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SCIENTIFIC REPORT OF EFSA

Scientific and technical assistance on *Echinococcus multilocularis* infection in animals[1]

European Food Safety Authority[2, 3]

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SUMMARY

Echinococcus multilocularis is a tapeworm occurring throughout the Northern Hemisphere, which is transmitted primarily between wild definitive carnivorous hosts (in Europe mainly red foxes and raccoon dogs) and wild intermediate hosts (small mammals, mainly arvicolid rodents). Dogs and cats can also be infected by ingestion of an intermediate host harbouring the larval form of the parasite, and may act then as definitive host. Humans can become accidentally infected by ingesting tapeworm eggs excreted by the definitive host and the resulting infection, alveolar echinococcosis (AE), is considered one of the most severe human parasitoses in non-tropical regions. In Europe, *E. multilocularis* is found in foxes in a range of central European countries, but has never been found in Finland, Ireland, Malta and the United Kingdom.

In order to ensure continuous protection of Finland, Ireland, Malta and the United Kingdom, that claim to have remained free of the parasite *Echinococcus multilocularis* (*EM*) as a result of applying national rules until 31 December 2011, Regulation (EU) No 1152/2011 of 14 July 2011 was adopted by the Commission as regards preventive health measures for the control of *Echinococcus multilocularis* infection in dogs. The Regulation includes, among other obligations for these Member States, the requirement to implement a pathogen-specific surveillance programme aimed at detecting the parasite, if present in any part of those Member States, and provides the specifications for these programmes. The results of the pathogen-specific surveillance programmes must be reported to the Commission by 31 May following the end of each 12-month surveillance period.

The Commission has requested EFSA to analyse and critically assess the sampling strategy considered for these programmes, the data collected as well as the detection methods used in the framework of these programmes and to produce a report the outcome of this assessment each year in October.

The first surveillance reports are due by 31 May 2013. This report proposes a harmonised reporting system for surveillance systems in compliance with Regulation (EU) No 1152/2011 to facilitate reporting as well as assessment of reports. The proposal is based on the guidelines for animal health surveillance of the World Organisation for Animal Health (OIE). The OIE Terrestrial Animal Health Code 2012 specifies in chapter 1.4 critical elements that need to be addressed in assessing the quality

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of a surveillance system (OIE, 2012). These elements were specified for *E. multilocularis* surveillance in the context of Regulation (EU) No 1152/2011, consulting two recent EFSA publications on *E. multilocularis* (EFSA, 2007, Boué et al., 2010) and additional relevant scientific literature.

The approach proposed for the reporting *E. multilocularis* surveillance system results consists of two parts. First, a tool for the description of the surveillance system has been developed specifically for this purpose by EFSA. The tool requires the specification of all parameters needed for describing the surveillance system of the respective Member State, such as design prevalence, population size and sensitivity of diagnostic tests used. Once the necessary information has been entered, the tool allows the calculation of the appropriate sample size and the assessment of the sensitivity of the surveillance system, i.e. whether the surveillance system fulfils the requirements for confidence outlined in Regulation (EU) No 1152/2011. Second, a data reporting framework is proposed indicating all relevant data that must be reported to enable assessment of the surveillance results. While the first part of the approach is generic and can be applied to any system designed to demonstrate absence of infection, the second part is specific for *E. multilocularis* surveillance in animals as requested by Regulation (EU) No 1152/2011.

The proposal for the *E. multilocularis* surveillance reporting format has been reviewed by four experts on *E. multilocularis* infection in animals and two experts on animal disease surveillance and modelling and was discussed and agreed with representatives of the four Member States[4].

KEY WORDS

Absence of infection, data collection, freedom from disease, reporting, risk-based, surveillance, wildlife

[4] Minutes of the 1st meeting of the AHAW Network sub-group on *Echinococcus multilocularis* infection in animals, http://www.efsa.europa.eu/en/events/event/121016c.htm

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BACKGROUND AS PROVIDED BY THE COMMISSION

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[5]. 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 *Echinococcus multilocularis* (*EM*) as a result of applying national rules until 31 December 2011.

This Regulation includes certain obligations for these Member States 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.

It also provides that the Commission is to review this Regulation no later than five years following the data of its entry into force, i.e. by December 2016, in the light of scientific developments regarding *EM* infection in animals and submit the results of the review to the European Parliament and to the Council.

Before a formal request for a scientific opinion on the infection with *Echinococcus multilocularis* infection in animals is addressed to EFSA to take account of the aforementioned deadline, 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:

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

1. Regular follow-up of the literature regarding *EM* 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 Member States concerned; (ii) the data collected in the framework of these programmes; (iii) the detection methods used.

EFSA is asked to produce a report regarding point 2 each year in October after reception of the Member States' reports by 31 May.

CONTEXT OF THE SCIENTIFIC OUTPUT

This report addresses ToR 2 of the mandate M-2012-0200 submitted to EFSA by the Commission. ToR 1 is being addressed by an Article 36 cooperation project of EFSA (in preparation).

[5] OJ L 296, 15.11.2011, p.6.

ASSESSMENT

1. Introduction

Echinococcus multilocularis is a tapeworm occurring throughout the Northern Hemisphere, which is transmitted primarily between wild definitive carnivorous hosts (in Europe mainly red foxes and raccoon dogs) and wild intermediate hosts (small mammals, mainly arvicolid rodents). Dogs and cats can also be infected by ingestion of an intermediate host harbouring the larval form of the parasite, and may act then as definitive host, contributing to the persistence of the parasite in urban and periurban areas. Humans are not part of the lifecycle, but can become accidentally infected (dead-end host) by ingesting tapeworm eggs excreted by the definitive host. The resulting infection in humans, alveolar echinococcosis (AE), typically presents as an infiltrative tumour-like growth in the liver, which at later stages may invade neighbouring organs. AE is considered one of the most severe human parasitoses in non-tropical regions. In Europe, *E. multilocularis* is found in foxes mainly in central Europe, from the north in Denmark, the Netherlands and Belgium, in the east to the Baltic States and Slovakia, in the south to north eastern Italy and Hungary, and in the west to central France (EFSA, 2007). Recently, *E. multilocularis* has been identified in the red fox (*Vulpes vulpes*) in Sweden (Osterman, 2011). It is also present in Belarus, Ukraine and Russia, but has never been found in Finland, Ireland, Malta and the United Kingdom.

