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Annual assessment of *Echinococcus multilocularis* surveillance reports submitted in 2018 in the context of Commission Regulation (EU) No 1152/2011

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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, Malta, the United Kingdom (UK) and Norway in their respective surveillance programmes. The surveillance programmes of these five countries were evaluated by checking the information submitted by each of them and verifying that the technical requirements laid down in Regulation (EU) No 1152/2011 were complied. The information was divided in four different categories for assessment: the type and sensitivity of the detection method, the selection of the target population, the sampling strategy and the methodology. For each category, the main aspects that need to be considered in order to accomplish the technical requirements of the legislation were checked against compliance of several criteria. All of the countries participating in this surveillance (Finland, the UK, Norway, Malta and Ireland) succeeded in the fulfilment of the technical legal requirements foreseen in Regulation (EU) No 1152/2011 concerning these four different categories. Northern Ireland (UK) fulfils those requirements only when assuming a diagnostic test sensitivity value of 0.99, which is higher than the sensitivity value suggested by EFSA (conservative value of 0.78). None of the five countries recorded positive samples in the 12-month reporting period.

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

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Summary

Following a request from the European Commission and, indirectly, from the European Free Trade Association (EFTA) Surveillance Authority, the Animal and Plant Health Unit (ALPHA) at the European Food Safety Authority (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 five countries which are listed in the Annex I of Regulation (EU) No 1152/2011.

The surveillance programmes performed by Finland, Ireland, the UK, Malta and Norway as reported in 2018 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 Regulation (EU) No 1152/2011 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 3' (Annex II): (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 four 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 *E. multilocularis* 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 of the countries selected adequate wild definitive hosts in order to perform the surveillance, with the exception of Malta, which, in the absence of wild hosts, selected dogs to perform the surveillance. Northern Ireland fulfils the requirements of Regulation (EU) No 1152/2011 related to the desired confidence level of 95% only when assuming a test sensitivity of 0.99, i.e. a value higher than the one recommended by EFSA in 2015 (0.78).

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

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1. Introduction

Human alveolar echinococcosis (AE), caused by the larval stage of the fox tapeworm *Echinococcus multilocularis* (*E. multilocularis*), is a serious parasitic zoonosis (Torgerson et al., 2010; EFSA AHAW Panel, 2015; EFSA and ECDC, 2017).

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 (Peregrine et al., 2012; CDC, online). 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 are able to 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		**Intermediate hosts**	
Red fox (*Vulpes vulpes*)	Considered the main DH	**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
Arctic fox (*Vulpes lagopus*)	In Europe, only relevant in Svalbard	**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
Raccoon dog (*Nyctereutes procyonoides*), **Wolf** (*Canis lupus*), **Golden jackal** (*Canis aureus*)	In presence of the red fox, they can act as DHs. There is no evidence supporting their ability to maintain the lifecycle in the absence of the red fox	**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)

Definitive hosts		Intermediate hosts	
Domestic dog and wild cat (*Felis s. silvestris*)	Overall, prevalence of **dogs** with the parasite is low. However, in experimental surveys, they become infected easily On the contrary, **cats** hardly get infected experimentally, but their natural infection has been reported in numerous occasions. For both species further information is needed	Suids, horses and domestic dogs	Only accidental or refractory intermediate hosts

DH: definitive host.

The distribution of the parasite seems[1] 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, E. multilocularis 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 twenty-four European countries have found the presence of *E. multilocularis* in the main definitive host, the red fox. In addition, human cases of AE are notified every year (ECDC, 2016) in some of these countries (**Table 2**). Overall, in 2015, 135 human cases of confirmed echinococcosis were attributed to *E. multilocularis*, including one death in Bulgaria (ECDC, 2017).

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 European Union 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).

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[(a)]
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, Netherlands, Romania and Ukraine	> 1% to < 10%	
Czech Republic, Estonia, France, Germany, Latvia, Lithuania, Luxembourg, Poland, Serbia, Slovakia, Liechtenstein and Switzerland	> 10%	

*: Excluding Svalbard.
(a): Only included the confirmed *E. multilocularis* species.

In order to guarantee the prevention of introduction of *E. multilocularis* through dogs (non-commercial movements only) into those European territories of the Member states, or parts thereof, that (i) have a lack of presence of the parasite in definitive host or (ii) have implemented an eradication

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

programme of the parasite in wild definitive hosts within a defined scale,[2] the European Union adopted Regulation (EU) No 1152/2011 'as regards preventive health measures for the control of *E. multilocularis* in dogs'.

On the one hand, this Regulation gives to those Member States (or parts thereof) the right to apply preventive health measures (see in Article 7) to dogs intended for non-commercial movements prior to their introduction.

On the other hand, this Regulation entails certain obligations for those countries (see Art. 5), including the implementation of pathogen-specific surveillance programmes, in accordance with Annex II, 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 shall be designed to detect, per epidemiologically relevant geographical unit in the Member State or part thereof, a prevalence of not more than 1% at confidence level of at least 95%;
2) The pathogen-specific surveillance programme shall use appropriate sampling, either risk-based or representative, that ensures detection of the *E. multilocularis* parasite if present in any part of the Member State at the design prevalence specified at point 1;
3) The pathogen-specific surveillance programme shall consist in the ongoing collection, during the 12-month surveillance period, of samples from wild definitive hosts or, in the case where there is evidence of the absence of wild definitive hosts in the Member State or part thereof, from domestic definitive hosts, to be analysed by examination of:
    a) intestinal contents for the detection of the *E. multilocularis* parasite by the sedimentation and counting technique (SCT) or a technique of equivalent sensitivity and specificity; or
    b) faeces for the 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.

The outcomes of the pathogen-specific surveillance programme of each Member State listed in the Annex I need to be annually submitted to the Commission by the 31 May.

At the moment, only four Member States (Finland, Ireland, Malta and the United Kingdom) are listed in the Annex I of Regulation (EU) No 1152/2011. The Decision of the EEA Joint Committee No 103/2012 of 15 June 2012 added also Norway to the list of countries complying with the conditions of Article 3 (Conditions for listing Member States of parts thereof in Part A of Annex I) of the legislation.

This report follows previous annual reports (EFSA, 2013, 2014, 2015, 2016, 2017) presented by EFSA to the European Commission which aim to analyse and assess the sampling strategy, data collection and detection methods used by these five countries in the context of Regulation (EU) No 1152/2011 in their respective *E. multilocularis* (pathogen-specific) surveillance programmes, and verify that the requirements laid down in this regulation are being complied with (EFSA AHAW Panel, 2015).

It is noted that the Commission Implementing Regulation (EU) 2018/878 is not applicable to the pre-July 2018 situation, meaning that Malta was still required to submit surveillance data.

1.1. Background and Terms of Reference as provided by 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[3] 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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[2] These territories are listed in Annex I of the legislation.

[3] 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.

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

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

1.2. Interpretation of the Terms of Reference

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

2. Data and methodologies

To address Terms of Reference (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 2018 surveillance reports of the four 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 Regulation (EU) No 1152/2011.

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 provided for in point c) of Article 3' (Annex II).

Table 3: Assessment categories and their equivalence in the Regulation (EU) No 1152/2011 (Annex II)

Information category	Main points considered in the assessment	Regulation (EU) No 1152/2011 reference
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 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 II – 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 host in the absence of the first	Annex II – Point 3
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 II – Point 2
	The sampling strategy was evaluated to ensure the fulfilment of the technical legal requirements regarding the 12-month surveillance period of data collection	Annex II – 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 II – Point 1

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

Table 4: Relevant elements checked for compliance of the technical requirements of Annex II of Regulation (EU) No 1152/2011

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 *E. multilocularis* 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 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
	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 II to Regulation (EU) No 1152/2011 and must be 1% or lower
	Geographic epidemiological unit	The geographic epidemiological unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification
	Methodology for calculation of area sensitivity	For the calculation of the area sensitivity, the diagnostic sensitivity should be set conservatively to the lowest value, excluding the lowest 20th percentile, from the ones reported in the scientific literature and related to the diagnostic tests implemented by the countries listed in Annex I of the Commission Delegated Regulation (EU) No 1152/2011. In this case, it is 78% (EFSA AHAW Panel, 2015)

A summary of the assessment of the relative elements of the different countries is given at the end of the document (see Annex A–E).

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, 2013) 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 twelve contiguous months over 2 years
2	**Actual sampling period**	Number of days from the first sampling collection date to the last sample date within the theoretical sampling period
3	**Summary dates**	Descriptive statistics of the sampling period
4	**Sampling period**	Total number of days sampled within the sampling period
5	**Number of samples**	Total number of samples collected during the theoretical sampling period
6	**Number of test results**	Total number of test results. If the number of test results is equal to the number of samples, none of the latter required further investigations (i.e. were negative at the first test)
7	**Laboratory test completion**	Comparison between the year when the samples are collected and the year when the test was completed
8	**Sensitivity**	Sensitivity of the diagnostic test
9	**Host**	Target population size (N); additional information on the host species
10	**Animal sample**	Type of sample collected
11	**Sampling Strategy and Design**	As reported (e.g. representative sample, risk based)
12	**Sampling point**	Activity adopted for the sample collection (e.g. hunting, veterinary activity, . . .)

3. Assessment

3.1. Finland

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

The Finnish Food Safety Authority (Evira) 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 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 (340,000) 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 Kahuala (2007). The population densities for both species are highest in the southern part of the country (see maps in Figure 1).

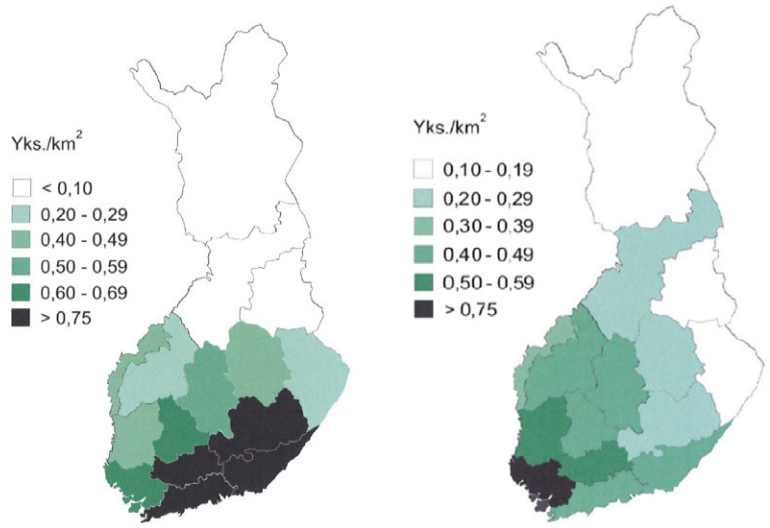


Figure 1: Finland – Raccoon dog densities (left) and red fox densities (right) according to Kahuala (2007) (Yks./km^2 = individuals/km^2)

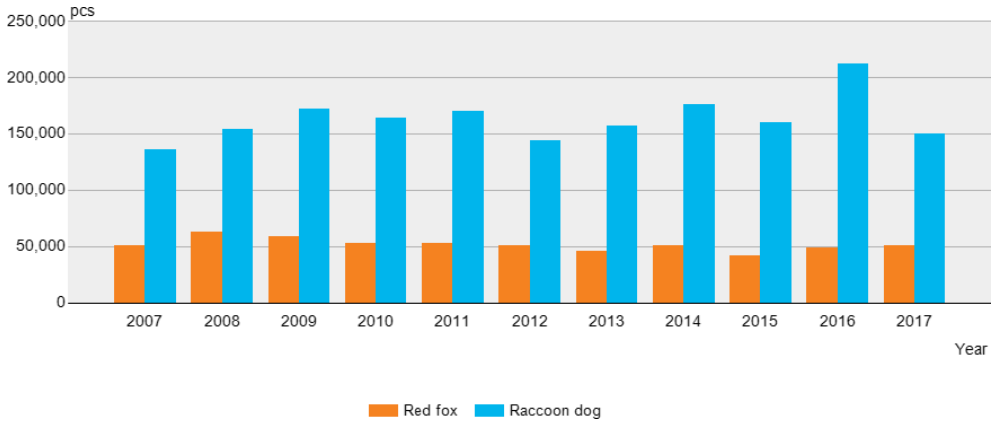


Figure 2: Finland – Annual hunting bag of foxes and racoon dogs (2007–2017) (Source: OSF Natural Resources Institute Finland). Pcs: number of animals

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 north-western taiga population).

