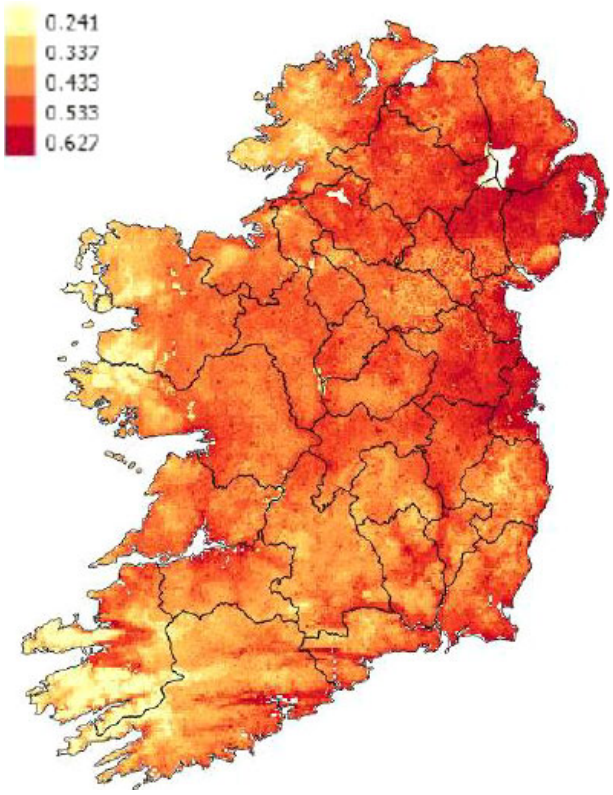


Figure 6: Ireland – Probability of presence per 1 km^2 from the final Maxent species distribution model (Phillips et al., 2006) for red fox (*Vulpes vulpes)*. Source: data up to 2015 provided by Dr. Tomás Murray, from National Biodiversity Data Centre (Ireland)

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 DP of 0.01, a survey sensitivity of 0.95, fox population size of 150,000 and test sensitivity of 0.78.

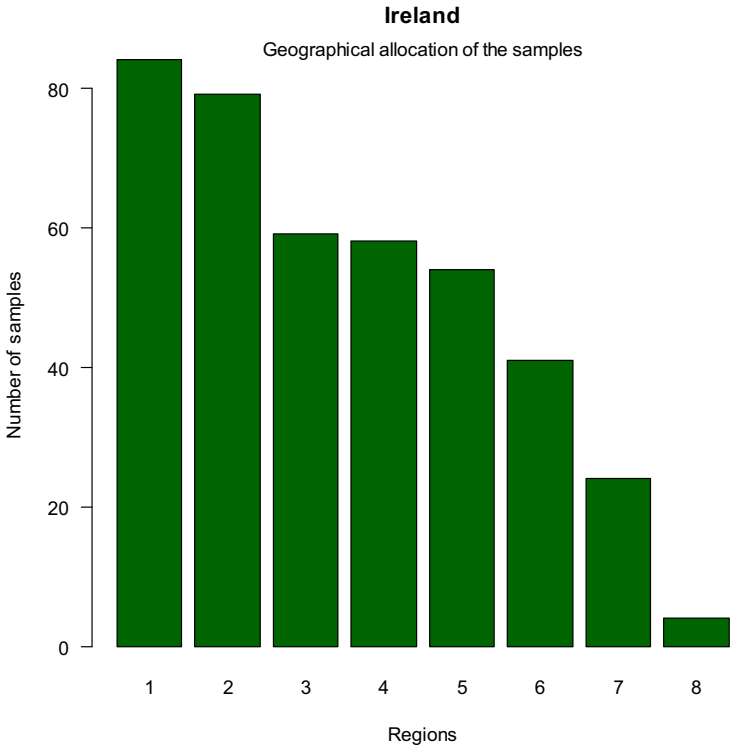
The epidemiological unit was defined as the individual animal (the individual red fox, *Vulpes vulpes*).

The geographical epidemiological unit used was the same geographical area as that of the member state Ireland. The rationale for selecting this area as the geographical epidemiological unit was in order to comply with the conditions of the Commission Delegated Regulation (EU) 2018/772.

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 finally 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 403 samples was reported by Ireland.

Samples were collected throughout 2018. 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 (Figure 7). Samples were obtained during 8 months of the year with intensification during winter, at the end of the available sampling period (see Figure 8). A greater number were collected from culling during October, November and December, to avoid culling of adult female foxes with fox cubs dependent on their dam to be fed. 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).

All the samples tested negative for *E. multilocularis* using the PCR Cest1-Cest2 NAD1 method.



	Region	# samples
1	West	84
2	Border	79
3	South-east (Irl)	59
4	South-west (Irl)	58
5	Mid-west	54
6	Mid-east	41
7	Midland	24
8	Dublin	4

Figure 7: Ireland – Sampling activity by regions

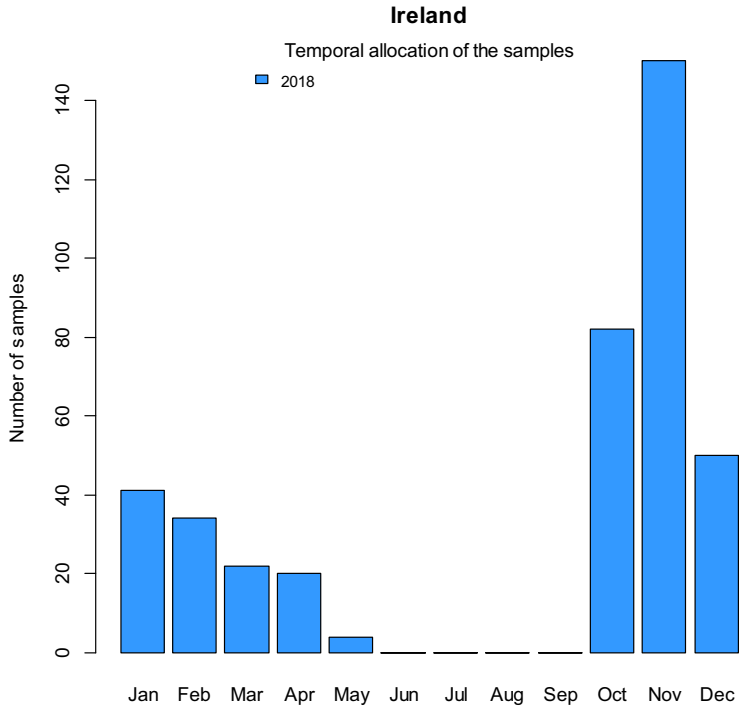


Figure 8: Ireland – Temporal distribution of samples

3.2.2. EFSA comments and considerations

3.2.2.1. Type and sensitivity of the detection method

Type of test: 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, 2017).

3.2.2.2. Selection of the target population

Definition of susceptible host population target by the system: The red fox has been recognised as the main wildlife definitive host species for this parasite (EFSA AHAW Panel, 2015). The selection of this species to perform the pathogen surveillance is well explained and referenced. The absence of other important definitive wild hosts (raccoon dogs and wolves) is also supported by scientific literature.

Regarding the age or gender of the target population, their role in the epidemiology and in the lifecycle of 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 statistical confidence of less than 1% (0.01) prevalence at a specific test sensitivity (EFSA, 2014). Therefore, fluctuations in the previous population size of 150,000 do not significantly alter the sample size required (EFSA, 2014).

3.2.2.3. 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) overall sensitivity of 0.78 (as recommended by EFSA AHAW Panel, 2015) and (b) 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 403, 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 DP of 1% (0.01). 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 (see Figure 9). The sampling activity per 1,000 km^2 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.

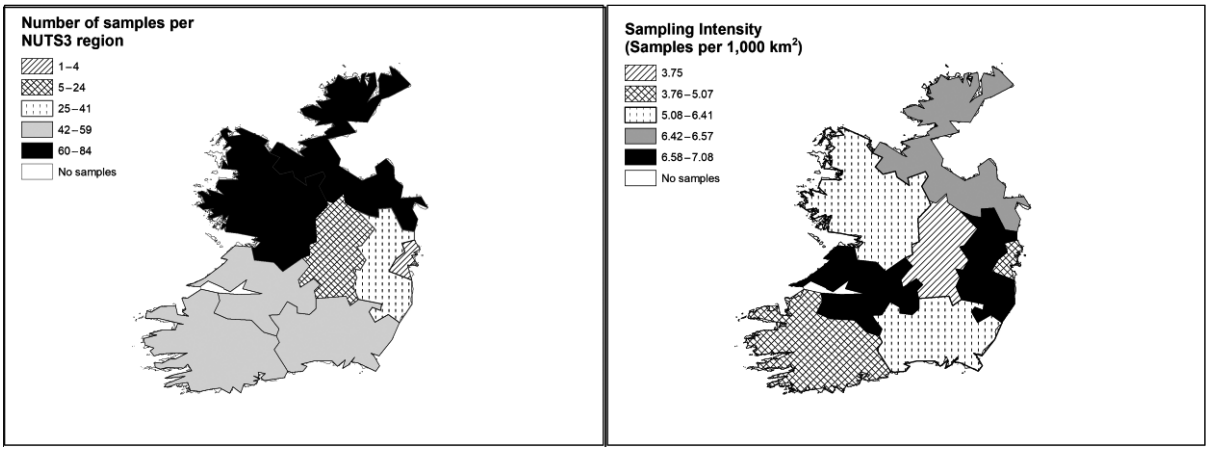


Figure 9: Ireland – Sampling activity and intensity by NUTS 3 region

Samples were obtained during the whole year excluding June and July (see Figure 8). The reduction of collection of samples during spring and summer is justified to avoid culling adult female foxes which have fox cubs dependent on their dam to be fed. 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).

3.2.2.4. Methodology

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

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

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

- DP of 1 %,
- test sensitivity of 0.78,
- population size of 150,000 and
- sample size of 403.