EFSA is asked to analyse and critically assess the sampling strategy considered, the data collected, and the detection methods used in Member States' *E. multilocularis* surveillance programmes in the context of Regulation (EU) No 1152/2011 regarding preventive health measures for the control of *E. multilocularis* infection in dogs.

Regulation (EU) No 1152/2011 provides that Member States listed in Annex 1 thereof, i.e. Finland, Ireland, Malta and the United Kingdom, shall have had in place for the last 10 years prior to applying (a) rules for *E. multilocularis* infection in host animals to be compulsorily notifiable under national law; and (b) an early detection system for *E. multilocularis* infection in host animals. These Member States shall also implement a pathogen-specific surveillance programme which is drawn up and carried out in accordance with Annex II and shall report to the Commission the results of the pathogen-specific surveillance programme referred to in paragraph 2 by 31 May following the end of each 12-month surveillance period. The objective of the pathogen-specific surveillance programme is to provide evidence for absence of *E. multilocularis* infection in the Member States listed in Annex 1.

The following requirements for the pathogen-specific surveillance programme are laid down in Annex II to Regulation (EU) No 1152/2011:

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 deoxyribonucleic acid (DNA) from tissue or eggs of the *E. multilocularis* parasite by polymerase chain reaction (PCR), or a technique of equivalent sensitivity and specificity.

Regulation (EU) No 1152/2011 applies since 1 January 2012. The first surveillance reports of the Member States listed in Annex 1 are therefore due by 31 May 2013.

Three of the four Member States listed in Annex 1 have submitted surveillance reports to the Commission in May 2012, covering the *E. multilocularis* surveillance activities carried out in 2011-12. The three reports differ considerably in the number of and detail on surveillance system parameters described regarding the sampling strategy used, the data collected, and the detection methods. Thus, there is a need to harmonise the reporting of *E. multilocularis* surveillance activities. This report proposes a harmonised reporting system for surveillance systems in compliance with Regulation (EU) No 1152/2011.

2. Approach

To address ToR 2 of the mandate submitted to EFSA by the European Commission, EFSA has identified the elements to be assessed in surveillance reports following the guidelines for animal health surveillance of the World Organisation for Animal Health (OIE). The OIE Terrestrial Animal Health Code 2012 specifies in chapter 1.4 critical elements that need to be addressed in assessing the quality of a surveillance system. These include the susceptible host population, the timeframe or temporal values of the surveillance data, the relevant epidemiological unit of the surveillance system, the geographical clustering of infection, the case definition, the sensitivity and specificity values of the tests used, and the methodologies for analysis of the surveillance data. Additional critical elements to be considered for structured population based surveys are the type of the survey, the survey design, the sampling methods and the sample size. For structured non-random surveillance, the coverage of the susceptible host population, and any duplication of data are additional critical elements to be considered (OIE, 2012).

The elements relevant for surveillance reports outlined in the OIE guidelines for animal health surveillance have been specified for *E. multilocularis* surveillance in the context of Regulation (EU) No 1152/2011. To this end, the EFSA opinion regarding the assessment of the risk of echinococcosis introduction into the UK, Ireland, Sweden, Malta and Finland as a consequence of abandoning national rules (EFSA, 2007), the external scientific report on the development of harmonised schemes for the monitoring and reporting of *Echinococcus* in animals and foodstuffs in the European Union (Boué et al., 2010) and additional relevant scientific literature were consulted (section 3 of this report).

The statistical and epidemiological concepts relevant to demonstration of freedom from infection have been identified from scientific literature and specified for *E. multilocularis* surveillance reports in compliance with Regulation (EU) No 1152/2011 (section 4 of this report).

The above described elements and concepts were used to develop a proposal for *E. multilocularis* surveillance report models and data report models that could be used by MS in order to harmonise the *E. multilocularis* surveillance reports and to facilitate reporting and assessment of reports (section 5 of this report).

The list of relevant elements created by consulting these resources, the surveillance options, as well as the proposal for the *E. multilocularis* surveillance reporting format have been reviewed by four experts on *E. multilocularis* infection in animals and two experts on animal disease surveillance and modelling and were discussed and agreed with representatives of the four Member States[6].

[6] Minutes of the 1st meeting of the AHAW Network sub-group on *Echinococcus multilocularis* infection in animals, http://www.efsa.europa.eu/en/events/event/121016c.htm

3. Relevant elements that need to be addressed in assessing the quality of *E. multilocularis* surveillance reports in the context of Regulation (EU) No 1152/2011

3.1. Susceptible host population

Ideally, all species susceptible to the infection should be taken into account for the surveillance. If the surveillance is conducted on a subpopulation, inferences from the results should be made carefully (OIE, 2012). Regulation (EU) No 1152/2011 defines that samples should be collected 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.

As regards the wild definitive hosts, *E. multilocularis* is primarily found in red foxes (*Vulpes vulpes*), which therefore is the best species for monitoring. Raccoon dogs (*Nyctereutes procyonoides*) can also harbour infection and may be sampled where they exist (Boué et al., 2010).

Regarding domestic definitive hosts, dogs and cats become infected by predating on infected rodents e.g. in city parks and gardens. The occurrence of *Echinococcus* spp. infections in dogs and cats was investigated in a cross-sectional survey analysing faecal samples of domestic dogs (n=21,588) and cats (n=10,650) from Germany and other European countries routinely submitted to a private veterinary laboratory between June 2004 and June 2005 using the polymerase chain reaction technique. The overall prevalence of *E. multilocularis* infection observed was 0.20 % in dogs and 0.23 % in cats. However, in upper Bavaria, prevalences of 0.54 and 0.45 % in dogs and cats respectively were found (Dyachenko et al., 2008).