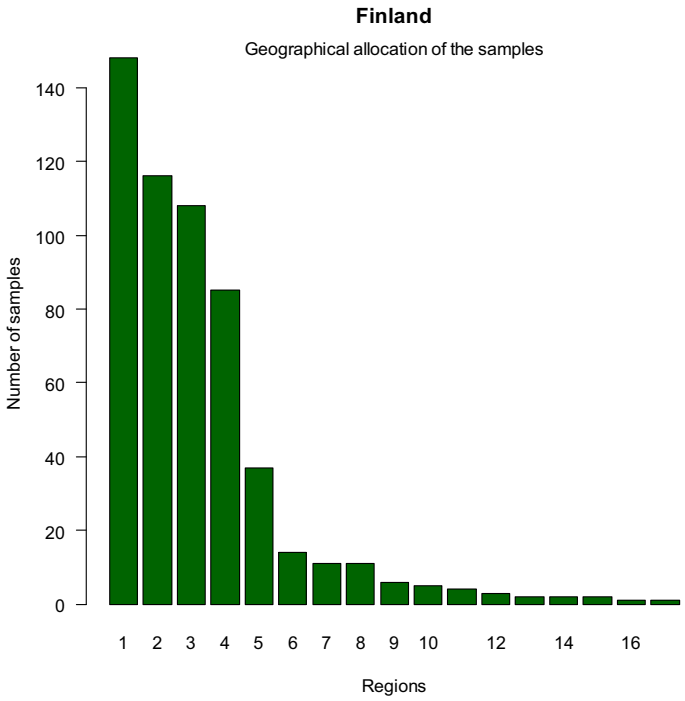
The sample size was calculated by Finland using an overall sensitivity of the diagnostic approach of 0.78 and the design prevalence of 1% prescribed in Regulation (EU) No 1152/2011 using the RiBESS tool.

The samples were collected by hunters on a voluntary basis. Hunters were informed of the sample collection by press releases in Evira's website 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 217 and 339 samples were collected from foxes and raccoon dogs, respectively (N = 556). Gender ratio was male-biased in foxes (1:1.4) while it was close to equal in raccoon dogs (1:1.06). Of the animals that could be classified by age (N = 483), 53% were juveniles. The proportion of juveniles was 56% in raccoon dogs and 47% 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 E. multilocularis due to geographical closeness of infected areas in the south. Also, south-east Finland has the highest density of raccoon dogs in Finland

(Kahuala, 2007). A large sample of foxes (21% of all animals) was received from Lappi where active red fox population reduction to protect the arctic fox was ongoing (see **Figure 3**).



ID	Region
1	Etelä-Karjala
2	Lappi
3	Kymenlaakso
4	Pohjois-Karjala
5	Satakunta
6	Helsinki-Uusimaa
7	Etelä-Savo
8	Varsinais-Suomi
9	Keski-Suomi
10	Kanta-Häme
11	Pohjois-Pohjanmaa
12	Pohjanmaa
13	Päijät-Häme
14	Pirkanmaa
15	Pohjois-Savo
16	Kainuu
17	Keski-Pohjanmaa

Figure 3: Finland – Geographical distribution of samples

Samples were collected throughout 2017 (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 due to the fact that the fox and female raccoon dogs with pups are protected and consequently, hunting is only focused on diseased or injured individuals.

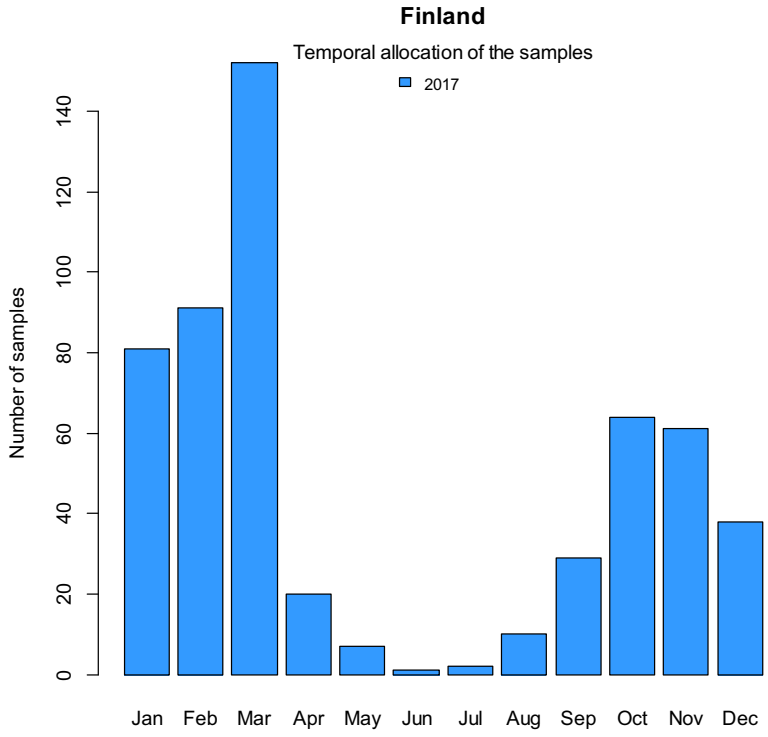


Figure 4: Finland–Temporal distribution of samples

All 556 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 in 2014 (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).

3.1.2.2. Selection of the target population

Definition of susceptible host population target by the system: The selection of racoon dogs and red fox species as target populations was based on their role as definitive hosts in the cycle. This is an assumption also confirmed by the EFSA Scientific opinion on *E. multilocularis* infection in animals (EFSA AHAW Panel, 2015).

It is not possible to conclude on the role of the age and gender composition of the target population in the epidemiology and the lifecycle of E. multilocularis, 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 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 0.76 (see Section 3.1.2.1) and a population size of 380,000 (sum of red fox and raccoon dog population). The sample size required in this case is 393. For both sensitivity estimates, the sample size collected (N = 556) is sufficient to satisfy the requirements.

Implementation of the sampling activity: The geographical information shows that 17 (15 in 2016) 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.

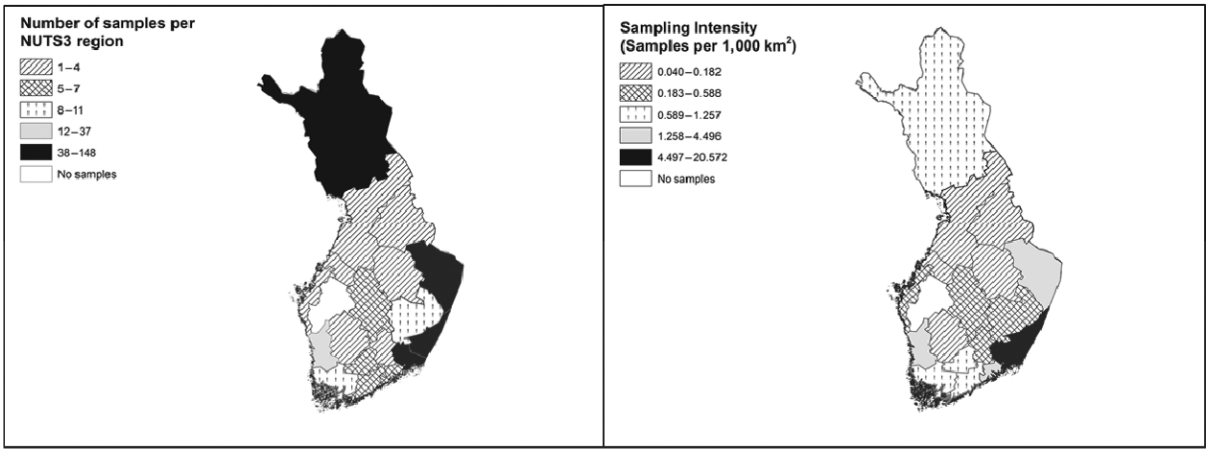


Figure 5: Finland – Sampling activity and intensity by NUTS 3 region

The surveillance strategy as described in the Finnish report cannot be considered a simple random sample. 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 (DP): The DP was equal to 1% (0.01), as it is specified in Annex II to Regulation (EU) No 1152/2011.

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:

- design prevalence of 1% (0.01),
- test sensitivity of 0.76,
- population size of 460,000 (raccoon dogs + red foxes) and
- sample size of 556.

The value of the area sensitivity (0.986) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements of Regulation (EU) No 1152/201.

In summary, the set of data relative to the surveillance activity in 2017 ensures the fulfilment of all the technical legal requirements included in the Annex II of Regulation (EU) No 1152/2011.

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 = TGCTGATTTG TTAAAGTTAGTGATC 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 organized 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 Regulation (EU) 1152/2011, 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 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 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 7 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).

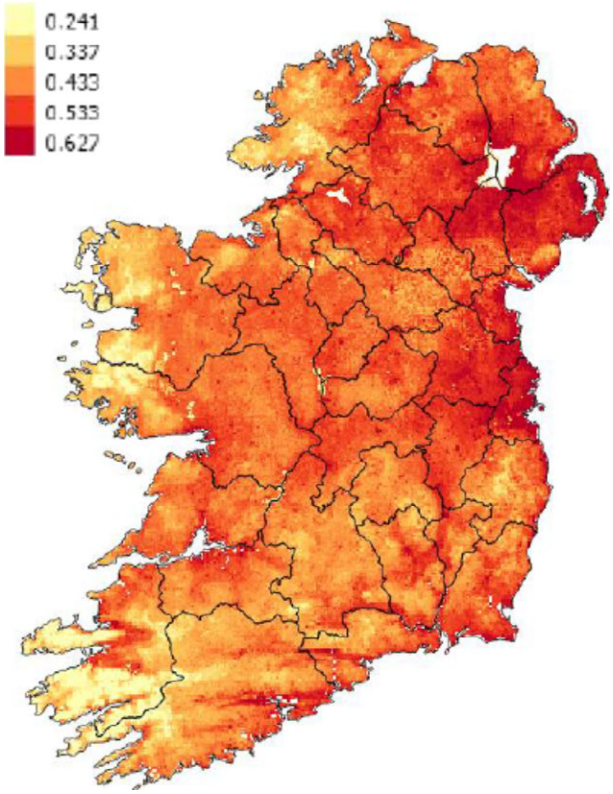


Figure 6: Ireland – Probability of presence per 1 km^2 from the final Maxent species distribution model (Phillips et al., 2006) for red fox (*Vulpes vulpes)*. Source: data up to 2015 provided by Dr. Tomás Murray, from National Biodiversity Data Centre (Ireland)

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

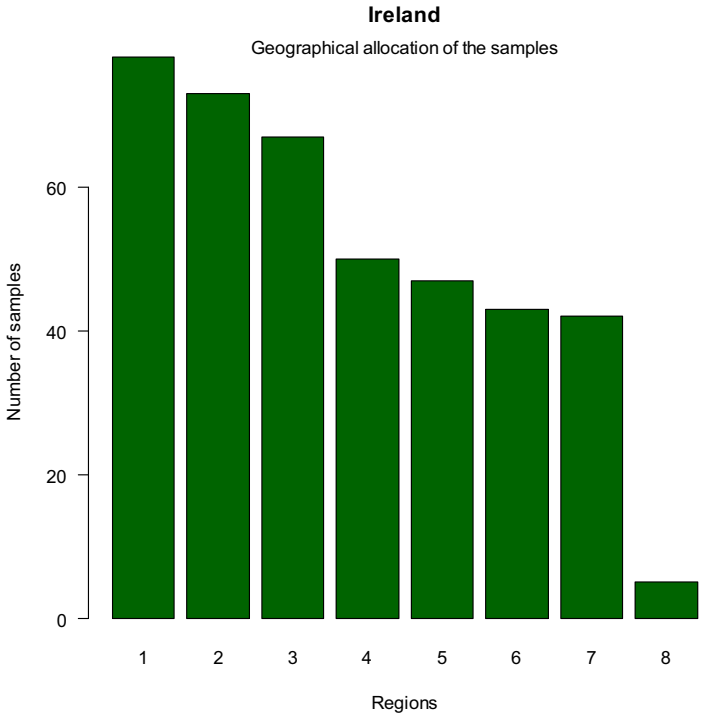
The epidemiological unit was defined as the individual animal (the individual fox, *V. vulpes*).

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 Regulation 1152/2011 for Member States listed in Annex I.

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 from all of the 16 regions available. 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 405 samples was reported by Ireland.

Samples were collected throughout 2017. The sampling intensity was undertaken to reflect the distribution throughout Ireland and further adjusted to reflect the geographical variation in density of fox population distribution (Figure 7). Samples were obtained during 9 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.