The value of the area sensitivity 0.958 (> 0.95) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements described in Commission Delegated Regulation (EU) 2018/772. With a population size of 200,000, the value of the area sensitivity would also reach this CL 0.957 (> 0.95).

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

3.3. United Kingdom

3.3.1. Information as submitted in the report by the Member State

In Great Britain (GB), a PCR test (PCR Cest1-Cest2 NAD1) was used to detect *E. multilocularis* DNA in rectal content (post-mortem sampling) (Mathis et al., 1996; Dinkel et al., 1998). The method is based on the concentration of helminth eggs by a combination of sequential sieving of faecal samples and flotation of the eggs in zinc chloride solution. DNA of the taeniid eggs retained in the 20 microns sieve was obtained after alkaline lysis and nested PCR was performed using *E. multilocularis* species-specific primers against the mitochondrial 12S rRNA gene. Test sensitivity for the PCR is between 85 and 99% depending on the laboratory. The sensitivity of the proposed method is further determined using spiked faecal samples and the specificity is tested with other taeniid species. In the case of the APHA/FERA laboratory, 78% sensitivity was used as the lowest possible sensitivity, based on successful ring trial participation.

In Northern Ireland (NI), a SCT test was used to detect *E. multilocularis* eggs 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 egg counting method sensitivity varies between laboratories. Eckert's suggestion to consider a Se of 99% was used (Eckert, 2003). In NI, AFBI participated in the last proficiency testing in 2015 and participated again in 2018.

The red fox (*Vulpes vulpes*) is the only wild definitive host for *E. multilocularis* in the UK (both GB and NI). No other wild definitive host is present. GB and NI are island populations with no access for other wild carnivores from other parts of Europe.

The fox population size (pre-breeding adults) has been estimated at 240,000 by wildlife experts, and the numbers were published in 2013 (Defra, 2013). The population does fluctuate from year to year, but is believed to be relatively stable, if marginally increasing. The urban/suburban fox population is now estimated at ~ 33,000 (up from 15,000) (~ 13%). The variation in abundance is likely correlated with food resources, so while the density in hill areas of Scotland have been estimated at one breeding pair every 40 km^2, the highest density recorded was in the urban areas of 30 foxes in a single km^2 (http://www.lhnet.org/red-fox/; Croft et al., 2017). The rapid spread of sarcoptic mange in

the red fox population and lack of geographic barriers demonstrates that there is considerable mixing of the red fox population within GB and within the island of Ireland, despite the variation in abundance. The average range of a red fox in the UK in open farm land is considered to be ~200 to 600 ha (2–6 km^2). There is good evidence that the total abundance has not changed in the last decade (Wright et al., 2014; Croft et al., 2017) as measured on BTO survey squares (mostly rural), and as predicted. The urban fox distribution has changed in recent years with almost all urban areas now having foxes present (Scott et al., 2014). A map of systematically estimated fox distribution and abundance using NBN data and published density information and a small project using public sighting data to estimate fox abundance in all urban areas was provided (see Figure 10).

For NI, an estimate of 14,000 is given, which is equivalent of 1 fox per km^2 and accounts for the large area of rural land in contrast to the urban land use (Conserve Ireland, 2009).

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.

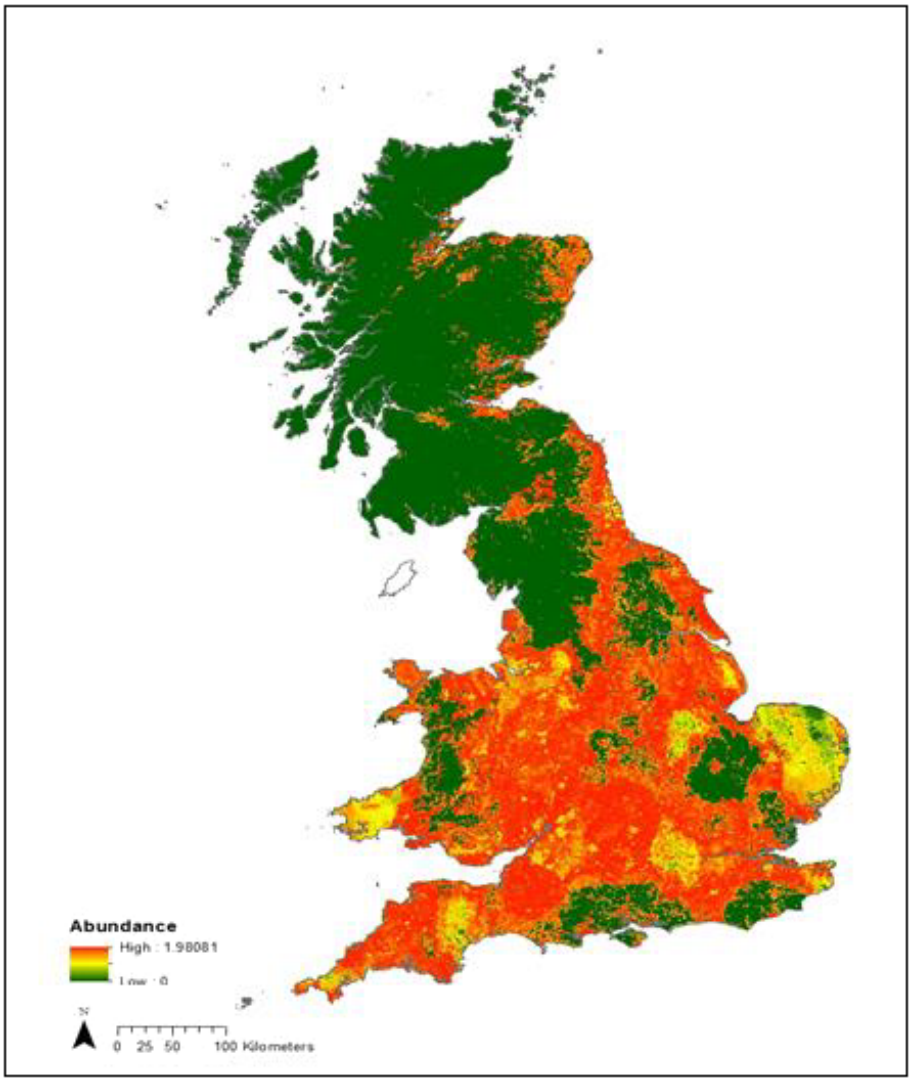


Figure 10: Great Britain – Map estimating fox density in the UK. This is a systematic approach using NBN presence data and published density data and provides a confidence interval of 120–280,000 foxes. Some areas have few data as permission was not given to use the records. For more information, see Croft et al. (2017)

The UK was divided into two surveillance regions for the purpose of this report: NI and GB (England, Scotland and Wales).

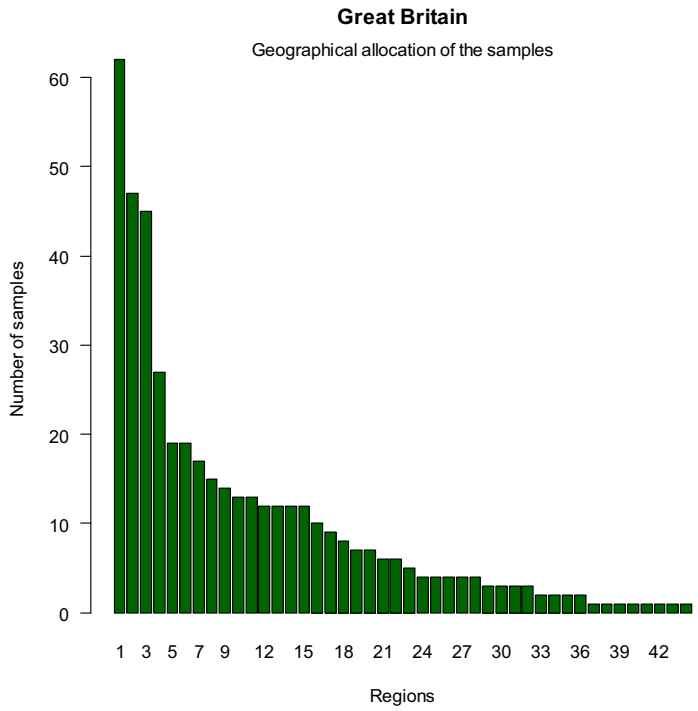
The sample size was calculated using the EFSA RiBESS tool. Random sampling – not risk based – sampling, is carried out at certain times of the year – the target is the wild population and therefore hunting is not permitted during the breeding season.

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. No issues resulted in deviation from the sampling plan.

Reports were made at NUTS 3 level (the lowest level of NUTS; in GB individual counties or upper-tier authorities, unitary authorities or districts; districts in NI). The NUTS boundaries are only rarely amended and therefore comparisons could be made from one year to the next in terms of distribution.

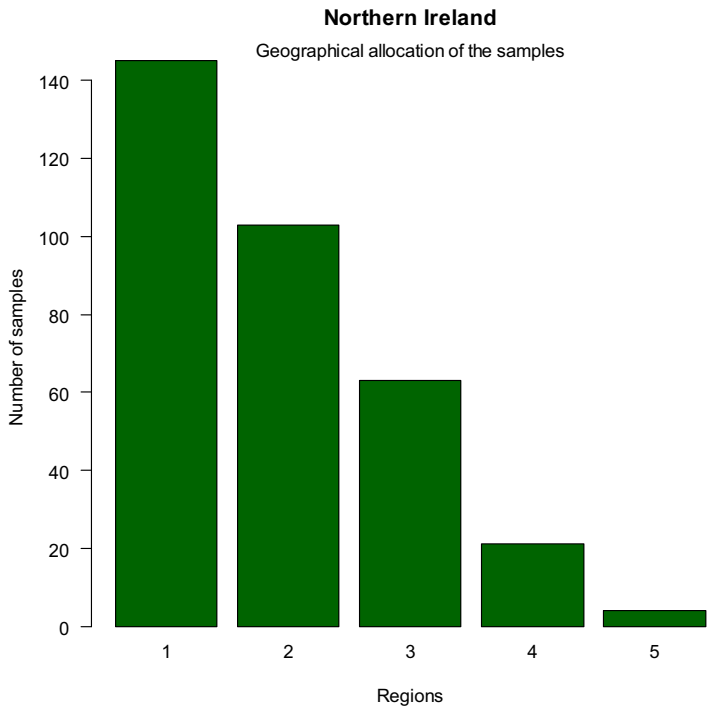
The map in Figure 10 shows that there is an uneven distribution of the wild host population – some areas have less dense fox populations than others – for example, the highest density is in urban areas in the south-west of England, the least dense are rural areas in Northern Scotland (see map) and that this distribution has not changed significantly in the last ten years. This uneven distribution means sampling of animals is also uneven. GB consists of islands, surrounded by sea with no land bridges for foxes to arrive; therefore there is a constant population (which varies during the year according to whether the females have given birth). Population size is based on numbers of breeding females. For NI, 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.*

In GB, 437 samples were collected and tested. In NI, 336 samples were collected and tested. The sampling activity targeted the regions with higher fox density, according with the red fox population density map provided (See Figures 10–12).



