SCIENTIFIC OPINION

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Echinococcus multilocularis infection in animals

Panel on Animal Health and Welfare

Abstract

The European Food Safety Authority (EFSA) was required to support the European Commission in preparing the review of Regulation (EU) No 1152/2011. In Europe, red fox (*Vulpes vulpes*) is the main definitive host of the *Echinococcus multilocularis* (EM) lifecycle. There is no evidence that any other carnivore species can maintain the lifecycle in the absence of red fox, and this makes it to most relevant target species for surveillance. Movement of infected definitive hosts is an important introduction pathway. The knowledge on the geographical distribution of the environmental factors for the persistence of the lifecycle is scarce. In areas where no suitable autochthonous wild canid hosts and no highly suitable intermediate hosts are present, e.g. Malta, establishment of the EM cycle is considered close to impossible. Such countries do not need to carry out surveillance on domestic dogs to substantiate absence of EM in the relevant animal population. Reconsideration of some aspects of the current legislation regarding surveillance activities might be relevant; for example the identification of epidemiologically relevant units should be independent from political borders. Studies to improve the knowledge on epidemiological risk factors should be encouraged to enable risk-based sampling. *Echinococcus* notification should always be done at species level in order to discriminate between the more severe alveolar echinococcosis and the cystic echinococcosis. Praziquantel is the substance of choice for the treatment of dogs. However, the treatment window should be reconsidered to reduce the risk of re-infection: a general rule is to treat as close as possible to entry into a non-infected country. There is a lack of standardization of the diagnostic methods between laboratories. The diagnostic sensitivity of the tests should be established in accordance to the World Organisation for Animal Health (OIE) standards for validation. For the time being, the diagnostic sensitivity can be set conservatively to 78%.

Keywords: *Echinococcus multilocularis*, echinococcosis, hosts, risk factors, diagnosis, surveillance, treatment

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Summary

Human alveolar echinococcosis (AE) is caused by the larval stage of the fox tapeworm *Echinococcus multilocularis*. It is amongst one of the most dangerous zoonoses. Naturally the parasite transmits between foxes or dogs and small mammals whilst humans are aberrant intermediate hosts. In rodents, the larval mass proliferates rapidly by exogenous budding of germinative tissue and produces an alveolar-like pattern of microvesicles filled with protoscolices. In humans, the larval mass resembles a malignancy in appearance and behavior, because it proliferates indefinitely by exogenous budding and invades the surrounding tissues (Moro et al., 2008). Transmission of AE to humans is by consumption of parasite eggs which are excreted in the faeces of foxes and dogs. Human infection can be through direct contact with the definitive host or indirectly through contamination of food or possibly water with parasite eggs (Torgerson et al., 2010).

The Commission adopted Commission Delegated Regulation (EU) No 1152/2011 of 14 July 2011 supplementing Regulation (EC) No 998/2003 of the European Parliament and of the Council as regards preventive health measures for the control of *Echinococcus multilocularis* (EM) infection in dogs. This was to ensure continuous protection of Finland, Ireland, Malta and the United Kingdom which claim to have remained free of EM as a result of applying national rules until 31 December 2011. The Regulation includes certain obligations for the Member States (MSs) listed under Regulation (EU) No 1152/2011 to implement a pathogen-specific surveillance programme aimed at detecting the parasite, if present in any part of those MSs, in accordance with certain requirements regarding sampling, detection techniques and reporting. On 31 May 2015, Finland, Ireland, Malta and the United Kingdom submitted documentation supporting the evidence for the absence of EM for three consecutive surveillance periods in accordance with Regulation (EU) No 1152/2011, and EFSA issued three scientific assessments of the submitted reports analysing the sampling strategy, the data collected and the detection methods used in the surveillance programmes in view of verifying compliance with the requirements laid down in Regulation (EU) No 1152/2011. The Regulation also provides that the Commission should review it no later than 5 years following the date of its entry into force, *i.e.* by December 2016, in the light of scientific developments regarding EM infection in animals. The review shall in particular assess the proportionality and the scientific justification of the preventive health measures. In order to meet the aforementioned deadline, updated scientific evidence from EFSA is required to support the Commission in preparing the review of Regulation (EU) No 1152/2011.

In view of the above, and in accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asked EFSA: to describe EM infection in animals in the European Union (EU) and adjacent countries; to assess the current situation in the EU and adjacent countries regarding the monitoring and surveillance programmes of EM infection in definitive and intermediate hosts, the probability of detection if EM is introduced into areas where it has never been recorded and the programmes for the eradication of EM in wildlife host species; to describe the current situation in the European Union and adjacent countries regarding the risk factors associated with human alveolar echinococcosis (AE) and the impact of EM infection in animals on public health; to describe the efficacy of available EM drugs and the effectiveness of the current species-specific treatment protocols to protect domestic species against the parasite and to assess the laboratory techniques for the detection of EM in live and dead animals, in terms of sensitivity, specificity, predictive values and practicability (i.e. rapidity, large scale use, ease of use).