ID	Region
1	West
2	Border
3	South-East (Irl)
4	South-West (Irl)
5	Mid-West
6	Midland
7	Mid-East
8	Dublin

Figure 7: Ireland – Sampling activity by regions

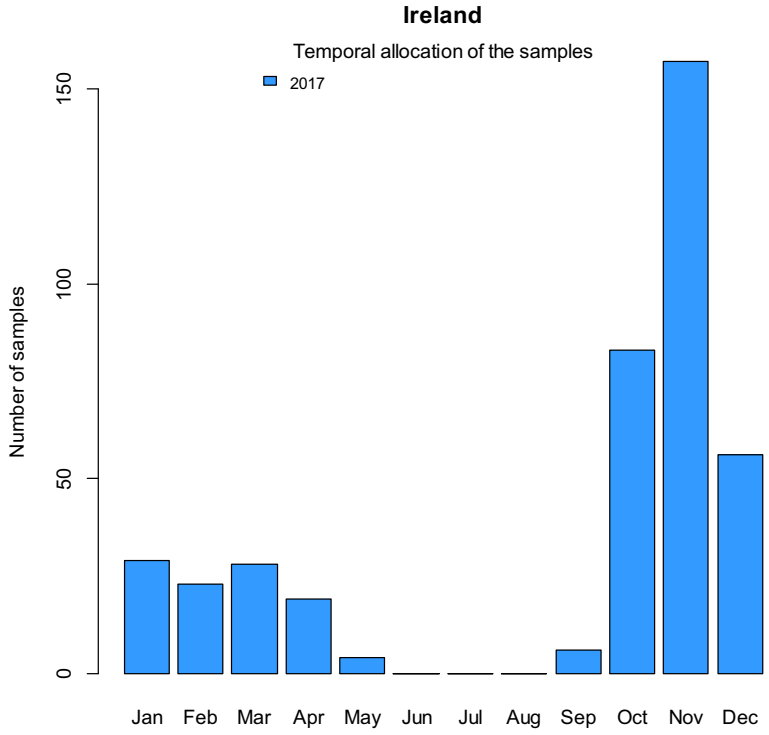


Figure 8: Ireland – Temporal distribution of samples

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 wolfs) is also supported by scientific literature.

Regarding the age or gender of the target population, their role in the epidemiology and in the lifecycle of E. multilocularis is not known due to the lack of appropriate data and studies (EFSA AHAW Panel, 2015).

Size of susceptible host population targeted by the system: Although the original information regarding the red fox population size was published in 2000 and 2009 (Hayden and Harrington, 2000; Marnell et al., 2009), Dr. Tomás Murray, of the National Biodiversity Data Centre, Ireland, specifically provided additional information regarding the Irish fox population in 2015, including more recent data on the relative population density distribution based on ongoing observation records. Nevertheless, at a population size greater than 10,000, moderate fluctuations in the population size would not significantly change the sample size required to achieve the same statistical confidence of less than 1% (0.01) prevalence at a specific test sensitivity (EFSA, 2014). Therefore, fluctuations in the previous population size of 150,000 do not significantly alter the sample size required (EFSA, 2014).

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 405, which ensures the fulfilment of the technical legal requirements in Regulation (EU) No 1152/2011 concerning a confidence level of at least 0.95 against a design prevalence of 1% (0.01). Although EFSA would recommend to consider 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 homogeneous 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.

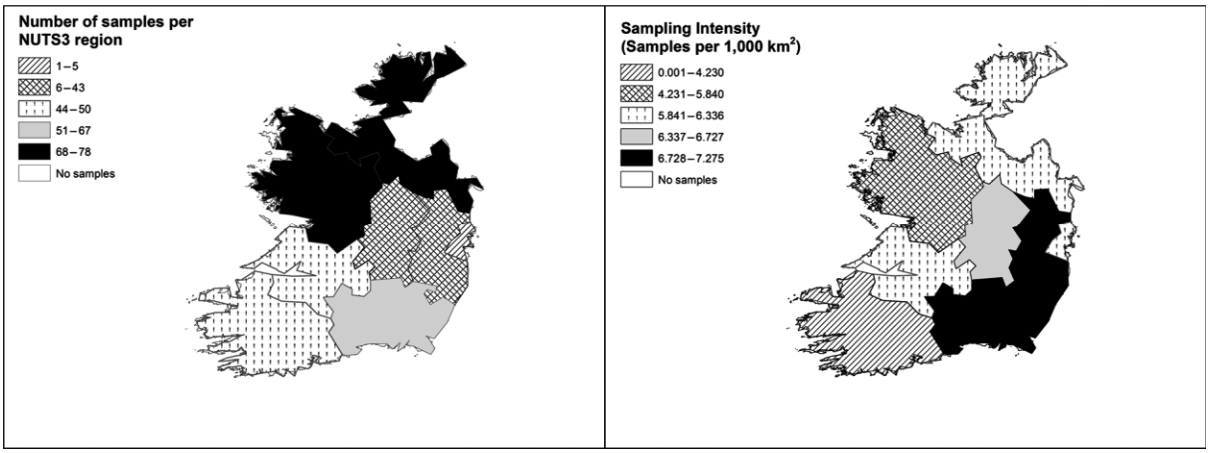


Figure 9: Ireland – Sampling activity and intensity by NUTS 3 region

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 (DP): The DP was equal to 1% (0.01), as it is specified in Annex II to Regulation (EU) No 1152/2011.

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 sub-areas within the Irish territory.

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

- design prevalence of 1%,
- test sensitivity of 0.78,
- population size of 150,000 and
- sample size of 405.

The value of the area sensitivity 0.959 (> 0.95) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements described in Regulation (EU) No 1152/2011. With a population size of 200,000, the value of the area sensitivity would also reach this confidence level (CL); 0.954 (> 0.95).

In summary, the set of data relative to the surveillance activity in 2017 ensures the fulfilment of the technical legal requirements included in all the paragraphs in Annex II of Regulation (EU) No 1152/2011.

3.3. Malta

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

In the Maltese *E. multilocularis* surveillance system, the microscopy/PCR RNAsn U1 method was used to analyse faecal samples from live animals. According to the article of Mathis et al. (1996), microscopy/PCR analytical method has a specificity of 100% and a sensitivity of 94% compared to the parasitological findings after examination of the small intestines.

The initial phase in the identification of the agent was carried out at the National Veterinary Laboratory in Malta. Laboratory personnel from the National Veterinary Laboratory followed a short hands-on training course at the Department of Infectious, Parasitic and Immunomediated Diseases of the Istituto Superiore di Sanità in Rome, Italy. The faeces samples were examined for worm eggs using the flotation and concentration method. All the worm eggs microscopically identified as *Taenia* spp. were then stored in 75% alcohol for further identification by PCR. The National Veterinary Laboratory in Malta is not accredited for the flotation method on faeces and the method is not yet validated.

The faeces positive for the presence of *Taenia* spp. eggs were sent to the Department of Infectious, Parasitic and Immunomediated Diseases of the Istituto Superiore di Sanità in Rome, Italy, for identification of *Echinococcus granulosus*, *E. multilocularis* and *Taenia* spp. eggs by means of multiplex-PCR analysis.

In Malta, there are no wild foxes or raccoon dogs and the only carnivore that is present is the weasel (*Mustela nivalis*). The population of this animal is considered to be very low and it is also worthy of note that *M. nivalis* is not considered to be an elite definitive host for E. multilocularis. Furthermore, the risk of transmission of the disease through *M. nivalis* is considered to be very remote due to their nocturnal and retrieval behaviour. The presence of wildlife definitive host (*V. vulpes*) worldwide is described by the International Union for Conservation of Nature and Natural Resources – Species Survival Commission (SSC), which has been assessing the conservation status of species, subspecies, varieties and even selected subpopulations on a global scale in order to highlight taxa threatened with extinction, and therefore promote their conservation (Macdonald and Reynolds, 2008). Red fox is described as a species not present in Malta as showed in the map of the distribution of the species available on IUCN website (http://www.iucnredlist.org/details/23062/0). Considering the absence of the definitive wild host population in Malta (including the island of Gozo), dogs may play a

role as potential definite hosts in maintaining the life cycle of the parasite, through possible contact with the rodents. The target populations for the purpose of this study consisted of dogs ('hunting dogs', 'stray dogs' in sanctuaries and 'rural dogs'). The main risk groups identified were 'Rural dogs' and 'Stray dogs'.

Dog registration and microchipping in the Maltese Islands is governed by a legal notice LN 199/2011 which obliges all dog owners to microchip and register their animals with the competent authority. The registration is undertaken and managed by the Veterinary Regulation Department.

The total number of registered dogs in 2017 was 66,731; out of which 33,511 were female and 33,220 were male. The age distribution young to adult dogs was 7,924 young dogs (≤ 2 years) and 58,807 adult dogs (> 2 years). This data was obtained from National Database used to register dogs for microchipping.

There is no classification of the dog population into pets, hunting or rural dogs in the National Veterinary Information System where information connected to the identified dogs is registered.

Estimates of stray dogs were supplied by the six dog sanctuaries present in the Maltese islands, showing that that the number of stray dogs collected vary from 1,000 to 2,000 per year.

Given the high population density of people in the Maltese Islands, the distribution of dogs is relatively homogeneous in Malta. The existence of strictly rural areas is subjective due to the fact that urban areas are within very close proximity to these areas.

Considering the very small territory of the country (316 km^2), and that rural areas are limited, a geographic distribution of the rural dog population was considered as not relevant for the purpose of the surveillance programme.

The surveillance followed a risk based approach through the sampling of dogs (hunting dogs, dogs in the sanctuaries and rural dogs). The sample size was set up using the tool RIBESS + provided by EFSA, EFSA Statistical Model, in order to detect a prevalence of 1% (0.01) with CL 95% within the population at risk. The sample size was identified in 383 samples. The estimated dog population, divided into the categories considered for the risk assessment, was the following: pets = 59,000; rural dogs (farm dogs; known history) = 4,000; stray dogs (sanctuary dogs; unknown history) = 2,000 for a total of 65,000 animals. The rural dog population was estimated to range between 3,500 and 4,000 considering that the number of farms present in the country are 2,050 (107 pig farms, 266 bovine, 1,669 sheep and goat farms, including those with < 3 animals). An average of two dogs for each farm was assumed. The estimation done was confirmed by information available at different NGOs operating in Malta and offering free neutering and microchipping for all dogs whose owners receive benefits, as well as for all farm, factory and hunters' dogs. Records available at the six sanctuaries present in the country show that the stray dogs collected vary from 1,000 to 2,000 per year. Dogs in this category are identified as non-pet animals within this surveillance programme. The sample size consisted of 383 samples, divided into 234 from rural dogs and 149 from domestic dogs.

The categories more at risk were identified as hunting dogs and rural dogs. The dogs held on the farms (rural dogs) could be considered at higher risk due to contact with the rodents, with particular reference to dogs present in pig and sheep farms. An unknown history (stray dogs) of the animal was considered a risk factor for the stratification of the sample, as it might indicate a possibility of having been in areas not free from the parasite or in areas with high risk. The dogs present in the sanctuaries were identified as animals with unknown history. All the categories considered with high risk because of their possibility of having been in contact with the intermediate host or for their possibility of having been in areas considered not free from the disease or at risk, were included in the surveillance programme, to optimise the likelihood of detection of E. multilocularis (EFSA AHAW Panel, 2015).

Sampling was carried out in two ways: samples from farms were collected by sampling teams carrying out *Brucella*, TB testing, Animal Welfare inspections and other on-farm inspections, while the samples from sanctuaries/stray dogs were collected by a dedicated *Echinococcus* sampling team. Samples were collected from the ground. To ascertain their provenience, sampling officers sampled dogs which were kept tide up on farms, while the sampling of faeces from the sanctuaries were collected when the dogs were first admitted and thus being kept isolated.

A total of 383 samples were collected throughout 2017 (234 rural dogs and 149 stray dogs). Samples were collected in both Malta and Gozo. In Gozo, samples were collected from 9 out of the 14 localities. These localities represent the major rural areas in the island of Gozo. A dog pound is also located in one of these localities, were stray dogs from the island of Gozo are collected. In Malta, 23 localities were sampled, across the island; the sampling area included four dog sanctuaries that collect stray dogs from all Malta. The distribution of the samples collected by locality is shown in Figure 10.