Thompson et al., who studied the development of *E. multilocularis* (growth, segmentation and maturation) in experimental infections of foxes, racoon dogs, dogs and cats beyond the prepatent period, found that maturation and proportion of worms with thickshelled eggs recovered from cats were consistently lower than those of worms recovered from the foxes, racoon dogs and dogs studied (Thompson et al., 2006). In the same study, the experimentally infected cats produced only small numbers of worm eggs, and 95 % of the total egg mass produced by infected cats was expelled by day 13 post infection (Kapel et al., 2006).

Other wildlife species with confirmed susceptibility to *E. multilocularis* infection like wolf (*Canis lupus*), lynx (*Lynx* spp.), wild cat (*Felis silvestris*) and jackal (*Canis aureus*) are of limited or no importance as definitve hosts in Europe. There are numerous records of *E. multilocularis* infection in the arctic fox (*Alopex lagopus*) outside Europe, e.g. in Siberia and Alaska. In Europe the first record of *E. multilocularis* in arctic fox came recently from the Norwegian arctic island of Spitsbergen (Svalbard), (EFSA, 2007). A recent study investigating the genetic diversity of *E. multilocularis* in Svalbard by genotyping found that *E. multilocularis* arctic populations were closely related to St. Lawrence Island samples from Alaska, which is consistent with the hypothesis that Arctic foxes introduced *E. multilocularis* to Svalbard (Knapp et al., 2012).

In view of the above it is suggested that results from sampling of red foxes, as they are the most suitable wild definitive hosts, or, from areas where foxes are not present, results from sampling of raccoon dogs are reported. If evidence for the absence of these wild definitive host species exists, results from sampling of dogs or, alternatively, of cats should be reported. However, regarding sampling of domestic host species, it is important to note that only samples collected from individuals having access to outdoors, i.e. having the potential for predating on infected rodents, are relevant for the surveillance systems in compliance with Regulation (EU) No 1152/2011.

3.2. Timeframe of the surveillance data

The surveillance should be carried out at a frequency reflecting the biology of infection and the risk of its introduction (OIE, 2012).

Prevalence and host density show temporal dynamics, which needs to be considered when comparing data from different regions obtained in different periods (EFSA, 2007).

It is thus recommended that the sampling time (year, month, if possible also the day of collection) is reported for each individual sample. If sampling takes place only during a part of the 12-month sampling period prescribed by Regulation (EU) No 1152/2011, a justification for this should be provided and the effect this has on the predictive value of the surveillance system should be explained.

3.3. Relevant epidemiological unit of the surveillance system

The epidemiological unit must be appropriate to achieve the objective of the surveillance. It must take account of carriers, reservoirs, vectors and other factors such as host criteria (OIE, 2012).

The relevant epidemiological unit for the *E. multilocularis* surveillance reports is the individual animal. As outlined in Regulation (EU) No 1152/2011 these individual animals could be wild definitive host animals or, in the case where there is evidence of the absence of wild definitive hosts in the Member State or part thereof, domestic definitive host animals. As an alternative to sampling individual host animals, individual faecal samples can constitute a useful sampling unit. The technique has the advantage of not being invasive and has been successfully applied in epidemiological surveys on *E. multilocularis* infections in foxes in Japan (Morishima et al., 1999) and Europe (Raoul et al., 2001). However, carnivore faeces are quite similar morphologically and it has been observed that applying only morphological identification methods to differentiate fox faeces from those of sympatric carnivores leads to misinterpretations (Davison et al., 2002). To overcome this problem, several molecular methods have been developed for species identification of faeces (Farell et al., 2000, Dalen et al., 2004, Nonaka et al., 2009), some of them combining species identification with simultaneous *E. multilocularis* detection (Dinkel et al., 2011). 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.

3.4. Geographical clustering of infection

If geographical clustering of infection in the population is present, it should be taken into account in the design of the surveillance system and the analysis of the surveillance data, at least at the most significant level of clustering (OIE, 2012).

Various studies indicate that landscape patterns and agricultural land use have an influence on *E. multilocularis* prevalence in foxes. In a focal endemic region in the northwest of Brandenburg in Germany, it was found that infected foxes were more frequently shot near water, in areas of high soil humidity and on pastures than in forest areas (Staubach et al., 2001). The presence of permanent grassland (meadows, pastures) favours populations of the parasite's most important intermediate hosts (common voles and water voles) and is likely to be of primary importance for transmission (EFSA, 2007). In a study carried out from 2001 to 2005 in north-eastern France, Guislan et al. found that high *E. multilocularis* prevalence in foxes were associated with fragmented grasslands (Guislan et al., 2008).

It is thus recommended that the sampling location (NUTS level 3[7]) is reported for each individual sample.

3.5. Case definition

A case should be defined for each infection under surveillance using clear criteria. For wildlife surveillance, it is essential to correctly identify and report host animal taxonomy including genus and species (OIE, 2012).

[7] Nomenclature of Territorial Units for Statistics, regional level, http://europa.eu/legislation_summaries/regional_policy/management/g24218_en.htm

For the purposes of the surveillance programme reports, a case is defined as any definitive host animal confirmed positive for *E. multilocularis* based on the results of the diagnostic tests described in Annex II to Regulation (EU) No 1152/2011 and having epidemiological information consistent with infection in the country.

If individual faecal samples have been selected as the sampling unit, a case is defined as any faecal sample of a definitive host confirmed positive for *E. multilocularis* based on the results of the diagnostic tests described in Annex II to Regulation (EU) No 1152/2011. For faecal samples from dogs or cats with a history of recent travel to an *E. multilocularis* endemic country, a case is defined as a faecal sample confirmed positive for *E. multilocularis* based on the results of the diagnostic tests described in Annex II to Regulation (EU) No 1152/2011 in the absence of anthelmintic treatment in the 120 h prior to sample collection.

3.6. Sensitivity and specificity values of the tests used

The sensitivity and specificity values of the tests used should be specified for each species in which they are used. The method used to estimate these values should be documented. Alternatively, where sensitivity or specificity values of a particular test are specified in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, these may be used as a guide (OIE, 2012).