	Region	# samples		Region	# samples
1	Gloucestershire	62	23	Essex Cc	5
2	Worcestershire	47	24	Blackburn With Darwen	4
3	North Yorkshire Cc	45	25	Cambridgeshire Cc	4
4	South West Wales	27	26	Glasgow City	4
5	Cheshire Cc	19	27	Inverness & Nairn And Moray, Badenoch & Strathspey	4
6	Somerset	19	28	Monmouthshire And Newport	4
7	Devon Cc	17	29	Lochaber, Skye & Lochalsh, Arran & Cumbrae And Argyll & Bute	3
8	East Cumbria	15	30	Medway	3
9	Greater Manchester North	14	31	North And North East Lincolnshire	3
10	East Riding Of Yorkshire	13	32	Perth & Kinross And Stirling	3
11	East Sussex Cc	13	33	Durham Cc	2
12	Caithness & Sutherland And Ross & Cromarty	12	34	Herefordshire, County Of	2
13	Halton And Warrington	12	35	Leicestershire Cc And Rutland	2
14	Hampshire Cc	12	36	Nottingham	2
15	Lincolnshire	12	37	Aberdeen City And Aberdeenshire	1
16	York	10	38	East Lothian And Midlothian	1
17	East Merseyside	9	39	Inverclyde, East Renfrewshire And Renfrewshire	1
18	Staffordshire Cc	8	40	Leicester	1
19	Bath And North East Somerset, North Somerset And South Gloucestershire	7	41	South And West Derbyshire	1
20	Shropshire Cc	7	42	Suffolk	1
21	Lancashire Cc	6	43	Surrey	1
22	South Nottinghamshire	6	44	Warwickshire	1

Figure 11: Great Britain – Geographical distribution of samples



	Region	# samples
1	West And South Of Northern Ireland	145
2	East Of Northern Ireland	103
3	North Of Northern Ireland	63
4	Outer Belfast	21
5	Belfast	4

Figure 12: Northern Ireland – Geographical distribution of samples

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 13 and 16).

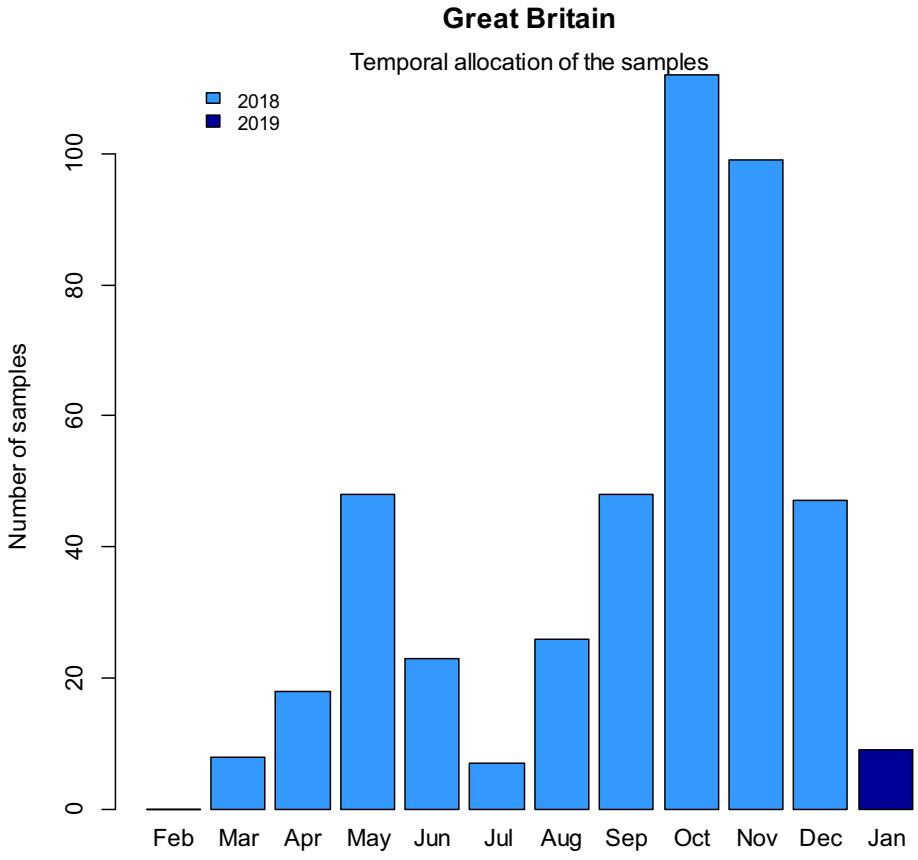


Figure 13: Great Britain – Temporal distribution of samples

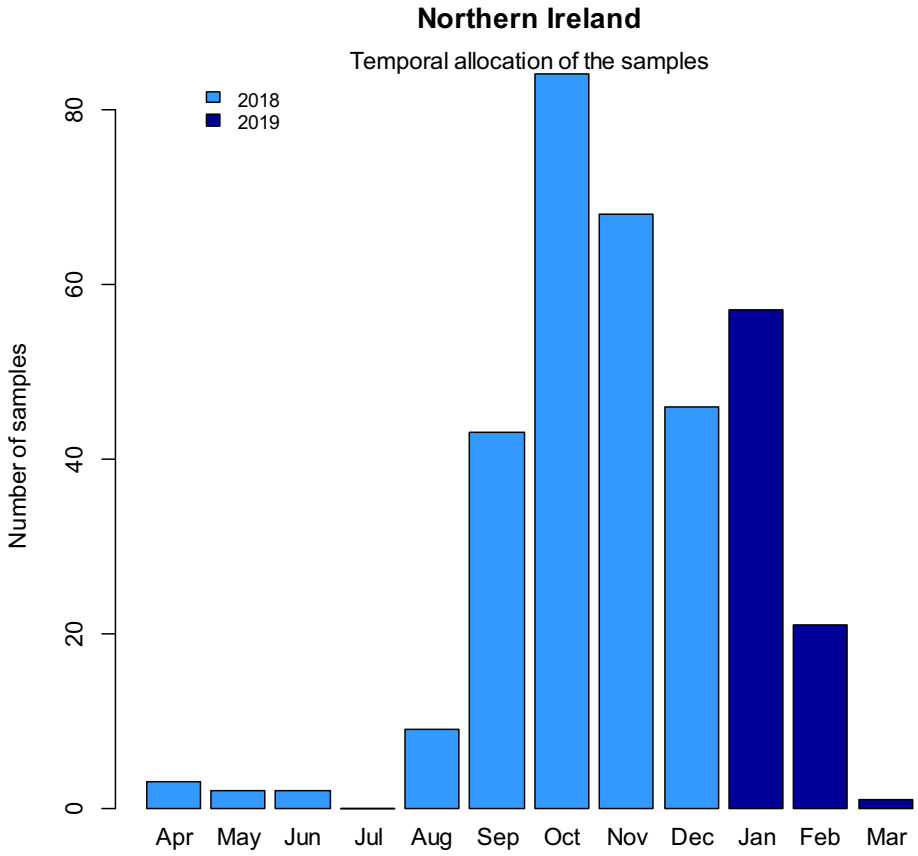


Figure 14: Northern Ireland – Temporal distribution of samples from NI

3.3.2. EFSA comments and considerations

3.3.2.1. Type and sensitivity of the detection method

Type of test: Both methods used for detection of *E. multilocularis* in the UK were well described. GB selected a PCR Cest1-Cest2 NAD1 test (Mathis et al., 1996; Dinkel et al., 1998) for detection of *E. multilocularis* in rectal content. In NI, the SCT test (Eckert, 2003), considered as the reference standard for detection of *E. multilocularis* eggs from individual intestinal content, was used.

Test sensitivity: The diagnostic technique used by GB has been found to range from 88% to 95.7% (Casulli et al., 2015). APHA/FERA laboratory used a sensitivity of 78% considering the lowest possible sensitivity based on successful ring trial participation (EFSA, 2017). This value also corresponds with the EFSA's recommended value of the sensitivity.

According to Casulli et al. (2015) and Conraths and Deplazes (2015), the method selected by NI (SCT) has a sensitivity of 98% and 83.8%, respectively. The analyses performed at the AFBI considered a Se of 99% (Eckert, 2003). 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. Here it is reported that no sample classified as negative by the SCT was detected positive by the intestinal scraping technique (IST), which could theoretically lead to the conclusion that the SCT has a sensitivity close to 100%, but in reality, there is no information on the real state of the sample (contaminated/not contaminated) nor is there any data on the IST technique. Therefore, the only possible conclusion is that the IST sensitivity is not higher than the one of the SCT. The almost perfect sensitivity of the SCT is actually an assumption. The EFSA recommendation would be to use a test sensitivity of 0.78 as a more risk averse option.

3.3.2.2. Selection of the target population

Definition of susceptible host population target by the system: The selection of red fox to perform the pathogen surveillance seems appropriate, as this species has been recognized 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 (240,000) is well documented and has been recently updated.