With the objective of improving the readability of this scientific opinion, the adjective 'free' will be used hereinafter to indicate an area or a country 'where, at present, no findings of the parasite have been recorded and / or reported'. Similarly, the term 'freedom' was used to indicate 'absence of infection'.

Until the 1990s, only a 'core' area consisting of Eastern France, Southern Germany and parts of Switzerland and Austria were known to be endemic. Since 1980, EM-infections in animals have been recorded in 17 countries, in Central-Eastern Europe, previously thought to be free. The observed prevalence of EM-infected animals as well as the abundance of host species increased in the Baltic areas, Denmark, Netherlands, Poland, Romania, Slovakia and Slovenia during the 1990s and have continued to increase since. The distribution of EM is not homogeneous, and there are areas of high and low prevalence of EM infection, with values ranging from close to 0% (e.g. Denmark, northeast Germany, Sweden), to values close to 50% (e.g. part of France, southern Germany, part of

Switzerland). These differences in prevalence levels in foxes, among the countries where EM has been reported, have been linked most frequently to the use and structure of landscape, which influences the species range and abundance of rodents as intermediate hosts and to the microclimatic conditions necessary for the transmission and establishment of the parasite. Accessible data indicate that, within the Russian Federation, EM-infection in animals and human Alveolar Echinoccosis (AE) are frequent in parts of Siberia and the Russian Far East. No scientific literature is available to conclude on the EM prevalence in the areas of the Russian Federation adjacent to the Eastern border of neighbouring EU MS.

Due to its high population densities, high susceptibility to EM infection, high worm burden in infected animals, and higher infection prevalence compared to other potential definitive hosts, the red fox (*Vulpes vulpes*) is considered to be the main definitive host in temperate parts of Europe, Asia and (probably) North America. The prevalence of infected animals in raccoon dog populations in Eastern Europe and in Eastern Germany has been shown to reach levels similar to those observed in red foxes. Where they occur, raccoon dog (*Nyctereutes procyonoides*), golden jackal (*Canis aureus*) and grey wolf (*Canis lupus*) can act as definitive hosts, but there is no evidence that they can maintain the lifecycle in the absence of red foxes.

Regarding domestic carnivores, there is no evidence that dogs and cats can maintain the lifecycle in the absence of red foxes. The prevalence of EM in the general dog population is very low. No systematic assessment has been done anywhere on the quantitative contribution of dogs to the infection of intermediate host populations. Living and working with dogs might be or become relevant as potential risk factors for AE in Europe. Cats show low susceptibility to experimental infection. However, natural infection of cats has been recorded in several countries. Nevertheless, current knowledge suggests that the contribution of cats to the EM lifecycle is low. There are currently no evidences to support the inclusion of cats in the scope of the legislation.

In the EU, various vole species of the genera *Microtus*, *Arvicola*, *Myodes* and *Lemmus* are confirmed as suitable intermediate hosts based on field studies and/or experimental infections. The common vole, *Microtus arvalis*, is the most important intermediate host in areas such as Northeast France (Ardennes) and Switzerland, while water voles *Arvicola* spp. may maintain transmission in Hungary and urban areas of Central Europe. Muskrats (*Ondatra zibethicus*), nutria/coypu (*Myocastor coypus*) and beaver (*Castor fiber*) are suitable intermediate hosts, but are likely to be infrequent prey for foxes due to their large size and habitat specificity. In conclusion, the relative importance of different rodents and other small mammal species for maintenance of the lifecycle differs according to geographical areas, the type of environment, prevalence of infection and other parameters. This extreme variability does not make any of those potential IH particularly suitable for surveillance purposes.

Concerning the probability of introduction, transmission and establishment it can be concluded that movement of definitive hosts with a pre-patent or patent infection (*i.e.* infected domestic and wildlife species involved in the *E. multilocularis* lifecycle) is an important introduction pathway. In principle, EM can be introduced also by infected intermediate hosts that carry fertile larval stages (metacestodes) or infectious parasitic stages, or by other items, e.g. plants, contaminated with eggs into free areas. It is difficult to distinguish introduction of EM from its first detection (i.e. established, but not detected) if no adequate surveillance had been in place in areas deemed to be free.