Regulation (EU) No 1152/2011 specifies in Annex II that the sedimentation and counting technique (SCT), or a technique of equivalent sensitivity and specificity, should be used for the detection of *E. multilocularis* parasite in intestinal contents and that the polymerase chain reaction (PCR), or a technique of equivalent sensitivity and specificity, should be used for the detection of species-specific deoxyribonucleic acid (DNA) from tissue or eggs of the *E. multilocularis* parasite in faeces. An overview of analytical methods suitable for *E. multilocularis* detection and their sensitivity, specificity and application is provided in Annex B.

3.7. Type of the survey

OIE suggests that surveys to document infection freedom be conducted using probability based sampling methods so that data from the study population can be extrapolated to the target population in a statistically valid manner. The sources of information and the sampling strategy used for the selection of units for testing should be fully described (OIE, 2012).

3.8. Survey design

The design of the survey depends on the size, structure and degree of understanding of the population being studied, the epidemiology of the infection and the resources available. The size of the wildlife population should be determined to the extent possible before the survey is designed. Historical population data should be updated since these may not reflect current populations. The expertise of wildlife biologists may be sought in the gathering and interpretation of such population data. The applied survey design should be fully documented, including considerations regarding potential biases inherent in the survey design (OIE, 2012).

3.9. Sampling methods

Ideally, probability based sampling methods, such as simple random sampling, cluster sampling, stratified sampling, systematic sampling, should be used so that data from the study population can be extrapolated to the target population in a statistically valid manner. When this is not possible, sampling should provide the best practical chance of generating a sample that is representative of the target population. The sampling methods used should be fully documented including the related assumptions and uncertainties, and a justification for choosing the approach should be provided. When assessing reports from structured non-random surveillance, attention needs to be paid to whether the susceptible host population has been adequately covered and any duplication of data has been properly addressed (OIE, 2012).

3.10. Sample size

The method used to calculate sample size for surveys depends on the purpose of the survey, the expected prevalence (hereafter referred to as design prevalence), the level of confidence desired of the survey results and the performance of the tests used. The method used in the system should be fully documented (OIE, 2012).

4. *E. multilocularis* surveillance options in compliance with Regulation (EU) No 1152/2011

Regulation (EU) No 1152/2011 deals with the concept of "absence of infection" which is interpreted as "freedom from infection" in this report. There are two main approaches that can fit this purpose and are both given in the Regulation:

(a) a *simple random sampling* from the population (see Section 4.2.), where the sample size is calculated in order to obtain at least one positive test in case the prevalence would be above a given threshold. When the sample is really random, it is representative of the entire population as all the components will be represented with the same proportions. Other options of achieving a representative sample might be used, such as subdividing the geographical area of interest by means of a grid and ensuring that samples are taken from each sub-area in the grid, but these are only approximations of a simple random sampling;

(b) a *risk based surveillance* (see Section 4.3.), where the calculation of the sample size and the choice of the sampling population depend on risk indicators (Willeberg et al., 2012), identified and quantitatively estimated, playing a role in the epidemiology of *E. multilocularis*. This approach must be implemented in each of the epidemiologically relevant geographical unit identified within a MS.

4.1. Sample size calculation

The formulae used to calculate the sample size needed to detect an infection when its prevalence is at or above a so called Design Prevalence (1% in this case) are based on the principles developed by Cannon (Cannon, 2002) and are based either on the binomial or the hypergeometric probability distributions, according to the size of the population under investigation.

If the population can be considered *infinite*[8] (i.e. the individual probability of being positive does not change along the sampling exercise; also referred to as "sampling with replacement"), the Binomial distribution can be used:

$$RSe^{9} = 1 - (1 - P \cdot TSe)^{n} \quad (1)$$

where *RSe* is the sensitivity of a round of tests, *P* is the Design Prevalence, *TSe* is the sensitivity of the test and *n* is the sample size.
From which *n* can be derived as follows:

$$n = \frac{log(1 - RSe)}{log(1 - P \cdot TSe)} \quad (2)$$

While, if the population is *finite*, the Hypergeometric adjustment is needed. In this case, the Round of tests Sensitivity is given by:

[8] Though a universal definition of "infinite" and "finite" population does not exist (as it depends on the prevalence, the test sensitivity and the desired level of confidence) the *rule of thumb* is that a population can be considered "infinite" when n/N < 0.1 (Evans M.; Hastings N.; Peacock B.; Statistical Distributions, Third Edition; Wiley Interscience, 2000)

[9] Note: Round of tests Sensitivity (RSe) is a generic term. According to the different situations may be called ASe (Area Sensitivity) or GSe (Group Sensitivity), but the formula used in these cases is the same. On the contrary, the SSe (System Sensitivity) calculation is based on another formula (see formula (5))

$$RSe \cong 1 - \left(1 - \frac{n \cdot TSe}{N - 0.5 \cdot (N \cdot P \cdot TSe - 1)}\right)^{N \cdot P} \quad (3)$$

where *N* is the total population size.

In this case the sample size is given by:

$$n \cong \frac{\left(1 - (1 - RSe)^{1/(N \cdot P)}\right) \cdot \left(N - \frac{1}{2} \cdot (N \cdot P \cdot TSe - 1)\right)}{TSe} \quad (4)$$

It is essential to highlight that the formulae used for the sample size calculation assume a diagnostic test with 100% specificity (Sp=1). About this assumption Cannon states that in general, the design of any survey to demonstrate freedom from/ absence of infection should specify a sequence of further testing that would be done to clarify the true status when a positive reaction is detected and questioned (Cannon, 2002). Such a sequence would effectively result in a 100%-specific test. In addition, the assumption of perfect specificity has an important consequence: if a positive test result is returned by a system having 100% specificity, freedom from infection can no longer be claimed, as all positive results are true. Each surveillance system should be seen to encompass all necessary follow-up testing to resolve potential false positive results (Cannon, 2001; Dufour et al, 2001; Martin et al., 2007).