3.3.2.3. Sampling strategy

Epidemiological unit: For GB, the epidemiological unit (post-mortem faecal samples from individual animals of research stations) was well defined and ensures individuality. The same is agreed for NI, where intestinal contents from hunted or road kill individual animals were sampled.

Sample size calculation: The method used to calculate the sample size of GB was the RIBESS tool. The sample size was calculated with an overall sensitivity of the diagnostic approach of 0.78 and a population size of 240,000 (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 GB was 437, which ensures the fulfilment of the technical legal requirements of Commission Delegated Regulation (EU) 2018/772 regarding a confidence level of at least 0.95 against a DP of 1% (0.01).

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 336. 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 DP of 1% (0.01) increase to 379 (with 43 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 0.93, slightly lower than the value indicated among the technical legal requirements of Commission Delegated Regulation (EU) 2018/772 regarding a confidence level of at least 0.95 against a DP of 1% (0.01). 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 NI 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 15.

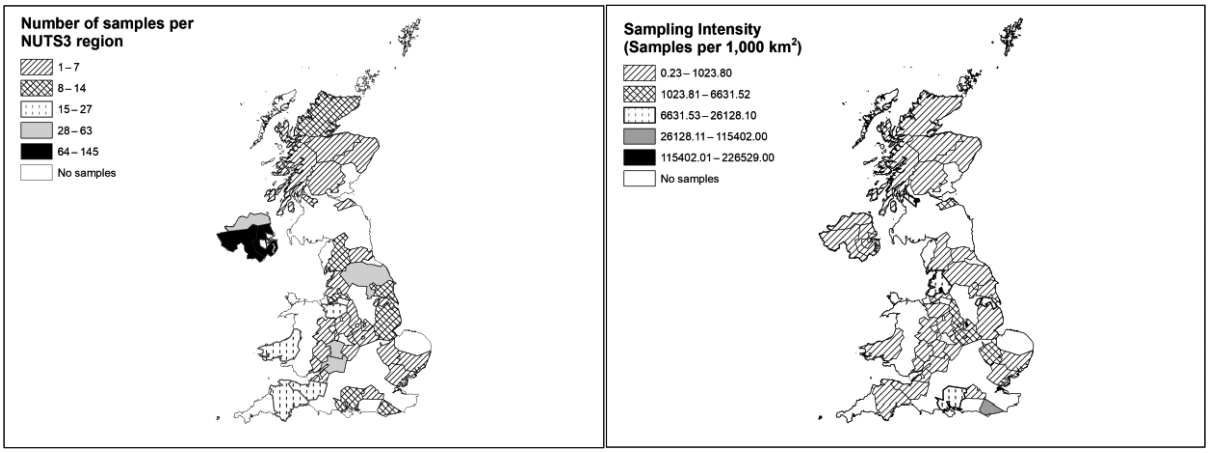


Figure 15: United Kingdom – Sampling activity and intensity by NUTS 3 region

The collection of samples was in both cases reduced during the spring-summer months when hunting is not allowed and the reason for this reduction has been well described.

3.3.2.4. Methodology

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

Epidemiological geographical unit: The UK was divided into two geographical epidemiological units, the whole territory of GB and NI.

Methodology for calculation of the area sensitivity:

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

- DP of 1 %,
- test sensitivity of 0.78,
- population size of 240,000 and
- sample size of 437.

The value of the area sensitivity (0.967) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements included in Commission Delegated Regulation (EU) 2018/772.

The area sensitivity for NI considering the following parameters:

- DP of 1 %,
- test sensitivity of 0.99,
- population size of 14,000 and
- sample size of 336,

With these conditions, area sensitivity was higher than 0.95 (0.966). However, if a test sensitivity of 0.78 is assumed, as suggested by EFSA (2015), the area sensitivity (0.93) is not sufficient to comply with the EU regulation in force (43 additional tests would be required).

In summary, the set of data from the surveillance activity in 2018/2019 for the UK, due to the use of a test sensitivity value not supported by strong scientific evidence by NI, does not ensure the fulfilment of the technical legal requirements of Commission Delegated Regulation (EU) 2018/772 regarding a confidence level of at least 0.95, against a DP of 1% (0.01) for one of its geographical epidemiological units (NI), unless the test sensitivity reported by the laboratory on a small set of spiked samples is used.

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	0.930	

3.4. Norway

3.4.1. Information as submitted in the report by the Member State

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). Test sensitivity was assumed to be at least 63% and the specificity 100% (see Øines et al., 2014 for details). Eggs/DNA extracted from whole

worms (*E. multilocularis* provided by the EURL) and MilliQ water is included as positive and negative control, respectively.

Red fox is the target species and practically, the only wild definitive host for *E. multilocularis* in Norway. There are only tiny populations of wolves and arctic foxes, whereas raccoon dogs are only occasionally reported. In 2018, a low number of samples (< 30) from wolves (*Canis lupus*), as part of our official surveillance programme, were included in the surveillance, but not in the *E. multilocularis* annual report.

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. The red fox is geographically distributed all over Norway, but the population densities during spring are (roughly estimated) varying from 1 red fox/10 km^2 (mountain areas), 3 red foxes/10 km^2 (forest/marsh) and 10 red foxes/10 km^2 (urban/agricultural areas; e.g. Akershus, Vestfold, Østfold) (pers.com. prof. Olav Hjeljord).

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 at with a confidence level of 95%. The goal was approximately 474 samples from red foxes in 2018, i.e. the epidemiological unit is the red fox.

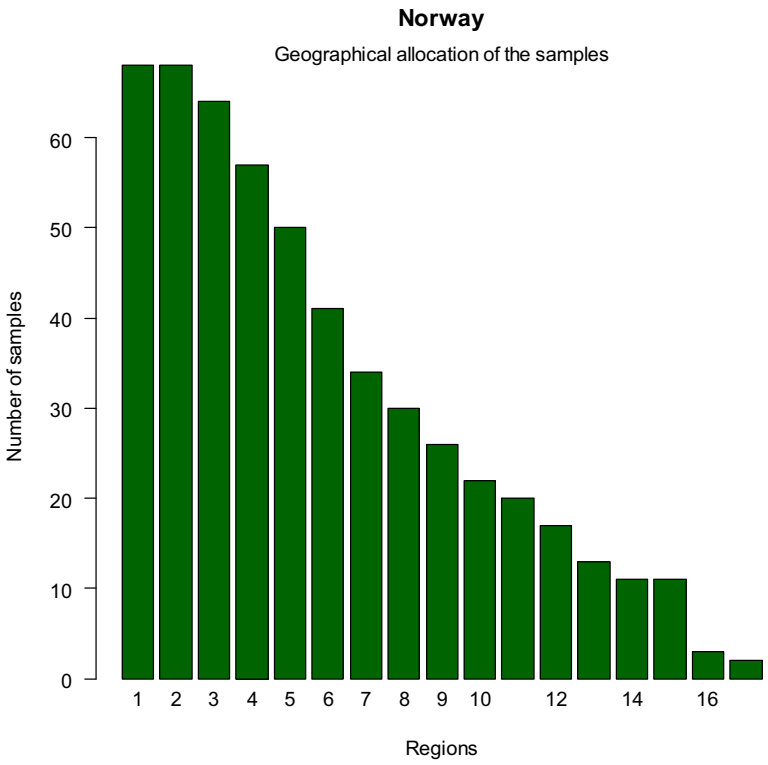
Red fox hunters from across the country were initially invited to participate based on a list obtained from The Norwegian Register of Hunters. In addition, previously participating red fox hunters received an invitation to attend the 2018-sampling season. Hunters were also recruited via the websites of the Norwegian Veterinary Institute and the Norwegian Association for Hunters and Anglers. The red foxes were all killed with firearms (shotgun or rifle), immediately followed by withdrawal of faeces from the rectum. A standard form that included information on where and when the fox had been killed, as well as the sex (male, female) and presumed age of the animal (juvenile, adult), was completed by each hunter. In addition to samples from foxes, samples from wolves (*Canis lupus*) killed legally or illegally during 2018 were tested for *E. multilocularis*.

Faecal samples were promptly mailed individually in pre-paid envelopes to the laboratory. To ensure the individuality of the samples, the hunters were also requested to submit the tongue from each fox together with the corresponding faecal sample. Upon arrival at the laboratory, samples were frozen at –80°C for at least 3 days before for the analysis commenced. Sampling provided by volunteering hunters is regarded to obtain a representative sampling of the national red fox population and no other superior alternatives of sampling under the demanding, both geographical and climatic, conditions in Norway are considered feasible.

The first Swedish case of *E. multilocularis* was reported from a red fox found near Uddevalla in southern Sweden in late 2011. Consequently, red fox hunters in the southern-eastern part of Norway along the border with Sweden were encouraged to increase hunting and to submit samples, since one could argue that the risk of introduction of the parasite to this part of Norway via foxes might be higher than for other parts of the country. Habitat use and extent of migration of red foxes in Sweden is, however, 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. Although the parasite is now approaching via migrating wildlife in neighbouring countries (Uddevalla in Sweden is about 80 km from the Norwegian border), lack of compliance with the anthelmintic treatment requirements for pets entering Norway is also a cause for concern. Thus, we have chosen to uphold the simple random sampling of red foxes.

In total, 537 samples were collected from red foxes in 2018 and all were negative in PCR.

Samples were collected throughout 2018. The spatial distribution of samples (see Figure 16) is somewhat uneven since the topography of Norway (large areas with mountains) entails scattered settlements and sampling is voluntary as performed by hunters that hunt in proximity to their homes. The temporal distribution of samples (Figure 17) is also somewhat uneven due to preferred hunting conditions during winter and banned hunting between April 15 and July 15. Samples were collected during the whole year with a decline of the sampling during the summer season.