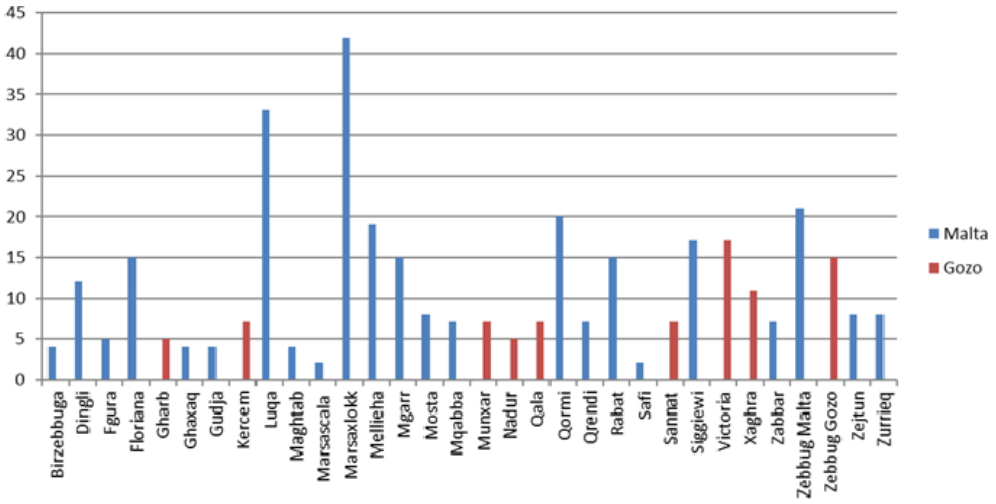


Figure 10: Malta – sample distribution by locality (plot as provided by the country)

The sampling activity was distributed over the full year (see Figure 11).

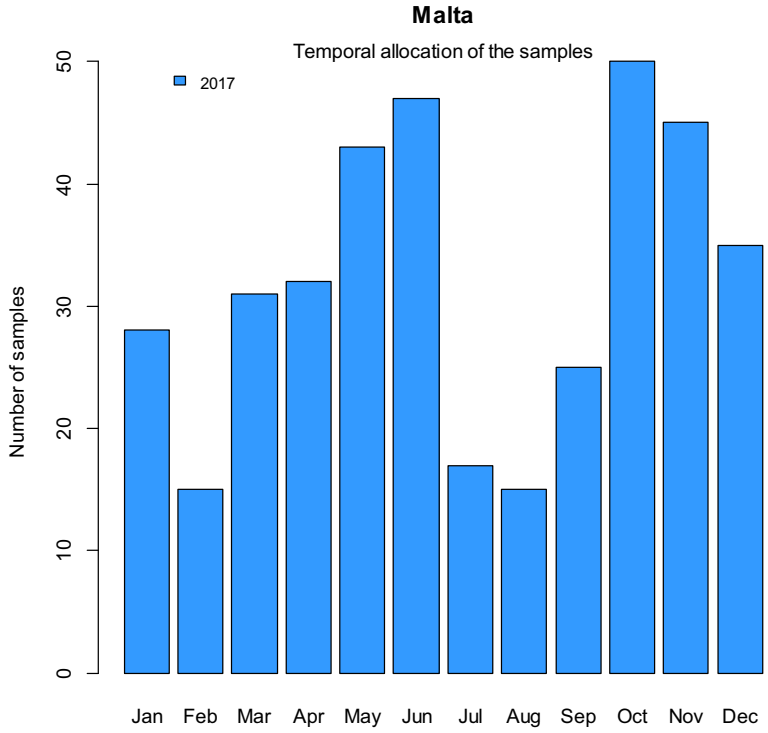


Figure 11: Malta – temporal distribution of samples

3.3.2. EFSA comments and considerations

3.3.2.1. Type and sensitivity of the detection method

Type of test: The method used by Malta in the surveillance of *E. multilocularis* (Microscopy/PCR RNAsn U1) is well described.

Test sensitivity: Malta followed EFSA's advice regarding the setting of the conservative, lowest value of the sensitivity (0.78) (EFSA, 2017).

3.3.2.2. Selection of the target population

Definition of susceptible host population target by the system: The selection of dogs as target species in order to carry on the surveillance is well described and justified. Although it is true that in

the map available on the IUCN website the red fox appears absent from Malta, in the text of the website is listed as native (http://www.iucnredlist.org/details/23062/0). However, the absence of the main wild definitive hosts is supported also from other sources of information (e.g. Fauna Europaea, online: https://fauna-eu.org/cdm_dataportal/taxon/84f2e35f-eea0-4289-99c9-e6262bb0e386). Malta selected domestic dogs, due to the fact that dogs have been reported occasionally as DH, to accomplish the rules of the Annex II in the legislation in order to be listed in Annex I: `The pathogen-specific surveillance programme shall consist in the ongoing collection, during the 12-month surveillance period, of samples from wild definitive hosts or, in the case where there is evidence of the absence of wild definitive hosts in the Member State or part thereof, from domestic definitive hosts'.

Although the selection of the population is adequate (dogs in the absence of red fox), the definition of the different categories, identified within the population, does not appear to be supported by evidences.

Size of susceptible host population targeted by the system: Dog population size is well described and has been updated since the last year. However, as discussed previously, the different categories in the classification are not always well defined and justified.

3.3.2.3. Sampling strategy

Epidemiological unit: The epidemiological unit is deduced to be the individual animal. Faeces samples were collected, presumably individually, from dogs of farms and sanctuaries (stray dogs).

Sample size calculation: The sample size of Malta was set up using the RiBESS+. For a prevalence of 1% (0.01) with a CL of 95%, the sample size was identified to be 383.

Implementation of the sampling activity: The geographical information shows that the samples were collected from both of the NUTS 3 regions.

The sampling activity was heterogeneously distributed over the full year with intensification in May–June and October–November. However, this fact may not affect the representativeness of the sample; 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 the whole year (EFSA, 2013).

3.3.2.4. Methodology

Design prevalence (DP): The DP used was equal to 1% (0.01), as it is specified in Annex II to Regulation (EU) No 1152/2011.

Epidemiological geographical unit: The whole territory of Malta (Maltese islands of Malta and Gozo) was considered as one epidemiological unit.

Methodology for calculation of the area sensitivity: The area sensitivity was estimated by Malta by three different method based on the risk categories identified. However, the assumptions made for this calculation do not appear to be supported by scientific evidences. Consequently, the assumption of a simple random sample is the safest option.

The parameters included for the calculation were the following:

- design prevalence of 1%,
- test sensitivity of 0.78,
- population size of 65,000 and
- sample size of 383.

The value of the area sensitivity 0.951 (> 0.95) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements described in Regulation (EU) No 1152/2011.

In summary, the set of data relative to the surveillance activity in 2017 ensures the fulfilment of the technical legal requirements included in all the paragraphs in Annex II of Regulation (EU) No 1152/2011.

3.4. United Kingdom

3.4.1. 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 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.

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). The egg counting method sensitivity is variable between laboratories. Eckert's suggestion to consider a Se of 99% was used (Eckert, 2003). In Northern Ireland, AFBI participated in the last proficiency testing in 2015 and will be participating again in 2018.

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

The fox population size (prebreeding adults) has been estimated at 240,000 by wildlife experts, and the numbers were published in 2013 (Defra, 2013) and has recently been modelled giving a predicted abundance as an average across several years (Croft et al., 2017) and gives a slightly lower prediction average of 230,000, but with a range of 70,000 to 385,000. 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 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 (see Figure 12).

For Northern Ireland, 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.

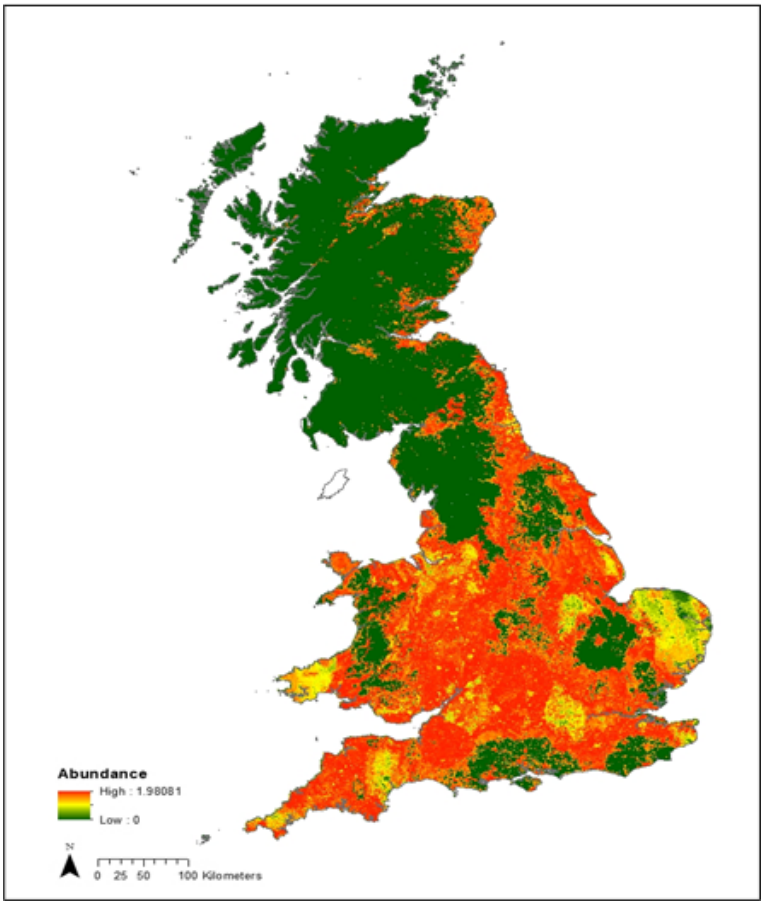


Figure 12: Great Britain – Map estimating fox density in the UK. This is a systematic approach using NBN presence data and published density data and provides a confidence interval of 120–280,000 foxes. Some areas have few data as permission was not given to use the records. For more information, see Croft et al. (2017)

The United Kingdom was divided into two surveillance regions for the purpose of this report: Northern Ireland and Great Britain (England, Scotland and Wales).

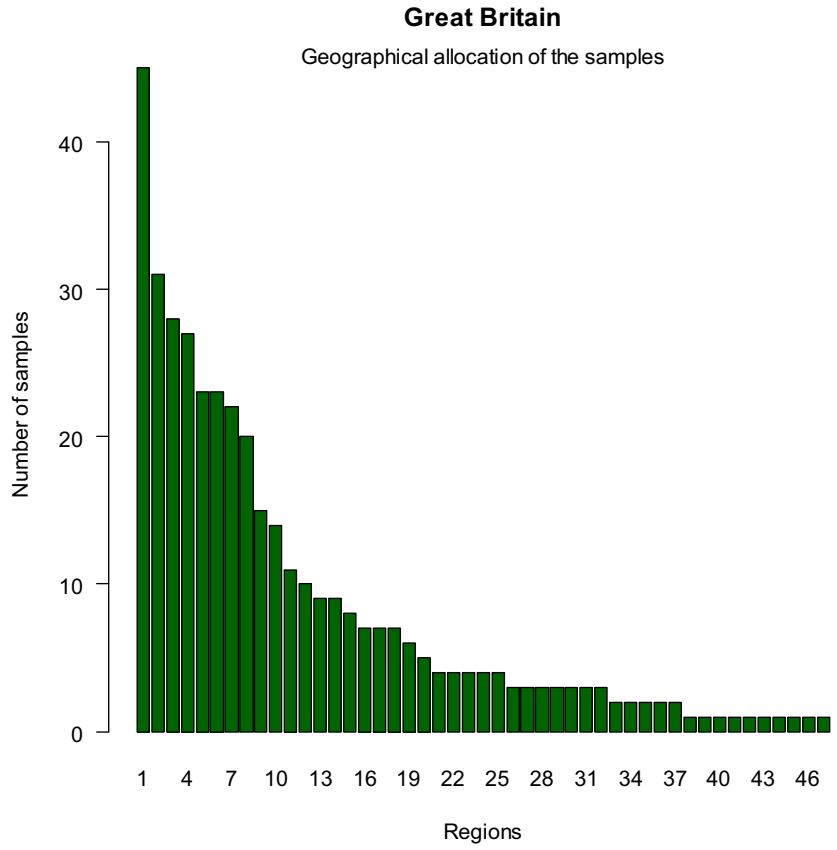
The sample size was calculated using the EFSA RiBESS tool. Random sampling – not risk based – sampling, is carried out at certain times of the year – the target is the wild population and therefore hunting is not permitted during the breeding season.

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 Northern Ireland). 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 12 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. Great Britain 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 Northern Ireland, there is a single land border with another EU Member State, which is the Republic of Ireland. This border is porous for wildlife; however, Ireland also has official disease free status for *E. multilocularis.*

In GB, 388 samples were collected and tested. In NI, 332 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 12, 13 and 14).