4.2. Representative survey (simple random sampling)

An important underpinning assumption that needs to be taken into consideration is that a simple random sampling is adequate when no risk factor plays a role in the distribution of the infection of concern. In practical terms, this approach assumes that the target population is homogeneously distributed in the study area (e.g. a Member State) and that the infected units are homogeneously distributed across the target population.

In addition, as the simple random sampling assumes that every unit in the target population has an equal probability of being included, a complete list of the source population is required and a formal selection process must be used (e.g., computer-generated random numbers)[10]. A violation of this assumption invalidates the results of the formulae (Cameron, 1998).

Considering the specific case of *E. multilocularis*, a formal random sampling seems to be difficult to implement if the target population is represented by wildlife for which, almost by definition, a complete list of the units does not exist. This may not be the case for domestic dogs, which would be the target population in case a MS does not have any wild red fox or raccoon dog in its territory.

However, as the two underpinning assumptions on homogeneity do not seem to be applicable, the simple random sampling approach is not recommended for the purpose of detecting an infection both in terms of efforts and reliability. Still, it represents an opportunity when no knowledge is available on possible risk indicators and on the characteristics of the target population (Blickenstorfer et al., 2011). As mentioned above, other options for achieving a representative sample might be used but these are only approximations of a simple random sampling. Nonetheless, a conservative approach should be adopted in this case to account for the potential bias given by the violation of the underpinning assumptions, e.g. by using a lower design prevalence and/or a higher confidence level.

4.3. Risk-based sampling (scenario-tree modelling)

Scenario-tree modelling techniques were introduced by Martin et al. (2007) to explicitly account for non-representative sampling approaches. These techniques captured the effect of differential sampling

[10] Among other references: Dohoo I., Martin W., Stryhn H.; Veterinary Epidemiologic Research, 2nd edition; VER Inc (2009)

from population strata with different risks of infection, allowing quantification of the benefits of risk-based sampling. The risk-based sampling, indeed, refers to the consideration of infection risk factors when determining the sampling pressure applied in different strata of a population under surveillance (Cameron, 2012).

The principle is that the design prevalence (DP), as a single value, implies that all units within the target population have the same average probability of being infected. Scenario-tree modelling effectively divides the population into multiple different risk groups, using the relative risk of infection in each group to adjust the DP to reflect the group-level probability of infection (Cameron, 2012).

Once the adjusted DP for each group is calculated, there are two possible ways of implementing a survey:

- To select the group where the adjusted DP is higher and calculate the sample size needed to detect the infection when this is present at or above the adjusted DP in that group (see Formula **(4)**). This option is based on the concept that it is more likely to find something where this is more likely to be: if all test results are negative in the highest risk group, this means that in the lower risk groups the infection (if present) would affect a smaller proportion of the population if compared to the high risk (i.e. infection is absent or below the DP);

- A second option is to collect sample from more than one group (for convenience matters, for instance). In this case, it is possible to calculate the *GSe* (Group Sensitivity) using the formulae reported in Section 4.1. The overall sensitivity of the surveillance is then calculated by formula **(5)**

$$SSe = 1 - \prod_{i=1}^{r}(1 - GSe_i) \qquad (5)$$

  where ***SSe*** is the System (overall) Sensitivity, ***GSe*** is the Group (or sub-area) Sensitivity and ***r*** is the number of groups/sub-areas included in the survey. The *SSe* represents the confidence of 95% required by the Regulation.

A prerequisite for the risk-based approach is the definition of the Risk Groups, which requires knowledge of the main risk indicators for *E. multilocularis* and the Relative Risk (RR) associated with each of them. This information, along with knowledge of the population of definitive hosts located in each Risk Group (i.e. the population fraction), allows calculating the Weighted Risk (WR) and, in turn, the Effective Probability of Infection (EPI) in each Risk Group. For each Risk Group either formula **(2)** or **(4)** can be used to estimate the sample size (EFSA, 2012, in preparation).

It is not necessary to have precise estimates of these parameters: the uncertainty around these estimates can be accounted for either using an *ad hoc* probabilistic model (in case evidence is available) or a what-if scenario approach. However, if there is no information available on the relevant risk indicators and the related quantitative information, the best option would be to implement a representative sample, bearing in mind that the underpinning assumptions will lead to an overestimation of the System Sensitivity (see Section 4.2).

In the context of Commission Regulation (EU) No 1152/2011, the principles of the risk-based approach, as described here, can be implemented in each of the epidemiologically relevant geographical units identified within a MS. In fact, for each of these geographical units, the design prevalence and the confidence level must be 1% and 95% respectively, but within each of them, the risk based principles can be applied (WR, EPI). As a consequence of having more than one

epidemiologically relevant geographical unit in the surveillance system, the overall sensitivity (at country level) will likely be higher than 95%.

5. Proposal for harmonisation of *E. multilocularis* surveillance reports in the context of Regulation (EU) No 1152/2011

In order to harmonise the reporting on *E. multilocularis* specific surveillance programmes operated by Member States in the context of Regulation (EU) No 1152/2011, and to facilitate both the submission and the analysis of the reports, EFSA is proposing a format for describing the surveillance systems (Part I of the surveillance report) and for reporting the data (Part II of the surveillance report) that Member States can use in fulfilment of the requirements laid down in Regulation (EU) No 1152/2011. This proposal is based on the elements considered relevant for *E. multilocularis* surveillance reports illustrated in section 3 and the surveillance options described in section 4 of this report.

5.1. Part I : Description of the surveillance system

In Regulation (EU) No 1152/2011 MS are given the possibility to choose between two main options: a survey based on a simple random sample or on a risk-based approach. The type of information that should be reported in the context of the two approaches is described in detail below.