	Region	# samples
1	Buskerud	68
2	Østfold	68
3	Trøndelag	64
4	Hordaland	57
5	Akershus	50
6	Nordland	41
7	Vest-Agder	34
8	Hedmark	30
9	Troms	26
10	Telemark	22
11	Oppland	20
12	Vestfold	17
13	Aust-Agder	13
14	Møre Og Romsdal	11
15	Oslo	11
16	Sogn Og Fjordane	3
17	Finnmark	2

Figure 16: Norway – Geographical distribution of samples in 2017–2018

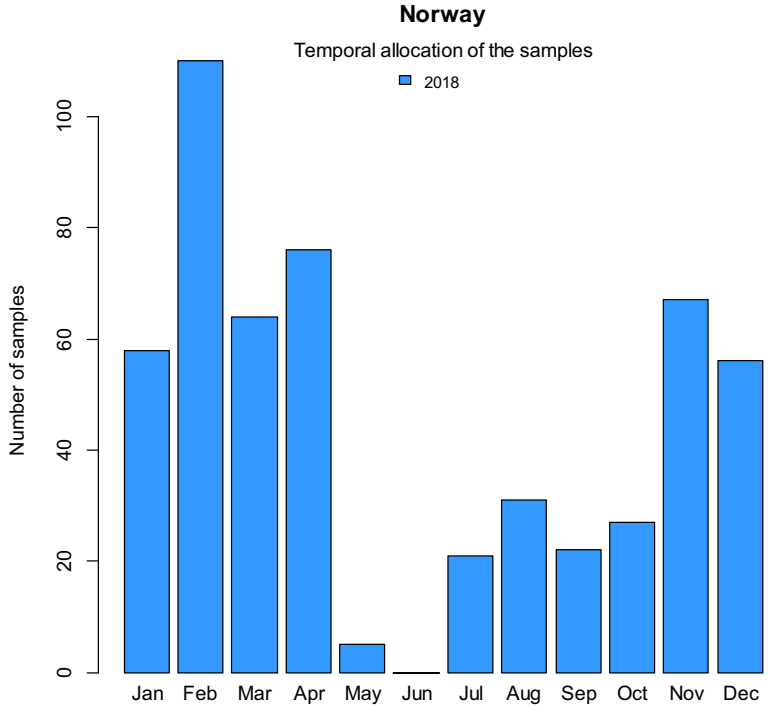


Figure 17: Norway – Temporal distribution of samples from 2018

3.4.2. EFSA comments and considerations

3.4.2.1. Type and sensitivity of the detection method

Type of test: 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: Despite internal trials seem to indicate a better performance of the test (Test Se = 0.8), 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.

3.4.2.2. 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. The reasons put forward by Norway to justify its decision of not including other wild definitive hosts (arctic foxes and raccoon dogs) are valid. Although no references were added, apparently their population densities do not reach high numbers (Environment.no, online: http://www.environment.no/topics/biodiversity/species-in-norway/threatened-species/arctic-fox-mainland-norway/; Florisson and Kreij, 2014).

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.

3.4.2.3. 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 DP of 1% (0.01), a test sensitivity of 0.63, and a population size of 151,000, the sample sized required is 474. The number of samples collected by Norway in 2018 (537 samples between January 2018 and December 2018) is more than required.

Implementation of the sampling activity: Samples were collected from all the 19 Norwegian NUTS3 regions with an increase of the sampling in the south-east of the country (Figure 18). The differences of sampling intensities among the different areas have also been justified in the report.

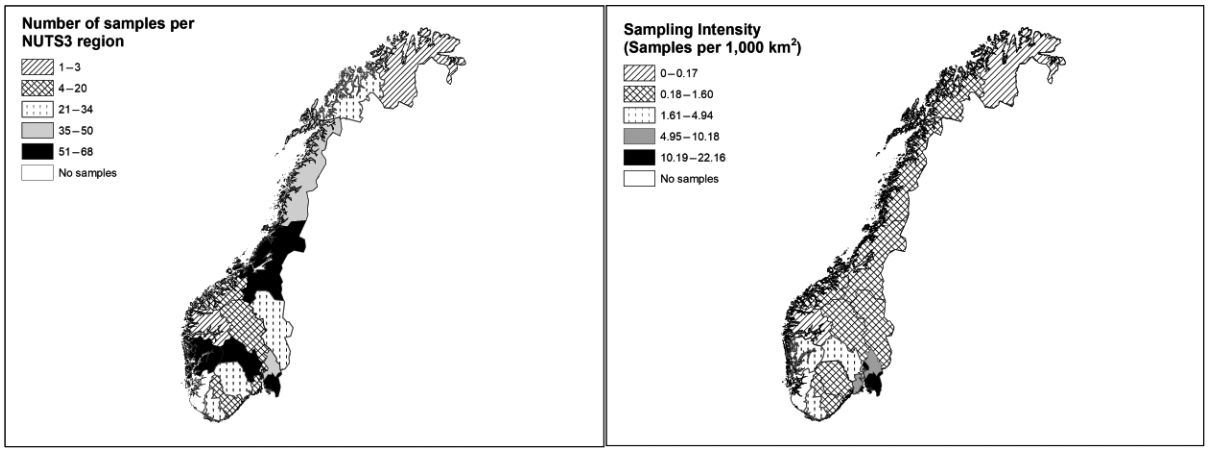


Figure 18: Norway – Sampling activity and intensity by NUTS 3 region in 2017

3.2.4.4. Methodology

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

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

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

- DP of 1 %,
- test sensitivity of 0.63,
- population size of 151,000 and
- sample size of 537,

The area sensitivity value is 0.967 (> 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 2018 ensures the fulfilment of the technical legal requirements of all the paragraphs included in the Annex I of Commission Delegated Regulation (EU) 2018/772.

4. Conclusions

- *E. multilocularis* was not detected in any of the samples from the four countries collected in the 12-months reporting period (2018 or 2018–2019).
- All the countries participating in this surveillance (Finland, the UK, 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. Each of them uses 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, the UK, Ireland and Norway) fulfil the technical legal requirements regarding the collection of samples from wild definitive hosts. The four countries (Finland, the UK, Ireland and Norway) selected adequate wild definitive hosts in order to perform the surveillance.
- All the countries participating in this surveillance (Finland, the UK, 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 DP of less than 1% (0.01). Although the surveillance strategies performed by Finland, the UK, Ireland and Norway cannot be considered 'based on a simple random sample', in the case of wildlife animals, convenience sampling is the method most frequently used. 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, the UK, Ireland and Norway) fulfil the technical legal requirements regarding the 12-month surveillance period collection. In general, the lower number of wild animal samples during spring and summer was well justified and historical data show that this lower number does not compromise the success of the detection of the parasite.
- All the countries participating in this surveillance (Finland, the UK, Ireland and Norway) fulfil the technical legal requirements regarding the confidence level of at least 0.95 against a DP of 1%.
- However, considering the EFSA recommendation of using a test sensitivity of 0.78 (under the assumption of a random sampling method), the sample size from the UK programme implemented in NI should increase by at least 43 additional animals testing negative in order to achieve the confidence level of at least 0.95 against a DP of 1% as described in the relevant legislation. The total number of samples collected by NI, assuming the theoretical safe value of 0.78 as test sensitivity, returns a confidence level equal to 0.93, slightly lower than the value indicated among the technical legal requirements of the Regulation. The UK should provide scientific evidence to support the test sensitivity value proposed in the surveillance.

5. Recommendation

- Based on the data provided by Finland, the sensitivity of the test used at the Finnish Food Authority seems to be characterised by higher values than the one suggested previously by EFSA (0.78) in absence of further specific evidence (EFSA, 2015). An exact binomial test (s = 196, n = 232, i.e. 196 correctly classified as 'positive' out of 232 spiked samples) and a Bayesian approach return consistent results, with a most likely value of 0.87 (0.82–0.91; CL = 95%). A conservative

approach would be to use the lower value of the confidence band, which is, however, higher than the suggest value of 0.78. The Evira/Finnish Food Authority should consider publishing the results of these internal trials for the benefit of the entire scientific community.

- In the framework of the UK programme, NI reported an assumed test sensitivity of 0.99. As explained in this and previous assessments of EFSA, this value is not supported by scientific literature. Similarly to Finland, the UK should perform some internal trials with more spiked samples: over time, the cumulative evidence will provide a scientifically based estimate of the test, with an uncertainty that will narrow over time. With the publication of these results, EFSA will have the elements for accepting the proposed test sensitivity values.

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Glossary

Alveolar echinococcosis	The human disease caused by infection with the larval stage (metacestode) of *E. multilocularis*. It is characterised by infiltrative, tumour-like growth, initially in the liver, potentially causing high fatality rates
EFSA Data Collection Framework (DCF)	The EFSA web interface accessible by most common web browsers through which data providers can submit their files. The system provides automatic feedback on errors in structure and content, and confirmation of successful submissions
Enzyme-linked immunosorbent assay (ELISA)	The test that applies the immunological concept of an antigen binding to its specific antibody, which allows detection of very small quantities of antigens such as proteins, peptides, hormones, or antibody in a fluid sample, utilising enzyme-labelled antibodies or antigens and a chromogenic substrate for the enzyme to detect the target molecules
Geographical epidemiological unit	The portion of territory within a given Member State characterised by a specific risk of presence which differs from others 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
NUTS	The Nomenclature of Territorial Units for Statistics (NUTS), or in French Nomenclature Unités Territoriales Statistiques, is a geocode standard for referencing the administrative divisions of countries for statistical purposes. The standard was developed by the European Union and subdivides the territory of the European Union into regions at three different levels (NUTS 1, 2 and 3, moving from larger to smaller territorial units (see also http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Glossary:NUTS)
Odds ratio (OR)	The ratio of the odds of an event occurring in one group to the odds of it occurring in another group. It estimates the probability of the event given exposure to a specific factor by measuring the probability of exposure given the presence of the event
Risk based Estimate of System sensitivity and Sample size (RiBESS) tool	The Microsoft Excel based tool developed by EFSA for the calculation of the sample size needed to substantiate absence of a given disease and/or to calculate the survey sensitivity (confidence) once the samples have been collected
Sedimentation and counting technique (SCT)	The technique for the quantitative assessment of the *E. multilocularis* burden of foxes or other definitive hosts, where intestinal material is washed and sedimented several times and the resulting sediment is examined under a stereomicroscope for the presence of the parasite