1	Worcestershire	25	York
2	Gloucestershire	26	Clackmannanshire And Fife
3	North Yorkshire Cc	27	Devon Cc
4	Cheshire Cc	28	Lochaber, Skye & Lochalsh, Arran & Cumbrae And Argyll & Bute
5	East Cumbria	29	Powys
6	Essex Cc	30	Staffordshire Cc
7	Lincolnshire	31	Surrey
8	South West Wales	32	Thurrock
9	East Riding Of Yorkshire	33	Edinburgh, City Of
10	Somerset	34	Inner London - East
11	Herefordshire, County Of	35	Inner London - West
12	North Lanarkshire	36	Inverness & Nairn And Moray, Badenoch & Strathspey
13	Caithness & Sutherland And Ross & Cromarty	37	Monmouthshire And Newport
14	Halton And Warrington	38	Angus And Dundee City
15	Cornwall And Isles Of Scilly	39	Cardiff And Vale Of Glamorgan
16	Shropshire Cc	40	Durham Cc
17	South Nottinghamshire	41	Leeds
18	Suffolk	42	North Nottinghamshire
19	Barnsley, Doncaster And Rotherham	43	Northumberland
20	Bournemouth And Poole	44	Outer London - West And North West
21	Bath And North East Somerset, North Somerset And South Gloucestershire	45	Perth & Kinross And Stirling
22	Dumfries & Galloway	46	South Lanarkshire
23	East Merseyside	47	Warwickshire
24	Greater Manchester North		

Figure 13: Great Britain – Geographical distribution of samples

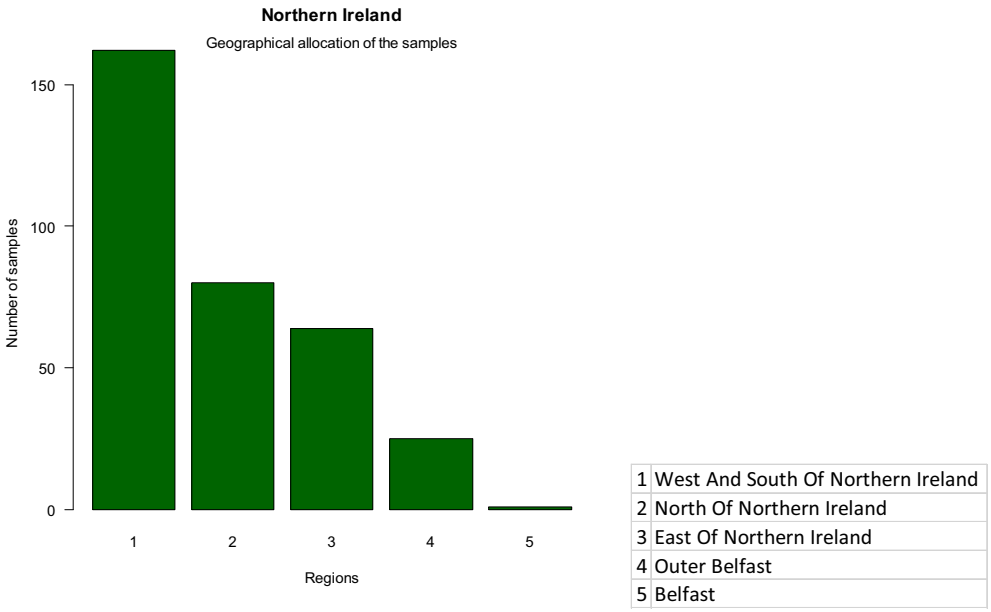


Figure 14: Northern Ireland – Geographical distribution of samples

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 15 and 16).

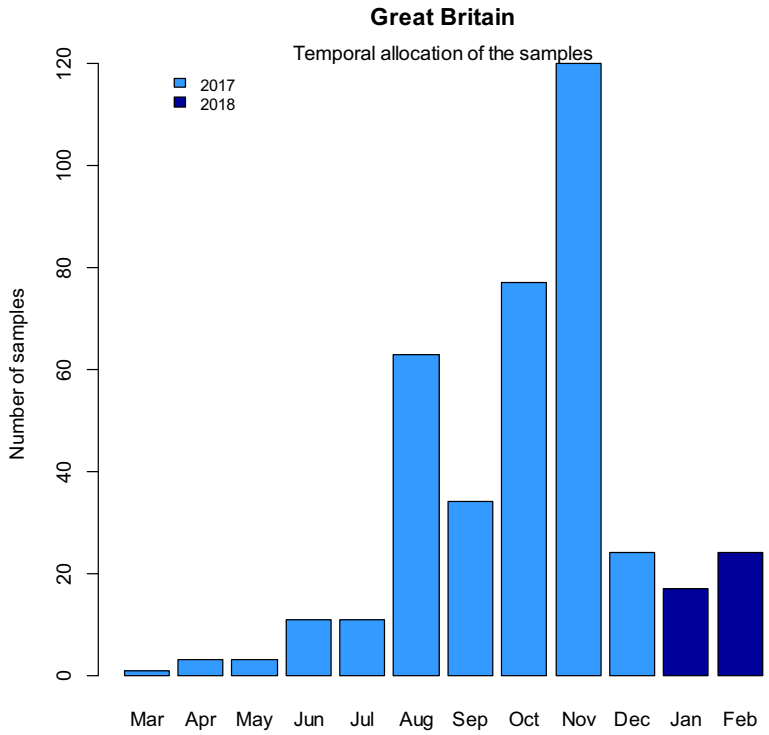


Figure 15: Great Britain – Temporal distribution of samples

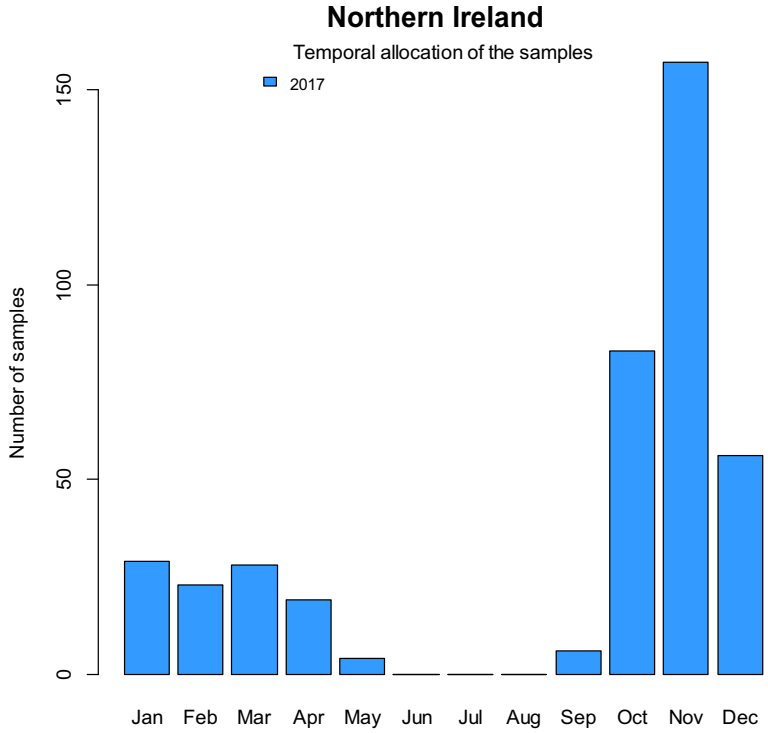


Figure 16: Northern Ireland – Temporal distribution of samples from NI

3.4.2. EFSA comments and considerations

3.4.2.1. Type and sensitivity of the detection method

Type of test: Both methods used for detection of *E. multilocularis* in the UK were well described. Great Britain (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 Northern Ireland (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. A safer option would be to follow the EFSA recommendation (Test Se = 0.78).

3.4.2.2. 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 recognised as the main wildlife definitive host species for this parasite (EFSA AHAW Panel, 2015). Regarding the absence of other potential wild definitive hosts (raccoon dogs, wolves) the information is consistent with the report of Ireland. However, no reference has been provided.

Size of susceptible host population targeted by the system: Data of fox population size (240,000) is well documented and has been recently updated.

3.4.2.3. 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. Also 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 388, which ensures the fulfilment of the technical legal requirements of Regulation (EU) No 1152/2011 regarding a confidence level of at least 0.95 against a design prevalence 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 332. However, if a sensitivity of 0.78 is considered, as suggested by EFSA as a worse-case scenario (EFSA, 2015), the required samples to fulfil the technical legal requirements regarding a confidence level of at least 0.95 against a design prevalence of 1% (0.01) increase to 379 (with 47 additional samples needed). As an internal validation of the test sensitivity has not been made (ideally it should be determined by each lab for the protocol used in house), a value of 0.78 would be the most suitable value in order to calculate the sample size. The total number of samples collected by NI, assuming the theoretical 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 Regulation (EU) No 1152/2011 regarding a confidence level of at least 0.95 against a design prevalence 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 Northern Ireland remains disease free this year, even if a lower test sensitivity were used for the sample calculation.

Implementation of the sampling activity: The sampling process has more 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 17).

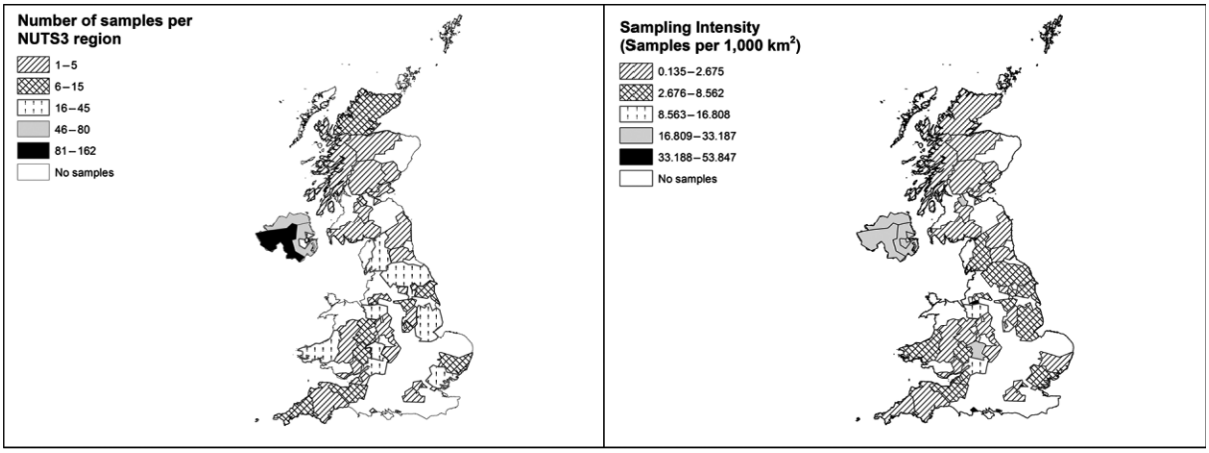


Figure 17: United Kingdom – Sampling activity and intensity by NUTS 3 region

The collection of samples was in both cases reduced during the spring-summer months and the reason for this reduction has been well justified.

3.4.2.4. Methodology

Design prevalence (DP): The DP used was equal to 1% (0.01), as it is specified in Annex II to Regulation (EU) No 1152/2011.

Epidemiological geographical unit: UK was divided in 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:

- design prevalence of 1%,
- test sensitivity of 0.78,
- population size of 240,000 and
- sample size of 388.

The value of the area sensitivity (0.952) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements included in Regulation (EU) No 1152/2011.

The area sensitivity for NI considering the following parameters:

- design prevalence of 1%,
- test sensitivity of 0.99,
- population size of 14,000 and
- sample size of 332.

With these conditions, area sensitivity was higher than 0.95 (0.965). However, if a test sensitivity of 0.78 is assumed, as suggested by EFSA (EFSA, 2015), the area sensitivity (0.927) is not sufficient to comply with the EU regulation in force (47 additional tests would be required).

In summary, the set of data from the surveillance activity in 2017/2018 for the United Kingdom, due to the use of a test sensitivity value not supported by scientific evidences by Norther Ireland, does not ensure the fulfilment of the technical legal requirements of Regulation (EU) No 1152/2011 regarding a confidence level of at least 0.95, against a design prevalence of 1% (0.01) in each of its geographical epidemiological units.

From a purely epidemiological point of view, to consider the whole island of Ireland as one epidemiological unit would be a scientifically sound approach. Population of foxes is widespread distributed in 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
EI	0.97	**0.9979**
NI	0.93	

3.5. Norway

3.5.1. Information as submitted in the report by Norway

In the Norwegian *E. multilocularis* surveillance system, a DNA-fishing technique was used (Isaksson et al., 2014), referred to as PCR 12S rRNA, which involves magnetic capture mtDNA extraction from samples applying specific DNA-hybridisation (Isaksson et al., 2014), followed by real-time PCR (CO1rtPCR) (Øines et al., 2014). Samples are also analysed in duplicates in the detection step to increase sensitivity, and to reduce chance of errors introduced by operator. Results from samples the very low target DNA has also 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/AATAAGAGTGATCCTA TTTTGTGGTGGGT/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 2017, samples from 11 wolves (*Canis lupus*), submitted for forensic post-mortem examination, were included in the surveillance. All tested negative for *E. multilocularis.*

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).