5.1.1. Simple Random Sample (Representative survey)

A representative survey is based on the assumptions that the population is distributed homogenously across the study area and that all individuals have the same probability of being infected across the area. The information that needs to be reported on representative surveys includes:

- **Design Prevalence (DP)**: this is the prevalence against which the surveillance system is implemented, i.e. the surveillance system must be able to give a positive result when the proportion of infected units will be at or above the DP. Regulation (EU) No 1152/2011 indicates a DP of 1%. Nevertheless, a lower DP can be used as it would be a more conservative approach. On the contrary, a higher DP will not be allowed.

- **Test Sensitivity (TSe)**: this is the ability of the analytical test used in the surveillance system to give a positive result when the tested unit is truly infected. It is likely that more than one laboratory is involved in the analysis of the specimens. Ideally, each laboratory estimates and certifies the sensitivity of the test in house, independently from the standards available in the published scientific literature: the closer to the specific field situation, the better the surveillance system can be developed. Either a point estimate (as the average of the Test Sensitivity values across the country) together with minimum and maximum values or a probability distribution[11] would fit the purpose.

- **Definition of the susceptible host population targeted by the system**: The susceptible host population(s) targeted by the surveillance system should be described and the choice justified.

- **Epidemiological unit**: the epidemiological unit used, i.e. individual animals or individual faeces samples collected from the environment should be clearly defined. It is important to bear in mind that no pooling is allowed: each sample should correspond to only one individual.

[11] The Beta distribution can be used as the description of uncertainty or random variation of a probability, fraction or prevalence. It is the conjugate prior of the Binomial distribution (Evans M.; Hastings N.; Peacock B.; Statistical Distributions, Third Edition; Wiley Interscience, 2000) and it is widely used for the description of sensitivity.

- **Size (N) of the susceptible host population targeted by the system**: This information is essential in case the calculation of the needed sample size is done using the Hypergeometric formula.

- **Methodology for sample size calculation**: two options are possible: (i) direct calculation or (ii) calculation via simulation, i.e. estimation of the sample size via calculation of the System Sensitivity. The formula used for the calculation should be specified. Classical formulae are based on the Binomial and the Hypergeometric distributions. If any other formula or any adjustment is made on the original formulae, the algebra of the formula should be given. Many tools and free software are available on the web, however, it is recommended to check how the calculations are made by those tools as quite often adjustments and corrections are included in the program that runs behind the interface. In case the full documentation is not available, it is recommended to cite the tool used for the exercise.

5.1.2. Risk-based surveillance

Risk-based sampling is based on the knowledge or assumption that within the target population the individual (unit) probability of being infected is not homogeneously distributed, due to the influence of one or more risk indicators. The individuals within the target population can be grouped by related risk indicators for *E. multilocularis* (Risk Groups), and within each group all individuals (units) have the same probability of being infected.

In the framework of Regulation (EU) No 1152/2011, *geographical* (i.e. related to the geographical area) and *non-geographical* (i.e. related to the individual within the target population) *risk indicators* can be distinguished.

Geographical risk indicators can be used to delineate "epidemiologically relevant geographical areas" (i.e. risk areas), which cannot be used to implement a risk-based approach[12].

On the other hand, non-geographical risk indicators may be used to define *risk groups*, which in turn allow for the implementation of the risk-based sampling principles mentioned in Regulation (EU) No 1152/2011.

Some examples of geographical and non-geographical risk indicators potentially relevant for *E. multilocularis* infection in animals are illustrated in Box 1. The list is not meant to be exhaustive and the examples are theoretical. If Member States implement a risk-based sampling approach, the non-geographical risk indicators used to define the risk groups have to be reported, along with robust evidence or appropriate justification for choosing these risk indicators.

Box 1. Examples of potential geographical and non-geographical risk indicators for *E. multilocularis* infection

Potential geographical risk indicators

1. *Density of definitive host population.* The overall size of the fox population in central Europe has increased since the early 1990s (e.g. due to the successful immunisation of foxes against rabies). In some urban habitats in south-central Europe fox population densities can be much higher than in rural habitats, due to abundant availability of anthropogenic food (EFSA, 2007).

2. *Proximity to endemic areas.* Trans-boundary movements of infected wildlife or domestic hosts can constitute an important route for the introduction of *E. multilocularis* infection in certain countries (EFSA, 2007).

[12] Regulation (EU) No 1152/2011 requires the criteria DP = 1% (95% confidence) to be fulfilled in each of the geographical epidemiological unit, regardless of the relative risk in each area

3. *Vegetation type*. Certain vegetation types will provide the habitat for large densities of suitable intermediate host species and, hence, be areas with a greater infection risk for definitive hosts. The presence of permanent grassland (meadows, pastures) favours populations of the parasite's most important intermediate hosts (common voles and water voles) and is likely to be of primary importance for transmission of *E. multilocularis* infection to definitive host animals (EFSA, 2007).

4. *Ground humidity*. Sufficient ground moisture will increase the survival period of eggs in the environment (EFSA, 2007).

Potential non-geographic risk indicators

5. *Dog category*. Such as hunting dogs, kennel dogs, stray dogs. The probability of being infected varies according to different probabilities of access to infected intermediate hosts.

6. *Age of animal*. The probability of being infected with *E. multilocularis* might differ according to age

As an example, a Member State could identify the proximity to the border as a relevant risk factor (e.g. when the neighbouring country is endemic for *E. multilocularis*). In this case, at least two epidemiologically relevant geographical units are identified and each must be defined precisely (e.g. Near: less than 5 km from the border; Far: more than 5 km from the border). Note that for each Risk Area, Regulation (EU) No 1152/2011 requires that the applied sampling strategy is able to detect a DP of 1% with a confidence of 95%.

However, within each Risk Area, one or more non-geographical risk indicators may influence the individual probability of being infected. For example, different dog categories may be present, each characterised by a different RR. This means that within each Risk Area, a risk-based sampling can be implemented. The required sample size will be smaller compared to a sample size based on simple random strategies, without affecting the sensitivity of the survey (see Section 4.3 and Figure 1).