Abbreviations

AE	alveolar echinococcosis
AFBI	Agri-Food and Biosciences Institute
ALPHA	Animal and Plant Health Unit
CE	cystic echinococcosis
CL	confidence Level
DAERA	Department of Agriculture, Environment and Rural Affairs
DH	definitive host
DP	design prevalence
EFTA	European Free Trade Association
EM	*Echinococcus multilocularis*
FI	Finland
GB	Great Britain (including England, Wales and Scotland)
IH	intermediate host
IST	intestinal scraping technique
N	target population size
NI	Northern Ireland
PCR	polymerase chain reaction
RR	relative risk
Se	sensitivity
Sp	specificity
TSe	test sensitivity

Appendix A – Finland. Assessment tables of the surveillance report

Table A.1: Assessment of the description of the surveillance system (Finland – Part I of surveillance report) for a representative sample survey

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	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	• The Finnish Food Safety Authority (Evira) used a PCR 12S rRNA (Isaksson et al., 2014) with a modification in the magnetic beads washing step (manual instead of automatic) described in the paper	Technique well described. A slight modification has been realised and it is indicated in the report
	Test sensitivity	The sensitivity and specificity of the test used in the surveillance system must be reported. This would ideally be estimates from each participating laboratory reported as a point estimate (average) of the values across the country with minimum and maximum values or a probability distribution. Alternatively, a value of 0.78, as recommended by EFSA (2015) shall be used	• **Test Se = 0.78 (78%)**	An exact binomial test indicates that the actual value may lie between 0.82 and 0.91 (95% CL). A Bayesian approach gives similar results. Therefore, the lowest value (0.82) may be the most conservative choice for estimating the overall system sensitivity considering a worst-case scenario
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	• Targeted host species: red fox (*Vulpes vulpes*) and raccoon dog (*Nyctereutes procyonoides*) • No information on age or gender structure of the target population is 1 available	The selection of raccoon dogs and red fox species as target populations was based on their role as definitive hosts in the cycle; assumption also confirmed by the EFSA Scientific opinion on *E. multilocularis* infection in animals (EFSA AHAW Panel, 2015) Regarding age or gender composition of the target population, it is not possible to conclude their role in the epidemiology and in the lifecycle of EM, due to lack of appropriate data and studies (EFSA AHAW Panel, 2015)

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	Comments
	Size of susceptible host population targeted by the system	The size of the targeted (wildlife) population should be reported, together with the evidence for this. Historical population data should be updated since these may not reflect current populations	• Raccoon dog more numerous (342,000) than red fox (120,000) • Population densities for both species highest in the southern part of the country • Population sizes were estimated by Kauhala (2007) using multiple methods and data, including radio tracking, hunting bag statistics, annual snow-track counts and knowledge on reproductive potential of each species. More recent estimates of the population sizes than Kauhala (2007) are not available • Data from annual hunting bag suggest some increase of the raccoon dog population and decrease of the fox population as compared to the situation 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 use the size of the population as published by Kauhala in the estimations is scientifically sound, considering that the sample size calculation is not heavily affected when the population size has large dimensions (see EFSA AHAW Panel, 2015). The fact of considering the sum of the red fox and raccoon dog populations as the target population size seems to be correct, as raccoon dogs can act as DHs in conjunction with the red fox (EFSA AHAW Panel, 2015)
Sampling strategy	**Epidemiological unit**	It should be clearly defined if individual animals or individual faeces samples collected from the environment constitute the epidemiological unit. If individual faeces samples are collected from the environment, the method applied to establish the species from which the faeces originated has to be reported	• The epidemiological unit was defined as the individual animal (red fox or raccoon dog)	The epidemiological unit appears in the report and is defined as the individual animal. Individual rectal contents were collected directly by hunters
	Sample size calculation	The applied survey design should be fully documented, including considerations regarding potential biases inherent in the survey	The required sample size has been calculated using the RiBESS tool with the following parameters: • Design prevalence = 0.01 (1%) • Test sensitivity = 0.78 (78%)	The area sensitivity was estimated by FI using the RiBESS tool. The parameters included for the calculation were the following, all fully documented

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	Comments
		design. The method and the formula used to calculate the sample size should be fully documented	• Target System Sensitivity = 0.95 (95%). • Target population size = 462,000 The sample size was estimated as being 383 (both binomial and hypergeometric)	
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must to be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be homogeneous	• Samples collected by hunters on a voluntary basis • Sampling targeted in the southern part of the country where populations are densest • The majority of the samples originated from south-east Finland; region with active monitoring rabies control programme and elevated risk of introduction of EM due to geographical closeness of infected areas, and also with highest density of raccoon dogs (Kauhala, 2007) • Large sample of foxes (17% of all animals) was received from Lappi where red fox population reduction to protect the arctic fox was ongoing • Samples were collected throughout 2018	In reality, the largest portion of samples originates from Etelä-Karjala region (south-east, 23% of the samples), followed by the Lappi region. There was a higher intensity of the sampling in the south-east of the country The surveillance strategy as described in the Finnish report cannot be considered a simple random sample, as claimed. Most of the samples were collected by hunters and efforts were concentrated the 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 (between April and September) 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; but a quantitative evaluation was not performed (EFSA Scientific Report, 2013)

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	Comments
Methodology	**Design prevalence (DP)**	DP is specified in Annex II to Regulation (EU) No 1152/2011 and must be 1% or lower	DP = 0.01 (1%)	
	Geographical epidemiologic unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification	The whole territory of Finland was considered as one epidemiological 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 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)	The system sensitivity was calculated by Finland using an overall sensitivity of the diagnostic approach of 0.78 and the design prevalence of 1 % prescribed in Regulation (EU) No 1152/2011 using the RiBESS tool SYSTEM SENSITIVITY CALCULATION DP = 0.01 TSe = 0.78 sample size for 2018 n = 529 The obtained system sensitivity was 0.987 (both binomial and hypergeometric)	Using the lowest bound of the credible interval estimated from the cumulative evidence provided by Finland about the outcome of internal trials (0.82), the confidence achieved is 0.99

Table A.2: Descriptive statistics for a representative survey (Finland - Part II of surveillance report)

Parameter	Evidence	Action
Theoretical sampling period	From 1 January 2018 to 31 December 2018	–
Actual sampling period	From January 2018 to December 2018	Exact sampling date not reported. Explain, if possible, what is the technical issue
Sampling period		–
Number of samples	529	
Number of test results	529 PCR 12S rRNA	
Laboratory test completion	529 results in 2018	–
Sensitivity	0.78	–
Host	203 *Vulpes vulpes*; 326 *Nyctereutes procyonoides*	–
Animal sample	529 Individual rectal content	–
Sampling strategy and design	Objective sampling – Simple random sample	–
Sampling point	529 hunting	–

Appendix B – Ireland. Assessment tables of the surveillance report

Table B.1: Assessment of the description of the surveillance system (Ireland – Part I of surveillance report) for a representative sample survey

Points addresses in Annex II	Element	Description of Element	Information provided in surveillance report	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	• Rectal contents from foxes were examined according to Trachsel et al. (2007); PCR Cest1-Cest2 NAD1 • DNA nucleotide sequences of primers were: Cest1=TGCTGATTTGTTAAAGTTAGTGATC Cest2 = CATAAATCAATGGAAACAACAACAAG	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	**Test Se = 0.78 (78%)** (based on EFSA AHAW Panel, 2015)	Ireland followed EFSA's advice regarding the setting of at least the conservative lowest value of the sensitivity (0.78)
Selection of the target population	**Definition of susceptible host population targeted by the system**	The susceptible wild definitive host population(s) targeted by the surveillance system should be described and the choice justified. If domestic host species are sampled, evidence for the absence of wild definitive hosts and for these domestic animals having had access to outdoors should be provided	• Because of the occurrence of red foxes throughout the country and no known occurrence of racoon dogs (Hayden and Harrington, 2000; Marnell et al., 2009), the former was selected as the wildlife definitive host species to survey for presence of *E. multilocularis* • The age structure of the red fox population varies depending on the time of year • There is little published scientific evidence of the gender structure of the Irish red fox population	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 is also supported by scientific literature Regarding 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)

Points addresses in Annex II	Element	Description of Element	Information provided in surveillance report	Comments
	Size of susceptible host population targeted by the system	The size of the targeted (wildlife) population should be reported, together with the evidence for this. Historical population data should be updated since these may not reflect current populations	• Red fox population has been estimated to be between 150,000 and 200,000 (Hayden and Harrington, 2000; Marnell et al., 2009) • Further information about red fox population distribution within Ireland has been produced in a report by Dr. Tomás Murray from the National Biodiversity Data Centre in 2015	Although the original information regarding the red fox population size was published in 2000 and 2009 (Hayden and Harrington, 2000; Marnell, 2009), Dr. Tomás Murray (National Biodiversity Data Centre), Ireland, provided additional information in 2015. Nevertheless, at a population size greater than 10,000, moderate fluctuations in the population size would not significantly change the sample size required to achieve the same statistical confidence of less than 1% (0.01) prevalence at a specific test sensitivity (EFSA, 2014). Therefore, fluctuations in the previous population size of 150,000 do not significantly alter the sample size required (EFSA, 2014)
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	The epidemiological unit was defined as the individual animal (the individual fox (*Vulpes vulpes*))	The epidemiological unit is defined in the report as the individual animal. Faeces samples were obtained post-mortem from culled or trapped animals
	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	The required sample size has been calculated using **the RiBESS tool** with the following parameters: • Design prevalence = 0.01 (1%) • Test sensitivity = 0.78 (78%) • Target System Sensitivity = 0.95 (95%). • Target population size = 150,000 The sample size was estimated as being 383	The total number of samples collected by Ireland was 403, which ensures the fulfilment of the technical legal requirements concerning a confidence level of at least 0.95 against a design prevalence of 1% (0.01)