EpiTools epidemiological calculators (http://epitools.ausvet.com.au/content.php?page=home), developed by AusVet Animal Health Services, was used to verify that the sample size is sufficient to claim a prevalence of not more than 1% at confidence level of at least 95%. The software uses a hypergeometric approximation when population size is provided. The goal was approximately 482 samples from red foxes in 2017, 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 invitation to attend the 2017-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. Faecal samples were promptly mailed individually in pre-paid envelopes to the laboratory. To ensure the individuality of the samples, the hunters were also request to submit either 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 might 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. The parasite is now approaching Norway (Uddevalla is about 80 km from the Norwegian border). For this reason, the sampling activity is more concentrated along the Swedish borders (performing a representative sampling, with convenience criterion).

Four hundred and thirty-eight samples were collected from red foxes in 2017 and all were negative in PCR.

Samples were collected throughout 2017. The spatial distribution of samples (see Figure 18) 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 19) is also somewhat uneven due to preferred hunting conditions during winter and banned hunting between 15 April and 15 July. Samples were collected during the whole year with a decline of the sampling during the summer season.

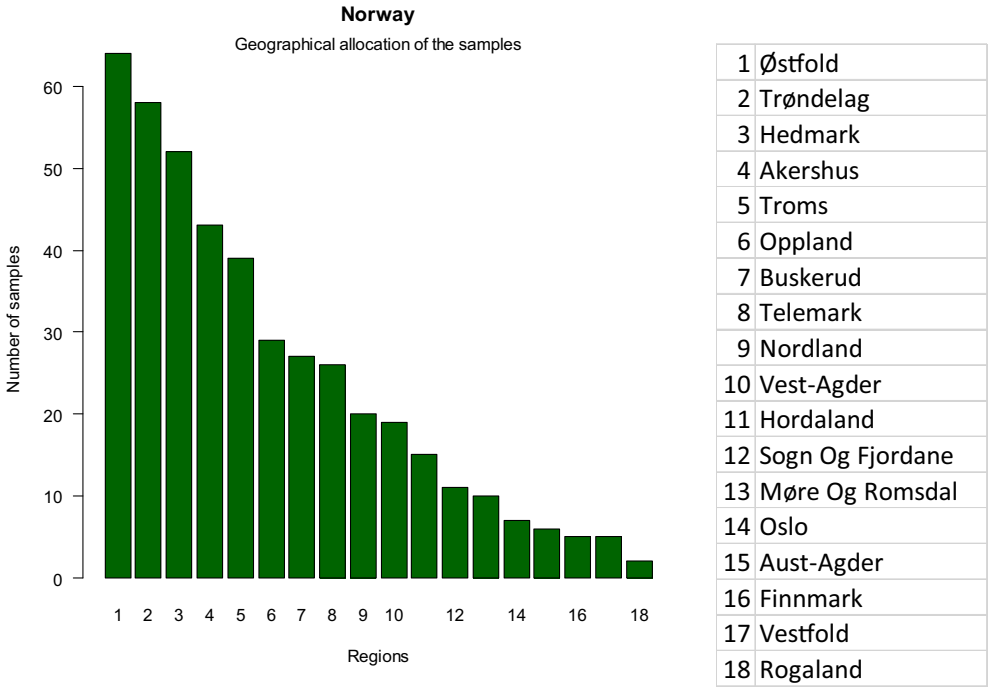


Figure 18: Norway – Geographical distribution of samples in 2017–2018

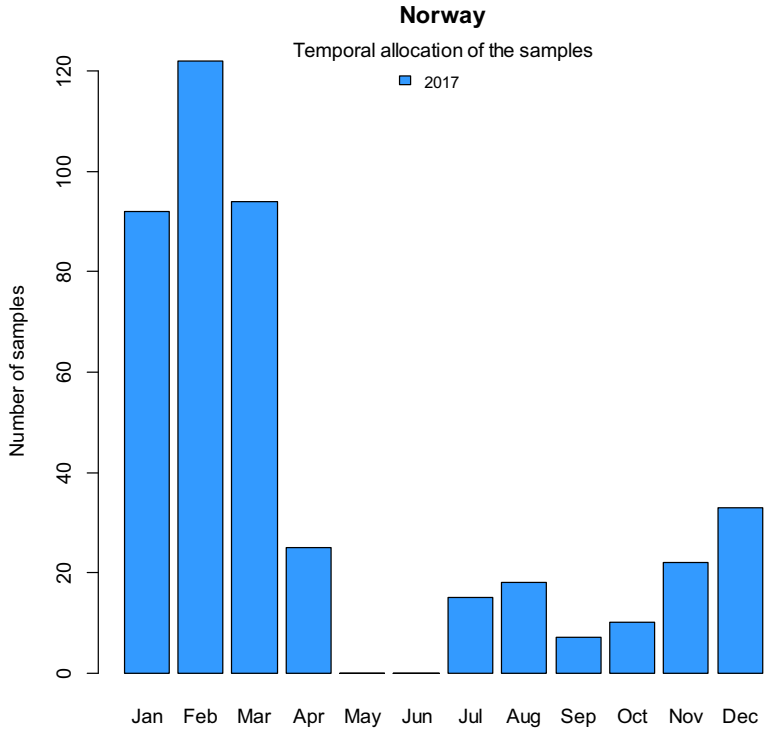


Figure 19: Norway – Temporal distribution of samples from 2017

3.5.2. EFSA comments and considerations

3.5.2.1. 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: The diagnostic sensitivity was set to the sensitivity obtained by Øines et al., 2014 (63%), a lower value than the minimum recommended by EFSA (0.78). Such 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. However, it has to be acknowledged that the choice of using a lower value than the one suggested by EFSA goes in a safe direction.

3.5.2.2. 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.

3.5.2.3. Sampling strategy

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

Sample size calculation: EpiTools epidemiological calculators, developed by AusVet Animal Health Services, were used to verify that the sample size is sufficient to claim a prevalence of not more than 1% at a confidence level of at least 95%. Using these values, the online tool returns a target sample size equal to 482. The goal set by Norway, however, was set to approximately 600 samples to be collected from red foxes in 2017. This number of samples would be also sufficient to meet the requirements if calculated with RiBESS tool. Using this application, and considering design prevalence of 1% (0.01), a test sensitivity of 0.63, and a population size of 151,000, the sample sized required is 474. In both cases, the number of samples collected by Norway in the 12-month sampling period (438 samples between January 2017 and December 2017) does not reach the target set. However, using the value suggested by EFSA for the test sensitivity (0.78), the required number of samples is equal to 383. In this latter case, the number of samples collected are higher than the minimum 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 20). The differences of sampling intensities among the different areas have also been justified in the report.

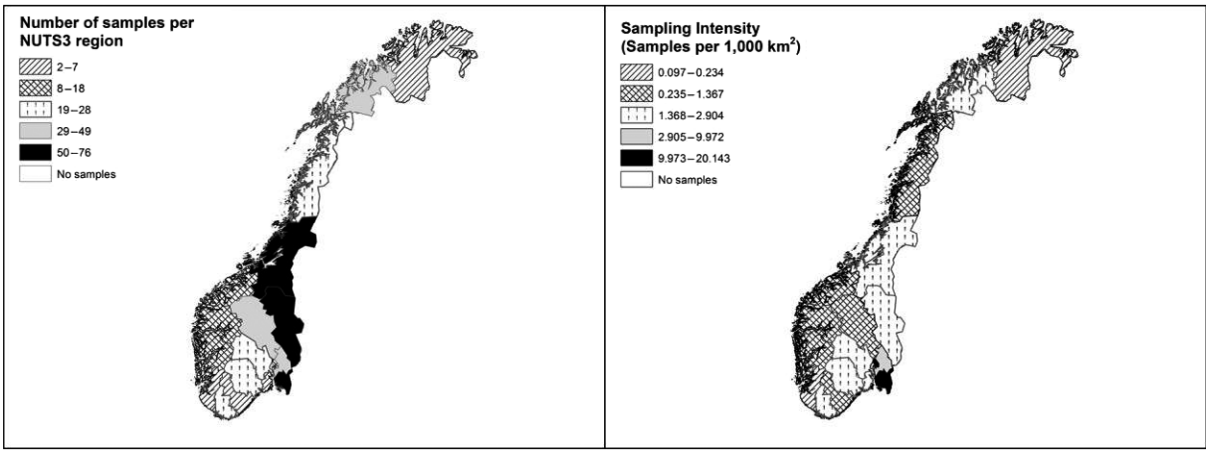


Figure 20: Norway – Sampling activity and intensity by NUTS 3 region in 2017

3.5.2.4. Methodology

Design prevalence (DP): The DP was equal to 1% (0.01), as it is specified in Annex II to Regulation (EU) No 1152/2011.

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 sub-areas within the Norwegian territory.

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

- design prevalence of 1%,
- test sensitivity of 0.63,
- population size of 151,000 and
- sample size of 438,

The area sensitivity value is 0.937 (< 0.95), which is below the established minimum value of 0.95 needed to fulfil the technical legal requirements of Regulation (EU) No 1152/2011.

However, using the value suggested by EFSA for the test sensitivity (0.78) the area sensitivity achieved is 0.968, which is above the established minimum value of 0.95 needed to fulfil the technical legal requirements of Regulation (EU) No 1152/2011.

In summary, the set of data relative to the surveillance activity in 2017 ensures the fulfilment of the technical legal requirements of all the paragraphs included in the Annex II of Regulation (EU) No 1152/2011.

4. Conclusions

- *E. multilocularis* was not detected in any of the samples from the five countries collected in the reporting period (2017).
- All of the countries participating in this surveillance (Finland, the UK, Norway, Malta and Ireland) fulfil the technical legal requirements regarding the use of appropriate techniques for the detection of *E. multilocularis* in intestinal contents or faeces (Annex II-paragraph 3 of Regulation (EU) No 1152/2011). Each of them uses different methods for detection of the parasite as described in the report. In addition, sensitivity (and specificity) values of the techniques have been indicated.
- All of the countries participating in this surveillance (Finland, the UK, Norway, Malta and Ireland) fulfil the technical legal requirements regarding the collection of samples from wild definitive hosts or domestic definitive host in the absence of the first (Annex II – paragraph 3 of Regulation (EU) No 1152/2011). Four of the countries selected adequate wild definitive hosts in order to perform the surveillance (Finland, the UK, Norway and Ireland). Malta, in the absence of wild animals that could act as definitive hosts, selected dogs to carry out the surveillance.
- All of the countries participating in this surveillance (Finland, the UK, Norway, Malta and Ireland) fulfil the technical legal requirements concerning an appropriate sampling for detection of the *E. multilocularis* parasite, if present in any part of the Member State, at the design prevalence of less than 1% (0.01) (Annex II – paragraph 2 of Regulation (EU) No 1152/2011). Although the surveillance strategies performed by Finland, the UK, Norway and Ireland cannot be considered `a surveillance strategy 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 to have those estimates, combining sampling activity results and modelling.
- All of the countries participating in this surveillance (Finland, the UK, Norway, Malta and Ireland) fulfil the technical legal requirements regarding the 12-month surveillance period collection (Annex II – paragraph 3 of Regulation (EU) No 1152/2011). 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 of the territories participating in this surveillance (Finland, the UK, Norway, Malta and Ireland) fulfil the technical legal requirements regarding the confidence level of at least 0.95 against a design prevalence of 1% (Annex II – paragraph 1 of Regulation (EU) No 1152/2011). 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 NI should increase by at least 47 additional animals testing negative in order to achieve the confidence level of at least 0.95 against a design prevalence of 1% as described in the Regulation. 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 Regulation (EU) No 1152/2011 regarding a confidence level of at least 0.95 against a design prevalence of 1%. Northern Ireland should provide scientific evidences to support the test sensitivity value proposed in the surveillance.

References

Casulli A, Possenti A, La Torre G, Boue F, Busani L, Colamesta V, Conraths FJ, D'Aguanno S, De Giusti M, De Vito C, Karamon J, Maas M, Mannocci A, Maffongelli E, Mipatrini D, Oksanen A, Probst C, Saulle R, Siles-Lucas M, Umhang G, vanden End S, van der Giessen J and Villari P, 2015. *E. multilocularis* infection in animals (GP/EFSA/AHAW/2012/01). EFSA Supporting Publication 2015; 12(12):EN-882, 168 pp. https://doi.org/10.2903/sp.efsa.2015.EN-882

CDC (Centers for disease control and prevention), online. Parasites-Echinococcosis. Available online: https://www.cdc.gov/parasites/echinococcosis/biology.html [Accessed: 5 July 2017].

Conraths FJ and Deplazes P, 2015. *Echinococcus multilocularis*: epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. Veterinary Parasitology, 213, 149–161.