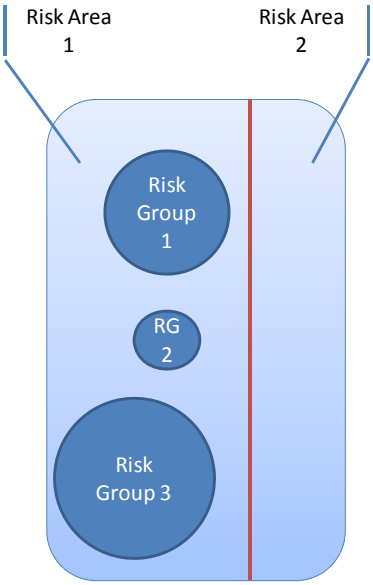

Figure 1: Schematic representation of a hypothetical scenario within a country. Two different epidemiologically relevant geographical areas are identified, based on a geographical risk factor (e.g. proximity to the borders of an endemic area/country). The RR for the 2 Risk Areas is not relevant as the target must be the same for both (DP=1%, Se=95%). Within Risk Area 1, three Risk Groups are identified (e.g. domestic dogs, stray dogs, kennel dogs) each

characterised by a different Relative Risk of being infected (e.g. 1, 3, 2 respectively). Once the proportion of the population allocated in each Risk Group is estimated, a risk based sampling can be implemented (see Section 4.3)

In addition to the information listed in Section 5.1.1, the following information should be provided in the surveillance reports for each risk area when the risk-based approach is applied:

- **Risk indicators**: all relevant risk indicators that influence the probability of an individual of the target population to be infected should be described. The information should be based on the actual situation in the areas where the survey will be implemented. As it is likely to contain continuous variables (e.g. population densities), it is suggested to categorise those variables using ranges. An example of some ranges is given in Table 1.

- **Relative Risk (RR)**: to each risk factor a Relative Risk should be associated. The evidence used to estimate the relative risk of a given risk factor should be documented as well.

Table 1: Example on how to categorise the relevant quantitative information on the risk indicators identified as playing a role in *E. multilocularis* infections: risk factor categories and related relative risk classes (the values used in the table are purely for illustrative purposes).

Risk Indicators			
	Levels	**Range**	**RR**
Population Density	**HD**	**> 500**	**3**
	MD	**300-500**	**2**
	LD	**< 300**	**1**
Proximity to endemic area	**Near**	**< 3 Km**	**3**
	Far	**> 3 Km**	**1**

- **Population Fraction**: an estimation of the proportion of the population allocated in each category of each risk factor is required. This is essential to balance the sample size calculation with the actual size of the population in a given risk area. Within a Risk Factor the sum of the population fractions must be 1 (100%)

Table 2: Example of categorising the relevant quantitative information on the risk indicators identified as playing a role in *E. multilocularis* infections: population fractions (the values used in the table are purely for illustrative purposes).

Population Proportion			
	Levels	**Range**	**popfr**
Population Density	**HD**	**> 500**	**0.1**
	MD	**300-500**	**0.2**
	LD	**< 300**	**0.7**
Proximity to endemic	**N**	**< 3 Km**	**0.8**
	F	**> 3 Km**	**0.2**

- **Implementation criteria**: once the adjusted risk for each risk area is estimated (see Section 4.3), it should be specified if the survey is implemented considering only the highest risk area (highest-risk criterion) or if more than one risk area (convenience criterion) will be considered. In this case the adjusted risk values are used to estimate the overall System Sensitivity. The choice should be justified.

In addition to the interpretation given to the term "confidence" used in the Regulation (see section 4.3.), an estimation of the Probability of Freedom can be included. In this case, some additional information should be reported:

- **Prior Probability of Freedom ($P_{free\ t}$)**: this is the negative predictive value calculated based on previous surveillance activities. In case no survey was implemented previously or no estimation is possible, this parameter can be assumed to be 0.5 (50%).

- **Probability of introduction (P_{intro})**: this probability is used to account for the decreased value of historical data. The calculation of the $P_{free\ t+1}$ should be always corrected by the P_{intro}. A full stepwise description of the methodology used to estimate this parameter should be given together with the relevant data used in the calculation.

5.2. Part II: Set of relevant data for the reports

It is suggested that in the second part of the surveillance reports the Member States provide the actual data collected during the surveillance period.

The OIE (2012) emphasises that "the success of a surveillance system is dependent on a reliable process for data collection and management. The process may be based on paper records or computerised. […] the consistency and quality of data collection and event reporting in a format that facilitates analysis is critical". According to the OIE, factors influencing the quality of collected data include the ability of the data processing system to detect missing, inconsistent or inaccurate data, and to address these problems; the maintenance of disaggregated data rather than the compilation of summary data; and the minimisation of transcription errors during data processing and communication.

In line with these OIE requirements and to facilitate and harmonise the reporting process, it is suggested that, to report their surveillance data, the Member States use the EFSA Data Collection Framework (DCF[13]), which is a web interface accessible by most of the common web browsers through which data providers can submit their files.

The system relies on controlled terminologies (e.g. ISO countries; NUTS geographical regions; methods for analytical measurement; EUROSTAT sampling strategy definitions; or parameter lists) which ensure that the data can be sorted, filtered and aggregated. By providing automatic feedback on errors in structure and content and confirmation of successful submissions, the system allows users to check the validation status of transmissions. Data providers are able to view error messages for a transmission when a validation failure occurs and can amend dataset and resubmit the data replacing the entire transmission. Data is available for analysis with SAS and ArcGIS.

The use of DCF would help to harmonise the reporting process as it ensures that all fundamental data are provided in a comprehensive and standardised way.

The data that should be reported for each individual definitive host sampled are illustrated in Table 3. More detailed information on the database structure and the related data catalogues can be found in a related EFSA report (EFSA, 2012, in preparation).

Table 3: Data to report for each individual definitive host sampled

#	Data to report	Description	Mandatory field (Y/N[14])

[13] http://www.efsa.europa.eu/en/datexsubmitdata/datexdatacollframework.htm
[14] "N" includes "not applicable".