Points addresses in Annex II	Element	Description of Element	Information provided in surveillance report	Comments
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must to be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be homogeneous	• Samples from culled foxes (by shooting) for pest and predator control reasons and foxes inadvertently captured in traps set for other wildlife as part of wildlife disease control measures • Each of the 16 Regional Veterinary Offices 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 throughout 2018 • Sampling intensity was undertaken to reflect the distribution throughout Ireland and further adjusted to reflect the geographical variation in density of fox population distribution	Samples were collected from all the available NUTS3 regions (8/8). The highest number of samples comes from the north of Ireland (bordering Northern Ireland). The sampling activity per 1000 km^2 (intensity) is higher in the south-east of Ireland. This distribution actually takes into account the geographical variation in density of fox population distribution Samples were obtained during the whole year excluding June, July, August and September. The reduction of collection of samples during spring and summer is justified. 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)
Methodology	**Design prevalence (DP)**	DP is specified in Annex II to Regulation (EU) No 1152/2011 and must be 1 % or lower	DP = 0.01 (1%)	
	Geographical epidemiologic unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification	The whole territory of Ireland was considered as one epidemiological 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

Points addresses in Annex II	Element	Description of Element	Information provided in surveillance report	Comments
	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)	SYSTEM SENSITIVITY CALCULATION DP = 0.01 TSe = 0.78 sample size for 2018 n = 403 The obtained system sensitivity was 0.958	The area sensitivity was estimated by Ireland using the RiBESS tool. The parameters included for the calculation were the following: a) design prevalence of 1%, b) test sensitivity of 0.78, c) population size of 150,000 and d) sample size of 403. The value of the area sensitivity 0.958 (> 0.95) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements

Table B.2: Descriptive statistics for a representative survey (Ireland – Part II of surveillance report)

Parameter	Evidence	Action
Theoretical sampling period	From 1 January 2018 to 31 December 2018	–
Actual sampling period	From 1 January 2018 to 19 December 2018	
Sampling period	353 days	–
Number of samples	403	
Number of test results	403 PCR Cest1-Cest2 NAD1	
Laboratory test completion	225 results in 2018 178 results in 2019	–
Sensitivity	0.78	–
Host	403 *Vulpes vulpes*	–
Animal sample	403 faeces post-mortem	–
Sampling strategy and design	Objective sampling – simple random sample	–
Sampling point	273 from hunting; 130 wildlife research station	–

Appendix C – United Kingdom. Assessment tables of the surveillance report

Table C.1: Assessment of the description of the surveillance system (Great Britain – Part I of surveillance report) for a representative sample survey

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	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	• A PCR test (PCR Cest1-Cest2 NAD1) to detect *E. multilocularis* DNA in rectal content (post-mortem sampling) was used (Mathis et al., 1996; Dinkel et al., 1998) • Method based on the concentration of helminth eggs by a combination of sequential sieving of faecal samples and flotation of the eggs in zinc chloride solution • DNA of the taeniid eggs retained in the 20 microns sieve was obtained after alkaline lysis • Nested PCR was performed using *E. multilocularis* species-specific primers against the mitochondrial 12S rRNA gene	The method used for detection of *E. multilocularis* in GB was well described and cited
	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	• Test sensitivity for the PCR is between 85% and 99% depending on the laboratory • The sensitivity of the proposed method is further determined using spiked faecal samples and the specificity is tested with other teaniid species • In the case of the APHA/FERA laboratory, 78% sensitivity was used as the lowest possible sensitivity, based on successful ring trial participation	APHA/FERA laboratory used a sensitivity of 78% considering the lowest possible sensitivity based on successful ring trial participation. This value also corresponds with the EFSA's recommended value of the sensitivity

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	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	• Red fox (*Vulpes vulpes*) the only wild definitive host for *E. multilocularis* in the UK. No other wild definitive host is present • Great Britain and Northern Ireland fox populations are isolated, with no access for wild definitive hosts from continental Europe • The rapid spread of sarcoptic mange in the red fox population and lack of geographic barriers demonstrates that there is considerable mixing of the red fox population within GB and within the island of Ireland, despite the variation in abundance • Uneven distribution of the wild host population – some areas less dense fox populations than others – e.g. the highest density is in urban areas in the south-west of England, the least dense are rural areas in Northern Scotland • Distribution has not changed significantly in the last ten years	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	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	• Great Britain consists of islands, surrounded by sea with no land bridges for foxes to arrive by, therefore there is a constant population (which varies during the year according to whether the females have given birth). Population size is based on numbers of breeding females • The fox population size (pre-breeding adults) estimated by wildlife experts (Defra, 2013) and recently modelled by Croft (Croft et al., 2017) is about 240,000 and is believed to be relatively stable, or marginally increasing • The urban/suburban fox population is now estimated at ~ 33,000 (~ 13%)	Data of fox population size (240,000) is well documented and has been recently updated

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	Comments
			• The variation in abundance is likely correlated with food resources (hill areas of Scotland estimated at 1 breeding pair every 40 km^2, the highest density recorded was 30 foxes in a single km^2 in urban areas) • The average range of a red fox in the UK in open farm land is considered to be ~200–600 ha (2–6 km^2) • There is good evidence that the total abundance has not changed in the last decade (Wright et al., 2014; Croft et al., 2017) as measured on BTO survey squares (mostly rural), and as predicted. The urban fox distribution has changed in recent years with almost all urban areas now having foxes present (Scott et al., 2014) • A map of systematically estimated fox distribution and abundance using NBN data and published density information and a small project using public sighting data to estimate fox abundance in all urban areas was provided	
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	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 epidemiological unit (post-mortem faecal samples from individual animals of research stations) was well defined and ensures individuality

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	Comments
	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	The sample size has been calculated using the EFSA RiBESS tool with the following parameters: • Design prevalence = 0.01 (1%) • Test sensitivity = 0.78 (78%) • Target System Sensitivity = 0.95 (95%) • Target population size = 240,000 The sample size was estimated as being 388 In GB, 437 samples were collected and tested	The total number of samples collected by GB was 388, which ensures the fulfilment of the technical legal requirements regarding a confidence level of at least 0.95 against a design prevalence of 1% (0.01)
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must to be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be homogeneous	• Post-mortem faecal samples of wild animals were collected from research stations -only an approximate location of the animal can be used • Reports were made at NUTS 3 level (the lowest level of NUTS; in GB individual counties or upper-tier authorities, unitary authorities or districts) • The uneven geographical distribution of the population means sampling of animals also uneven. The sampling activity targeted the regions with higher fox density • Sampling 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	The sampling process has more the characteristics of a convenience sampling, rather than a simple random sample. The difficulties in running such a sampling technique, however, are well known and are broadly discussed in previous reports. The temporal distribution of samples was reduced during the spring-summer months and the reason of this reduction of the sampling effort has been well justified
Methodology	**Design prevalence (DP)**	DP is specified in Annex II to Regulation (EU) No 1152/2011 and must be 1 % or lower	DP = 0.01 (1%)	

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	Comments
	Geographical epidemiologic unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification	The United Kingdom was divided into two surveillance regions for the purpose of this report: GB (England, Scotland and Wales) and NI	The whole territory of GB and NI were considered each as one epidemiological unit in their respective 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)	The system sensitivity was calculated by GB using an overall sensitivity of the diagnostic approach of 0.78 and the design prevalence of 1 % prescribed in Regulation (EU) No 1152/2011 using the RiBESS tool SYSTEM SENSITIVITY CALCULATION DP = 0.01 TSe = 0.78 sample size for 2018 n = 437 The obtained system sensitivity was 0.967	The area sensitivity was estimated by GB using the RiBESS tool. The parameters included for the calculation were the following: a) design prevalence of 1 %, b) test sensitivity of 0.78, c) population size of 240 000 and d) sample size of 437. The value of the area sensitivity (0.967; > 0.95) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements

Table C.2: Descriptive statistics for a representative survey (Great Britain – Part II of surveillance report)

Parameter	Evidence	Action
Theoretical sampling period	From 1 March 2018 to 28 February 2019	–
Actual sampling period	From 14 March 2018 to 16 January 2019	–
Sampling period	339 days	–
Number of samples	437	–
Number of test results	437 PCR Cest1-Cest2 NAD1	–
Laboratory test completion	22 in 2017; 423 in 2018	–
Sensitivity	0.78	–
Host	437 *Vulpes vulpes*	–
Animal sample	437 faeces post-mortem	–
Sampling strategy and design	Objective sampling – Simple random sample	–
Sampling point	Wildlife Research Station 388	–

Table C.3: Assessment of the description of the surveillance system (Northern Ireland – Part I of surveillance report) for a representative sample survey

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	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	• Sedimentation and counting technique (SCT) test • To detect *E. multilocularis* eggs from individual intestinal content • The analyses were performed at the Agri-Food and Biosciences Institute (AFBI)	The method used for detection of *E. multilocularis* in NI is cited
	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	• **Test Se = 0.99 (99%)**	The evidence provided to support the test sensitivity value for the SCT (Eckert, 2003) actually refers to a previous work (Hofer et al., 2000) focus on the prevalence in the target population and not in the sensitivity of the SCT. The almost perfect sensitivity of the SCT is actually an assumption. A safer option would be to follow the EFSA recommendation (Test Se = 0.78) As an alternative, NI should provide evidences 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	• Red fox (*Vulpes vulpes*) the only wild definitive host for *E. multilocularis* • No other wild definitive host is present • Great Britain and Northern Ireland fox populations are isolated, with no access for wild definitive hosts from continental Europe • Uneven distribution of the wild host population – some areas less dense fox populations than others – e.g. the highest density is in urban areas in the south-west of England, the least dense are rural areas in Northern Scotland • Distribution has not changed significantly in the last 10 years	The selection of red fox to perform the pathogen surveillance seems appropriate, as this species has been recognized 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