Conserve Ireland, 2009. Red Fox. Available online: http://www.lhnet.org/red-fox/

Croft S, Chauvenet A and Smith G, 2017. A systematic approach to estimate the distribution and total abundance of British mammals. PLoS ONE, 12, e0176399.

Davidson RK, Romig T, Jenkins E, Tryland M and Robertson LJ, 2012. The impact of globalisation on the distribution of *Echinococcus multilocularis*. Trends in Parasitology, 28, 239–247.

Defra (Department for Environment Food and Rural Affairs), 2013. Request for information: Bovine TB hotspots and population sizes of badgers and foxes. Available online: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/243894/5577.pdf

Deplazes P, 2006. Ecology and epidemiology of *Echinococcus multilocularis* in Europe. Parassitologia, 48, 37–39.

Dinkel A, von Nickisch-Rosenegk M, Bilger B, Merli M, Lucius R and Romig T, 1998. Detection of *Echinococcus multilocularis* in the definitive host: coprodiagnosis by PCR as an alternative to necropsy. Journal of Clinical Microbiology, 36, 1871–1876.

ECDC (European Centre for Disease Prevention and Control), 2016. Annual Epidemiological Report 2016 – Echinococcosis., Stockholm, Sweden. Available online: https://ecdc.europa.eu/en/publications-data/echinococcosis-annual-epidemiological-report-2016-2014-data

ECDC (European Centre for Disease Prevention and Control), 2017. Echinococcosis. In: ECDC. Annual epidemiological report for 2015. Stockholm.

Eckert J, 2003. Predictive values and quality control of techniques for the diagnosis of *Echinococcus multilocularis* in definitive hosts. Acta Tropica, 85, 157–163.

Eckert J and Deplazes P, 1999. Alveolar echinococcosis in humans: the current situation in Central Europe and the need for countermeasures. Parasitology Today, 15, 315–319.

EFSA (European Food Safety Authority), 2012a. Scientific and technical assistance on *Echinococcus multilocularis* infection in animals. EFSA Journal 2012;10(11):2973, 22 pp. https://doi.org/10.2903/j.efsa.2012.2973

EFSA (European Food Safety Authority), 2012b. 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 Supporting Publications 2012; 9(12):EN-366, 44 pp. https://doi.org/10.2903/sp.efsa.2012.EN-366

EFSA (European Food Safety Authority), 2013. Assessment of *Echinococcus multilocularis* surveillance reports submitted 2013 in the context of Commission Regulation (EU) No 1152/2011. EFSA Journal 2013;11(11):3465, 41 pp. https://doi.org/10.2903/j.efsa.2013.3465

EFSA (European Food Safety Authority), 2014. Assessment of *Echinococcus multilocularis* surveillance reports submitted in 2014 in the context of Commission Regulation (EU) No 1152/2011. EFSA Journal 2014;12(10):3875, 44 pp. https://doi.org/10.2903/j.efsa.2014.3875

EFSA (European Food Safety Authority), 2015. Assessment of *Echinococcus multilocularis* surveillance reports submitted in 2015 in the context of Commission Regulation (EU) No 1152/2011. EFSA Journal 2015;13(11):4310, 58 pp. https://doi.org/10.2903/j.efsa.2015.4310

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2015. Scientific opinion on *Echinococcus multilocularis* infection in animals. EFSA Journal 2015;13(12):4373, 129 pp. https://doi.org/10.2903/j.efsa.2015.4373

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA Journal 2017;15(12):5077, 228 pp. https://doi.org/10.2903/j.efsa.2017.5077

EFSA (European Food Safety Authority), Bau A, Palumbo R, Richardson J and Zancanaro G, 2016. Assessment of *Echinococcus multilocularis* surveillance reports submitted in 2016 in the context of Commission Regulation (EU) No 1152/2011. EFSA Journal 2016;14(12):4649, 55 pp. https://doi.org/10.2903/j.efsa.2016.4649

EFSA (European Food Safety Authority), Beltran-Beck B and Zancanaro G, 2017. Scientific report on the assessment of Echinococcus multilocularis surveillance reports submitted in 2017 in the context of Commission Regulation (EU) No 1152/2011. EFSA Journal 2017;15(11):5051, 75 pp. https://doi.org/10.2903/j.efsa.2017.5051

Environment.no. online. Arctic fox in Norway. Available online: http://www.environment.no/topics/biodiversity/species-in-norway/threatened-species/arctic-fox-mainland-norway/

Fauna Europaea, online. *Vulpes vulpes* (Linnaeus, 1758). Available online: https://fauna-eu.org/cdm_dataportal/taxon/84f2e35f-eea0-4289-99c9-e6262bb0e386

Florisson L and Kreij M, 2014. The raccoon dog: an exponential problem? Management methods used in Europe to control the raccoon dog population (*Nyctereutes procyonoides*). Available online: https://www.google.it/url?sa=t&rct=j&q=&esrc=s&source=web&cd=5&cad=rja&uact=8&ved=0ahUKEwiX7oHN5fzUAhUE8RQKHS7GAYIQFghIMAQ&url=http%3A%2F%2Fwww.samhao.nl%2Fwebopac%2FMetaDataEditDownload.csp%3Ffile%3D2%3A125524%3A1&usg=AFQjCNEMZdUQBO5HYnM34WSXNm-cbLyfAw

Hayden T and Harrington R, 2000. *Exploring Irish mammals*. Town House & Country House Ltd., Dublin, Ireland. 340 pp.

Hofer S, Gloor S, Muller U, Mathis A, Hegglin D and Deplazes P, 2000. High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zurich, Switzerland. Parasitology, 120, 135–142.

Isaksson M, Hagström Å, Armua-Fernandez MT, Wahlström H, Ågren EO, Miller A, Holmberg A, Lukacs M, Casulli A, Deplazes P and Juremalm M, 2014. A semi-automated magnetic capture probe based DNA extraction and real-time PCR method applied in the Swedish surveillance of *Echinococcus multilocularis* in red fox (*Vulpes vulpes*) faecal samples. Parasites & Vectors, 7, 583.

Kahuala K, 2007. Paljonko Suomessa on pienpetoja? Riista-ja kalatalous Selvityksiä 1. Riista-ja kalatalouden tutkimuslaitos, Helsinki 2007. Available online: http://jukuri.luke.fi/bitstream/handle/10024/532812/paljonko_suomessa_on_pienpetoja_julkaisu_nettiin.pdf?sequence=1

Kapel CMO, Torgerson PR, Thompson RCA and Deplazes P, 2006. Reproductive potential of *Echinococcus multilocularis* in experimentally infected foxes, dogs, raccoon dogs and cats. International Journal for Parasitology, 36, 79–86.

Lalošević D, Lalošević V, Simin V, Miljević M, Čabrilo B and Čabrilo OB, 2016. Spreading of multilocular echinococcosis in southern Europe: the first record in foxes and jackals in Serbia, Vojvodina Province. European Journal of Wildlife Research, 62, 793–796.

Macdonald D and Reynolds J, 2008. *Vulpes vulpes*. In: IUCN 2013. IUCN Red List of Threatened Species, Version 2013.1.

Marnell F, Kingston N and Looney D, 2009. Ireland Red List No. 3: Terrestrial Mammals, National Parks and Wildlife Service. Department of the Environment, Heritage and Local Government, Dublin, Ireland. Available online: https://www.npws.ie/content/publications/irish-red-list-no-3-terrestrial-mammals

Mathis A, Deplazes P and Eckert J, 1996. An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. Journal of Helminthology, 70, 219–222.

Mihmanli M, Idiz UO, Kaya C, Demir U, Bostanci O, Omeroglu S and Bozkurt E, 2016. Current status of diagnosis and treatment of hepatic echinococcosis. World Journal of Hepatology, 8, 1169–1181.

Moro P and Schantz PM, 2009. Echinococcosis: a review. International Journal of Infectious Diseases, 13, 125–133.

Øines Ø, Isaksson M, Hagström Å, Tavornpanich S and Davidson RK, 2014. Laboratory assessment of sensitive molecular tools for detection of low levels of *Echinococcus multilocularis*-eggs in fox (*Vulpes vulpes*) faeces. Parasites & Vectors, 7, 246.

Oksanen A, Siles-Lucas M, Karamon J, Possenti A, Conraths FJ, Romig T, Wysocki P, Mannocci A, Mipatrini D, La Torre G, Boufana B and Casulli A, 2016. The geographical distribution and prevalence of *Echinococcus multilocularis* in animals in the European Union and adjacent countries: a systematic review and meta-analysis. Parasites & Vectors, 9, 519.

Peregrine AS, Jenkins EJ, Barnes B, Johnson S, Polley L, Barker IK, De Wolf B and Gottstein B, 2012. Alveolar hydatid disease (*Echinococcus multilocularis*) in the liver of a Canadian dog in British Columbia, a newly endemic region. The Canadian Veterinary Journal, 53, 870–874.

Phillips SJ, Anderson RP and Schapire RE, 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling, 190, 231–259.

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

Scott DM, Berg MJ, Tolhurst BA, Chauvenet ALM, Smith GC, Neaves K, Lochhead J and Baker PJ, 2014. Changes in the distribution of Red Foxes (*Vulpes vulpes*) in Urban Areas in great Britain: findings and limitations of a media-driven nationwide survey. PLoS ONE, 9, e99059. https://doi.org/10.1371/journal.pone.0099059

Torgerson PR, Keller K, Magnotta M and Ragland N, 2010. The global burden of alveolar Echinococcosis. PLOS Neglected Tropical Diseases, 4, e722.

Trachsel D, Deplazes P and Mathis A, 2007. Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. Parasitology, 134, 911–920.

WHO (World Health Organization), 2017. Echinococcosis- Fact sheet 2017. WHO, Geneva, Switzerland. Available online: http://www.who.int/mediacentre/factsheets/fs377/en/

Wright LJ, Newson SE and Noble DG, 2014. The value of a random sampling design for annual monitoring of national populations of larger British terrestrial mammals. European Journal of Wildlife Research, 60, 213–221.

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

AFBI	Agri-Food and Biosciences Institute
CL	confidence level
DALY	disability-adjusted life-year
DCF	EFSA Data Collection Framework
DH	definitive host
DP	design prevalence
EFTA	European Free Trade Association
GB	Great Britain (including England, Wales and Scotland)
IST	intestinal scraping technique
N	target population size
NI	Northern Ireland
OR	odds ratio
PCR	polymerase chain reaction
RR	relative risk
SCT	sedimentation and counting technique
Se	sensitivity

SSC Species Survival Commission
SSe system sensitivity
Sp specificity
ToR Terms of Reference
TSe test sensitivity
UK United Kingdom (including Great Britain and Northern Ireland)

Appendix A – Assessment tables for the surveillance report of Finland

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 *E. multilocularis* must be defined. Modifications of the original method should be indicated	• The Finnish Food Safety Authority (Evira) utilises 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 0.76 and 0.87 (95% CL). A Bayesian approach gives similar results. Therefore, the lowest value (0.76) may be the safest 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 available	The selection of racoon 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 *E. multilocularis*, 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 (340,000) than red fox (120,000). • Population densities for both species highest in the southern part of the country • Population sizes were estimated by Kahuala (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 Kahuala (2007) are not available • Data from annual hunting bag suggest no major changes since 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

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 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 = 460,000 The sample size was estimated as being 383 (both binomial and hypergeometric)	Due to the results that were obtained in the estimation of the test sensitivity (see **test sensitivity**), EFSA investigated a worst-case scenario using a test sensitivity of 0.76. The sample size required in this case is 393
	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 *E. multilocularis* due to geographical closeness of infected areas, and also with highest density of raccoon dogs (Kahuala, 2007) • Large sample of foxes (21% of all animals) was received from Lappi where red fox population reduction to protect the arctic fox was ongoing • Samples were collected throughout 2017	In reality, the largest portion of samples originates from Etelä-Karjala region (south-east, 27% of the samples), followed by the Lappi region (north, 21%). 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

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	Comments
				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)
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 geographical epidemiologic 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, it 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 2017 n = 556 The obtained system sensitivity was 0.987 (both binomial and hypergeometric)	As mentioned earlier, EFSA investigated a worst case scenario using 0.76, i.e. the lowest value of the credible interval around the estimate of the test sensitivity (see Section 3.1.2.1). However, also in this case, the sample size required is sufficient to satisfy the technical legal requirements (area sensitivity = 0.986; > 0.95)