#	Data to report	Description	Mandatory field (Y/N[14])
1.	Definitive host sampled	Either wild (red fox or raccoon dog) or domestic host (i.e. domestic dog - stray dog/hunting dog/shelter dog; or cat – stray cat/ sanctuary cat/ cattery cat)	Y
2.	Sampling unit	Individual intestinal content/ faeces post-mortem/ individual faecal sample from environment	Y
3.	Method for faeces species identification	In case faeces are collected from the environment: Morphological methods/ molecular methods (provide reference)	Y
4.	Sampling area	Area where the sample was taken (NUTS 3 level)	Y
5.	Sampling time	Month and year (if possible, also day)	Y
6.	Sources of specimens from wildlife and domestic hosts	The possible sources of specimens for EM can be (from OIE 2011 and EUROSTAT Doc ESTAT/F5/155): 1. Hunting 2. Road-kills 3. Wildlife biologists and wildlife agency field personnel, farmers, and other landholders, naturalists and conservationists 4. Veterinary activities (e.g. sanitary inspection of hunted animals) 5. Support activities to agriculture 6. Game handling establishment 7. Other activities (e.g. surveys on Rabies) 8. "Catch" of stray dogs	Y
7.	Diagnostic technique	Sedimentation and Counting Technique (SCT), Polymerase Chain Reaction (PCR) or technique of equivalent Se and Sp	Y
8.	Sensitivity	Sensitivity of the diagnostic test (%)	Y
9.	Specificity	Specificity of the diagnostic test (%)	Y
10.	Method validation	Validation process and type of validation (i.e. ISO/IEC17025; other third party quality assessment procedure; internally validated; or according to OIE guidelines)	N
11.	Laboratory accreditation	Accredited; third party assessment; none	N
12.	Results	+/- (based on the case definition)	Y

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APPENDICES

A. REPORT PART I CHECK LIST

For each element all decisions and assumptions should be clearly justified and documented together with the related uncertainties.

1. Type of survey

a. Simple random sample (representative sample) – Go to section 2
b. Risk-based approach – Go to section 3

Justification:

2. Simple Random Sample

a. Design Prevalence (DP)
b. Test Sensitivity (average or probabilistic distribution) (TSe)
c. Target population definition
d. Target Population size (N)
e. Methodology for sample size calculation:
    i. Direct calculation of the sample size (not recommended)
        1. Binomial
        2. Hypergeometric
        3. Other (please specify)
    ii. Calculation via simulation and calculation of the System Sensitivity
        1. Binomial
        2. Hypergeometric
        3. Other (please specify)

3. Risk Based Sampling

a. Design Prevalence (DP)
b. Test Sensitivity (average or probabilistic distribution) (TSe)
c. Target population definition
d. Target Population size (N)
e. Methodology for sample size calculation:
    i. Direct calculation of the sample size (not recommended)
        1. Binomial
        2. Hypergeometric
        3. Other (please specify)
    ii. Calculation via simulation and calculation of the System Sensitivity
        1. Binomial
        2. Hypergeometric
        3. Other (please specify)
* Risk Indicators and categorisation
* Relative Risk
* Population Fraction
f. Implementation criteria:
    i. Highest-risk criterion
    ii. Convenience criterion

B. ANALYTICAL METHODS SUITABLE FOR *E. MULTILOCULARIS* DETECTION AND THEIR SENSITIVITY, SPECIFICITY AND APPLICATION

Analytical method	Sensitivity	Specificity	Application (sample materials)	Application result	Reference
Sedimentation and Counting Technique (SCT)*	99%	100%	Post mortem diagnosis, necropsy Intestines	Individuals Population level National prevalence	Eckert J, Deplazes P, Craig PS, Gemmel MA, Gottstein B, Heath D, Jenkins DJ, Kamiya M, Lightowlers M, 2001a.
Modified Sedimentation and Counting Technique (mSCT)	98%	100%	Post mortem diagnosis, necropsy Intestines	Individuals Population level National prevalence	Umhang G, Woronoff-Rhen N, Combes B, Boué F, 2011.
Intestinal Scrapping Technique (IST)	78%	100%	Post mortem diagnosis, necropsy Intestines	Individuals Population level National prevalence	Hofer S, Gloor S, Muller U, Mathis A, Hegglin D, Deplazes P, 2000.
Shaking in a Vessel Technique (SVT)	96%	100%	Post mortem diagnosis, necropsy Intestines	Individuals Population level National prevalence	Duscher G, Prosl H, Joachim A, 2005.
Coproantigen ELISA (Deplazes)	80%	95-99%	In vivo and post mortem diagnosis Faecal samples	Population level National prevalence	Deplazes P, Alther P, Tanner I, Thompson RCA, Eckert J, 1999.
PCR Target : RNAsn U1 Monnier	82%	100%	Faecal samples Liver and organ cysts	Individuals Population level National prevalence	Monnier P, Cliquet F, Aubert M, Bretagne S, 1996.
Microscopy/PCR Target : RNAsn U1 Mathis	94%	100%	Faecal samples Liver and organ cysts	Individuals Population level National prevalence	Mathis A, Deplazes P, Eckert J, 1996.
PCR H15-H17 Target: 12S rRNA Dinkel	89%	100%	Faecal samples Liver and organ cysts	Individuals Population level National prevalence	Dinkel A, Nickisch-Rosenegk MV, Bilger B, Merli M, Lucius R, Romig T, 1998.
PCR Target : 12S rRNA Van der Giessen	ND	100%	Faecal samples Liver and organ cysts	Individuals Population level National prevalence	Van Der Giessen JW, Rombout YB, Franchimont JH, Limper LP, Homan WL, 1999.
PCR Cest1-Cest2 Target : NAD1 Trachsel	ND	100%	Faecal samples Liver and organ cysts	Individuals Population level National prevalence	Trachsel D, Deplazes P, Mathis A, 2007.

* Used as gold standard for the other tests, whose sensitivity and specificity have been expressed relative to the sensitivity and specificity of the SCT.
Note: Sensitivity figures are taken from the publications. The test panels used to obtain these figures are heterogeneous, thus the figures given for different tests are not directly comparable. (adapted from Boué et al., 2010)