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	Comments
	Size of susceptible host population targeted by the system	The size of the targeted (wildlife) population should be reported, together with the evidence for this. Historical population data should be updated since these may not reflect current populations	• An estimate of 14,000 is given; 1 fox per km^2 and accounts for the large area of rural land in contrast to the urban land use (Conserve Ireland, 2009) • Great Britain consists of islands, surrounded by sea with no land bridges for foxes to arrive by, therefore there is a constant population (which varies during the year according to whether the females have given birth) • Population size is based on numbers of breeding females	
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	• The epidemiological unit was the individual animal. As animal carcasses rather than fox scat were collected, the results could be reported at the individual level with a high level of confidence	The epidemiological unit (intestinal contents from individual hunted or roadkill animals) was well defined and ensures individuality
	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	The sample size has been calculated using the EFSA RiBESS tool with the following parameters: • Design prevalence = 0.01 (1%) • Test sensitivity = 0.99 (99%) • Target System Sensitivity = 0.95 (95%). • Target population size = 14,000 The sample size was estimated as being 298	If a sensitivity of 0.78 is considered (as recommended by EFSA as a worst-case scenario), the required samples to fulfil the technical legal requirements regarding a confidence level of at least 0.95 against a design prevalence of 1% (0.01) increase to 379 (with 81 additional samples needed) 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

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	Comments
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must to be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be homogeneous	• Wild animal carcasses collected from hunting or road kills; 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, the number was low • Reports were made at NUTS 3 level (the lowest level of NUTS, districts in NI) • The uneven geographical distribution of the population means sampling of animals is also uneven • Sampling carried out at certain times of the year –hunting is not permitted during the breeding season	The sampling process has more 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. The collection of samples was in both cases reduced during the spring–summer months and the reason for this reduction has been well justified
Methodology	**Design prevalence (DP)**	DP is specified in Annex II to Regulation (EU) No 1152/2011 and must be 1 % or lower	DP = 0.01 (1%)	
	Geographical epidemiologic unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification	The United Kingdom was divided into two surveillance regions for the purpose of this report: GB (England, Scotland and Wales) and NI	GB and NI were considered each as one epidemiological unit in their respective 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)	The system sensitivity was calculated by NI using an overall sensitivity of the diagnostic approach of 0.99 and the design prevalence of 1 % prescribed in Regulation (EU) No 1152/2011 using the RiBESS tool SYSTEM SENSITIVITY CALCULATION DP = 0.01 TSe = 0.99 sample size for 2018 n = 336 The obtained system sensitivity was 0.965 (binomial) and 0.966 (hypergeometric)	If a test sensitivity of 0.78 is assumed, the area sensitivity (0.928, binomial; 0.93 hypergeometric) is not sufficient to comply with the technical legal requirements of the EU regulation in force (43 additional tests would be required)

Table C.4: Descriptive statistics for a representative survey (Northern Ireland – Part II of surveillance report)

Parameter	Evidence	Action
Theoretical sampling period	From 1 April 2017 to 31 March 2018	–
Actual sampling period	From 5 April 2018 to 26 March 2019	–
Sampling period	355 days	–
Number of samples	336	–
Number of test results	336 sedimentation and counting technique	–
Laboratory test completion	135 test results in 2018 201 test results in 2019	–
Sensitivity	0.99	Adopt the suggested value by EFSA (0.78) or provide scientific evidences on the proposed test sensitivity value of 0.99
Host	336 *Vulpes vulpes*	–
Animal sample	336 individual intestinal content	–
Sampling strategy and design	Objective sampling – Simple random sample	–
Sampling point	307 from hunting; 29 from road kills	–

Appendix D – Norway. Assessment tables of the surveillance report

Table D.1: Assessment of the description of the surveillance system (Part I of surveillance report) for a representative sample survey – Norway

Points addresses in Annex II	Element	Description of Element	Information provided in surveillance report	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	• DNA-fishing technique, PCR 12S rRNA (Isaksson et al., 2014) • 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 analysed in duplicates • Primers: 'EMrtCO1F' (5′-TGGTATAAAGGTGTTTACTTGG-3′) 'EMrtCO1Rew' (5′-ACGTAAACAACACTATAAAAGA-3′) 'Zen probe' 5′-56-FAM/TCTAGTGTA/Zen/ AATAAGAGTGATCCTATTTTGTGGTGGGT/ 3IABkFq/-3′ • Following a positive signal, samples 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) • Eggs/DNA extracted from whole worms (*E. multilocularis* provided by the EURL) and MilliQ water is included as positive and negative control, respectively	Method well described and appropriately referenced in the report
	Test sensitivity	The sensitivity and specificity of the test used in the surveillance system must be reported. This would ideally be estimates from each participating laboratory reported as a point estimate (average) of the values across the country with minimum and maximum values or a probability distribution. Alternatively, a value of	• Test Se = 0.63 (63%) • Test Sp = 100%	Despite internal trials seem to indicate a better performance of the test (Test Se = 0.8), 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

Points addresses in Annex II	Element	Description of Element	Information provided in surveillance report	Comments
		0.78, as recommended by EFSA (2015), should be used		the legislation, as a large sample size is required The choice of using a lower value than the one suggested by EFSA goes in a precautionary direction
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	• Red fox practically the only wild definitive host for *E. multilocularis* • Only tiny populations of wolves and arctic foxes, whereas raccoon dogs are only occasionally reported	The reasons provided by Norway to justify its decision of not including other wild definitive hosts (arctic foxes and raccoon dogs) are scientifically sound. Although no references were added, apparently their population densities do not reach high numbers (Environment.no, online; Florisson and Kreij, 2014)
	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	• No scientific studies describing red fox population size in the literature • Around 21,000 red foxes hunted annually (Statistics Norway) • In the absence of better alternatives, an updated estimated red fox population (partly based on the spatial distribution of preferred fox habitat and hunting statistics; provided by professor emeritus Olav Hjeljord) of 151,000 was used in the surveillance programme • Red fox geographically distributed all over Norway, but population densities during spring are (roughly estimated) varying from 1 red fox/10 km^2 (mountain areas), 3 red foxes/10 km^2 (forest/marsh) and 10 red foxes/10 km^2 (urban/agricultural areas; e.g. Akershus, Vestfold, Østfold) (pers.com. prof. Olav Hjeljord)	In absence of data on fox populations in Norway, the size was estimated considering the annual hunted foxes

Points addresses in Annex II	Element	Description of Element	Information provided in surveillance report	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	• The epidemiological unit was defined as the red fox	The epidemiological unit appears in the report and is defined as the red fox. Individual rectal contents were collected directly by hunters
	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	The sample size has been calculated using the EFSA RiBESS tool with the following parameters: • Design prevalence = 0.01 (1%) • Test sensitivity = 0.63 (63%) • Target System Sensitivity = 0.95 (95%). • Target population size = 151,000 The sample size was estimated as being 474	The sample size required using the value suggested by EFSA for the test sensitivity (0.78) is 383
	Implementation of the sampling activity	The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. Timeframe of the surveillance data and geographical clustering of the infection must to be reported. The sample collection period must comprise the whole year and the spatial distribution of the sampling must be homogeneous	• Hunters from across the country were initially invited to participate by different means • Red foxes were killed with firearms, but occasionally also caught in traps or killed in traffic accidents • A standard form that included information on place, time of dead, sex and presumed age was completed by each hunter. • Faecal samples were mailed individually to the laboratory with ear or tongue from each fox to ensure the individuality • At the laboratory samples were frozen at – 80°C for at least three days before analysis	Samples were collected from all the 19 Norwegian NUTS3 regions with an increase of the sampling in the south-east of the country. The differences of sampling intensities among the different areas have been justified in the reportSamples were collected during the whole year with a decline of the sampling during the summer season. The reasons are well justified

Points addresses in Annex II	Element	Description of Element	Information provided in surveillance report	Comments
			• Sampling provided by volunteering hunters is regarded to obtain a representative sampling of the national red fox population and no other superior alternatives of sampling under the demanding, both geographical and climatic, conditions in Norway are considered feasible • The sampling activity is more concentrated along the Swedish borders, without compromising the representativeness of the sample (performing a simple random sampling; convenience criterion) • Samples were collected throughout 2018 • The spatial distribution of samples is somewhat uneven since the topography of Norway (large areas with mountains) entails scattered settlements and sampling is voluntary as performed by hunters that hunt in proximity to their homes • The temporal distribution of samples is also somewhat uneven due to preferred hunting conditions during winter and banned hunting between April 15 and July 15	
Methodology	**Design prevalence (DP)**	DP is specified in Annex II to Regulation (EU) No 1152/2011 and must be 1% or lower	DP = 0.01 (1%)	
	Geographical epidemiologic unit	The geographic epidemiological unit (s) identified as target for the surveillance activity has to be clearly indicated and supported by justification	–	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

Points addresses in Annex II	Element	Description of Element	Information provided in surveillance report	Comments
	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)	SYSTEM SENSITIVITY CALCULATION DP = 0.01 TSe = 0.63 sample size for 2018 n = 537 The obtained system sensitivity is 0.967 (hypergeometric). Using the value suggested by EFSA for the Test Sensitivity (0.78) the system sensitivity achieved is 0.985	Using the RiBESS tool, and considering a test sensitivity of 0.63, a population size of 151000 and a sample size of 537, the value of the area sensitivity is 0.967, which is above the established minimum value of 0.95 needed to fulfil the technical legal requirements Using the value suggested by EFSA for the Test Sensitivity (0.78) the system sensitivity achieved is 0.985

Table D.2: Descriptive statistics for a representative survey (Part II of surveillance report) – Norway

Parameter	Evidence	Action
Theoretical sampling period	1 January–31 December 2017	–
Actual sampling period	11 January 2018–31 December 2018	–
Sampling period	354 days	–
Number of samples	537	–
Number of test results	537 PCR 12S rRNA	–
Laboratory test completion	537 reported in 2018	–
Sensitivity	0.63	–
Host	537 *Vulpes vulpes*	–
Animal sample	537 individual rectal content	–
Sampling strategy and design	Objective sampling – single random sampling	–
Sampling point	537 hunting	–