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

Parameter	Evidence	Action
Theoretical sampling period	From 1 January 2017 to 31 December 2017	–
Actual sampling period	From January 2017 to December 2017	Exact sampling date not reported. Report, if possible
Sampling period		–
Number of samples	556	
Number of test results	556 PCR 12S rRNA	
Laboratory test completion	556 results in 2017	–
Sensitivity	0.78	–
Host	217 *Vulpes vulpes*; 339 *Nyctereutes procyonoides*	–
Animal sample	556 individual rectal content	–
Sampling strategy and design	Objective sampling – Simple random sample	–
Sampling point	556 hunting	–

Appendix B – Assessment tables for the surveillance report of Ireland

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 *E. multilocularis* 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 *E. multilocularis* 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 et al., 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 405, which ensures the fulfilment of the technical legal requirements in Regulation (EU) No 1152/2011 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 2017 • 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 1,000 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 and August. 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 geographical epidemiologic 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 sub-areas 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, it is 78% (EFSA AHAW Panel, 2015)	SYSTEM SENSITIVITY CALCULATION DP = 0.01 TSe = 0.78 Sample size for 2017 n = 405 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 405. 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 2017 to 31 December 2017	–
Actual sampling period	From 5 January 2017 to 19 December 2017	
Sampling period	348 days	–
Number of samples	405	
Number of test results	405 PCR Cest1-Cest2 NAD1	
Laboratory test completion	225 results in 2017 180 results in 2018	–
Sensitivity	0.78	–
Host	405 *Vulpes vulpes*	–
Animal sample	405 faeces post-mortem	–
Sampling strategy and design	Objective sampling – Simple random sample 405	–
Sampling point	283 from hunting; 122 wildlife research station	–

Appendix C – Assessment tables for the surveillance report of Malta

Table C.1: Assessment of the description of the surveillance system (Malta - 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 *E. multilocularis* must be defined. Modifications of the original method should be indicated	• The Microscopy/PCR RNAsn U1 method was used to analyse faecal samples from live animals (Mathis et al., 1996). • The initial phase in the identification of the agent was carried out at the National Veterinary Laboratory in Malta. Faeces samples were examined using the flotation and concentration method. All the worm eggs microscopically identified as *Taenia* spp. were stored in 75% alcohol. The National Veterinary Laboratory in Malta is not accredited for the flotation method on faeces and the method is not yet validated. • The faeces positive for presence of *Taenia* spp. eggs were sent to the Istituto Superiore di Sanità in Rome, Italy, for identification of *Echinococcus granulosus, Echinococcus multilocularis* and *Taenia* spp. eggs by means of multiplex-PCR analysis.	The method used by Malta in the surveillance of *E. multilocularis* (microscopy/PCR RNAsn U1) is well described
	Test sensitivity	The sensitivity and specificity of the test used in the surveillance system must be reported. This would ideally be estimates from each participating laboratory reported as a point estimate (average) of the values across the country with minimum and maximum values or a probability distribution. Alternatively, a value of 0.78, as recommended by EFSA (2015) shall be used	**Test Se = 0.78 (78%)**	Malta followed EFSA's advice regarding the setting of at least the conservative lowest value of the sensitivity (0.78)

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	• In Malta there are no wild foxes or raccoon dogs and the only carnivore that is present is the weasel (*Mustela nivalis*) • The population of *M. nivalis* is very low and is not considered to be an elite definitive host. Furthermore, transmission of the disease through *M. nivalis* is considered to be very remote due to their nocturnal and retrieval behaviour • The absence of wildlife definitive host (*Vulpes vulpes*) worldwide is described by the International Union for Conservation of Nature and Natural Resources - Species Survival Commission (SSC) (Macdonald and Reynolds, 2008) • Considering the absence of the definitive wild host population in Malta (including Gozo), dogs may play a role as potential definite hosts through possible contact with the rodents • Given the high population density of the Maltese Islands, distribution of dogs is relatively homogeneous in Malta • The target populations for the purpose of this study consisted of dogs (pets, hunting, rural and stray dogs): the main risk groups identified were 'Rural' dogs and 'Stray dogs'	The selection of dogs as target species in order to carry on the surveillance is well described and justified. The absence of the main wild definitive hosts is supported by IUCN and other sources of information (e.g. Fauna Europaea). Malta selected domestic dogs, due to the fact that dogs have been reported occasionally as DH, to accomplish the rules of the Annex II in the legislation in order to be listed in Annex I: `The pathogen-specific surveillance programme shall consist in the ongoing collection, during the 12-month surveillance period, of samples from wild definitive hosts or, in the case where there is evidence of the absence of wild definitive hosts in the Member State or part thereof, from domestic definitive hosts'.
	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	• Dog registration and microchipping in the Maltese Islands is governed by a legal notice LN 199/2011 which obliges all dog owners to microchip and register their animals with the	Dog population size is well described and has been updated since the last year. However, as discussed previously, the different categories in the classification are not always well defined and justified

Points addresses in Annex II	Element	Description of Element	Information provided in surveillance report	Comments
			competent authority. The registration is undertaken and managed by the Veterinary Regulation Department • The total number of registered dogs in 2017 was 66,731; out of which 33,511 were female and 33,220 were male. The age distribution young to adult dogs was 7,924 young dogs (≤ 2 years) and 58,807 adult dogs (> 2 years). This data was obtained from National Database used to register dogs for microchipping • There is no classification of the dog population into pets, hunting or rural dogs in the National Veterinary Information System where information connected to the identified dogs is registered • Estimates of stray dogs were supplied by the six dog sanctuaries present in the Maltese islands, showing that that the stray dogs collected vary from 1,000 to 2,000 per year	
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 is deduced to be the individual animal. Faeces samples were collected presumably individually from dogs of farms and sanctuaries (stray dogs)

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 was estimate by Malta using the RiBESS tool and the reported value is 383	It is not clear how the risk factors identified have played a role in the calculation of the sample size, as the reported value is the one obtained assuming no risk factors and no convenient samplingIn any case, the target sample size of 383 is the safest possible (no strong assumptions needed)
	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	• Sampling was carried out in 2 ways: (i) samples from farms were collected by sampling teams carrying out *Brucella*, TB testing, Animal Welfare inspections and other on-farm inspections, while (ii) the samples from sanctuaries/stray dogs were collected by a dedicated Echinococcus sampling team • Samples were collected from the ground. To ascertain their provenience, sampling officers sampled dogs which were kept tide up on farms, while the sampling of faeces from the sanctuaries were collected when the dogs were first admitted and thus being kept isolated • A total of 383 samples were collected throughout 2017 (234 rural dogs and 149 stray dogs) • Samples were collected in both Malta and Gozo. In Gozo, samples were collected from 9 out of the 14 localities. These localities represent the major rural areas in the island of Gozo. A dog pound is also located in one of these localities, were stray dogs from the island of Gozo are collected. In Malta, 23 localities were sampled, across the island, the sampling area included 4 dog sanctuaries that collect stray dogs from all Malta	The sampling activity was heterogeneously distributed over the full year with intensification in May–June and October–November. However, this fact may not affect the representativeness of the sample; 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 the whole year (EFSA, 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 geographical epidemiologic unit(s) identified as target for the surveillance activity has to be clearly indicated and supported by justification	The whole territory of Malta (Maltese islands of Malta and Gozo) was considered as one epidemiological unit	The geographical unit was specified to be the entire territory of Malta
	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, it is 78% (EFSA AHAW Panel, 2015)	SYSTEM SENSITIVITY CALCULATION DP = 0.01 TSe = 0.78 sample size for 2017 n = 383 The obtained system sensitivity was 0.951	The value of the area sensitivity 0.951 (> 0.95) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements described in Regulation (EU) No 1152/2011

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

Parameter	Evidence	Action
Theoretical Sampling period	From 1 January 2017 to 31 December 2017	–
Actual Sampling Period	From January 2017 to December 2017	Exact sampling date not reported. Report, if possible
Sampling period		–
Number of samples	383	
Number of test results	383 Microscopy/PCR RNAsn U1	
Laboratory test completion	All test results were reported in 2017	–
Sensitivity	0.78	–
Host	383 *Canis lupus familiaris*	–
Animal sample	383 faeces from live animal	–
Sampling Strategy and Design	Selective sampling – Risk Based Surveillance 383	–
Sampling point	383 Veterinary activities	–

Appendix D – Assessment tables for the surveillance report of United Kingdom

Table D.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 *E. multilocularis* 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	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 urban/sub-urban fox population is now estimated at ~ 33,000 (~ 13%) • 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 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, 384 samples were collected and tested	The total number of samples collected by GB was 388, which ensures the fulfilment of the technical legal requirements of Regulation (EU) No 1152/2011 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

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 geographical epidemiologic 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, it 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 2017 n = 388 The obtained system sensitivity was 0.952	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 388. The value of the area sensitivity (0.952; > 0.95) exceeded the established minimum value of 0.95 needed to fulfil the technical legal requirements

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

Parameter	Evidence	Action
Theoretical sampling period	From 1 March 2017 to 28 February 2018	–
Actual sampling period	From 27 March 2017 to 24 February 2018	–
Sampling period	334 days	–
Number of samples	388	–
Number of test results	388 PCR Cest1-Cest2 NAD1	–
Laboratory test completion	225 in 2017; 163 in 2018	–
Sensitivity	0.78	–
Host	388 *Vulpes vulpes*	–
Animal sample	388 faeces post-mortem	–
Sampling strategy and design	Objective sampling – Simple random sample 384	Provide evidences on the proportional sample size to the population density (high sample size should correspond to high density areas)
Sampling point	Wildlife Research Station 388	–

Table D.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 *E. multilocularis* 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 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

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/ 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 road kill 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 geographical epidemiologic 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

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, it 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 2017 n = 332 The obtained system sensitivity was 0.963 (binomial) and 0.965 (hypergeometric)	If a test sensitivity of 0.78 is assumed, the area sensitivity (0.926, binomial; 0.927 hypergeometric) is not sufficient to comply with the technical legal requirements of the EU regulation in force (47 additional tests would be required)

Table D.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 14 April 2017 to 23 February 2018	–
Sampling period	315 days.	–
Number of samples	332	–
Number of test results	332 sedimentation and counting technique	–
Laboratory test completion	96 test results in 2017 236 test results in 2018	–
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	332 *Vulpes vulpes*	–
Animal sample	332 individual intestinal content	–
Sampling strategy and design	Objective sampling – Simple random sample 320	–
Sampling point	304 from hunting; 28 from road kills	–

Appendix E – Assessment tables for the surveillance report of Norway

Table E.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 *E. multilocularis* 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

Points addresses in Annex II	Element	Description of element	Information provided in surveillance report	Comments
	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), should be used	• Test Se = 0.63 (63%) • Test Sp= 100%	The diagnostic sensitivity was set to the sensitivity obtained by Øines et al., 2014 (63%), a lower value than the minimum recommended by EFSA (0.78). Such 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. However, it has to be acknowledged that 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 (Florisson and Kreij, 2014; Environment.no, online)
	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	In the 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
			• 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)	
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	• EpiTools epidemiological calculators was used (http://epitools.ausvet.com.au/content.php?page=home), DP = 1% CL = 95%. The software uses hypergeometric approximation when population size is provided. The goal was approximately 482 samples from red foxes in 2017. In addition, in 2017, samples from 11 wolves (*Canis lupus*) were included in the surveillance	This number of samples would be also sufficient to meet the requirements if calculated with RiBESS tool. Using this application, and considering design prevalence of 1% (0.01), a test sensitivity of 0.63, and a population size of 151,000, the sample sized required is 474 The sample size required using the value suggested by EFSA for the test sensitivity (0.78) is 383

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	• 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 • 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 2017 and part of 2018	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 report Samples 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
			• 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 15 April and 15 July	
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 geographical epidemiologic 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
	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, it is 78% (EFSA AHAW Panel, 2015)	SYSTEM SENSITIVITY CALCULATION DP = 0.01 TSe = 0.63 Sample size for 2017 n = 438 The obtained system sensitivity is 0.937 (binomial and hypergeometric) Using the value suggested by EFSA for the test sensitivity (0.78), the system sensitivity achieved is 0.968	Using the RiBESS tool, and considering a test sensitivity of 0.63, a population size of 151,000 and a sample size of 495, the value of the area sensitivity is 0.937, which is below the established minimum value of 0.95 needed to fulfil the technical legal requirements of Regulation (EU) No 1152/2011 However, using the value suggested by EFSA for the test sensitivity (0.78), the system sensitivity achieved is 0.968 (> 0.95)

Table E.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	3 January 2017–29 December 2017	–
Sampling period	360 days	–
Number of samples	438	–
Number of test results	438 PCR 12S rRNA	–
Laboratory test completion	438 reported in 2017	–
Sensitivity	0.63	–
Host	438 *Vulpes vulpes*	–
Animal sample	438 individual rectal content	–
Sampling strategy and design	Objective sampling –Single Random Sampling 495	–
Sampling point	438 Hunting	–