Based on the model results, which do not represent any particular country as complete data are not available at present, the following general conclusions can be drawn: (i) the presence of the border compliance checks increases the number of dogs that need to pass the border by 1.75 to 4 times; (ii) if no border compliance check is in place for a country adjacent to an endemic area (prevalence in foxes equal to 16%) introduction would require 75 to 1200 times more dogs than foxes crossing the border. If border compliance checks are in place, the proportion increases (150 to 2550 times more dogs than foxes crossing the border); (iii) for a free country adjacent to an area with a very low prevalence in foxes (0.001%) the crossborder movement of dogs has a prominent role (1.16 to 2.31 fewer dogs coming from an endemic area relative to migrating foxes); (iv) the degree of non-compliance to treatment among dogs that are not checked at the border (because no border checks are in place or because the border checks have been evaded) plays a less important role on the probability of introduction of EM, compared to other parameters.; (v) despite the implementation of

appropriate mitigation measures, it is inevitable that infected dogs enter free countries. However, other factors in addition to introduction are important in establishing EM in free countries.

In fact, for the transmission and establishment of the lifecycle, appropriate definitive and intermediate hosts must be present. Environmental factors influence the persistence of the lifecycle; therefore the probability of EM becoming established will vary from one area to another. However, the knowledge on the potential role of environmental factors for the persistence of the life cycle is scarce. Studies to improve the knowledge on the probability of transmission and establishment of new EM introduction in free countries should be encouraged.

In areas where no suitable autochthonous wild canid hosts and no highly suitable intermediate hosts are present, e.g. Malta, establishment of the EM cycle is considered close to impossible. Such countries do not need to carry out surveillance on domestic dogs to substantiate EM-freedom. The option of making the treatment non compulsory anymore for dogs entering such country is a public health issue and relates to the risk of humans getting infected by the parasite by means of contaminated dog faeces.

Only the countries listed under Commission Delegated Regulation (EU) No 1152/2011 are obliged to implement surveillance activities. The legislation lists, among the obligations for those MS, the need of having in place 'an early detection system for Echinococcus multilocularis infection in host animals'. In order to allow early detection of EM infection, a very low design prevalence of e.g. 0.1% is required, as it may take many years for EM to reach a prevalence of 1% in the population. However, such a low design prevalence may make surveys for early detection impracticable due to the large sample size required. In addition, the following critical aspects have been identified: (i) the diagnostic sensitivity of the tests used in these EM surveillance programmes is not supported by robust scientific evidence and the tests are not validated according to World Organisation for Animal Health (OIE); (ii) obtaining a representative sample from host populations is hampered by the impossibility of implementing a representative random sample in wildlife and the scarcity of knowledge on the distribution of red fox populations at regional level. Reconsideration of some aspects of the current legislation to optimise the surveillance activities might be relevant. In detail: (i) the identification of epidemiologically relevant units should be independent from the political borders; (ii) for the purpose of demonstrating absence of infection, the inclusion of the concept of the Bayesian Probability of Freedom in the regulation, may allow a reduction of the sample size. Finally, studies to improve the knowledge on epidemiological risk factors, including geographical risk factors, should be encouraged to enable well-founded risk-based sampling in geographical subpopulations of hosts to improve the detection.

Aside from the technical aspects of the implementation of the surveillance systems, it has to be highlighted that the detection of the parasite is currently not notifiable in non-free MS. Occurrence may be reported at genus or species level. However, *E. multilocularis* and *E. granulosus*, although they belong to the same genus, have different lifecycles and cause completely different pathologies in humans. *Echinococcus* notifications should always be done at species level to enable an understanding of the actual trend and geographical distribution of these infections. In fact, considering the spatial and temporal heterogeneity in the EM distribution within a country, the results of local or regional surveys cannot be extrapolated to a whole country.

Eradication of EM in the European wildlife could theoretically be achieved by means of baites, at least in small areas, where foxes are present, but the intervention needs to be perpetuated to maintain the status. In large areas, long term control - but not elimination - of the parasite may be possible by baiting. Control by baiting campains requires more knowledge about how and where to control the parasite in a cost-efficient way. Increased fox hunting/trapping is not considered to be effective in controlling the parasite.

Concerning human AE, dog ownership, cat ownership, living in a rural area, having a kitchen garden, occupation (farming), haymaking in meadows not adjacent to water, going to forests for vocational reasons, chewing grass and handling foxes were identified as potential risk factors in Europe. Particular Human leukocyte antigen (HLA) types have been found to be protective against AE. However, the presumably very long incubation period of the human AE makes the study of risk factors extremely difficult which makes the uncertainty on the risk factors considerably high. In addition, the true number of cases of AE in Europe is not known mainly because of under-reporting. There has been an increase in the number of reported AE cases in new areas, such as Lithuania and Latvia, and an increase in the incidence of human AE in endemic countries, such as Austria, France, Poland and

Switzerland, which suggests a geographic spread and an increase of this disease/infection in Europe. If early detection does not become more effective, the European health system might face costs in the order of billions of euros to care for the number of AE patients expected in the next two decades. The public health risk associated with human cases of AE is the reason behind the EU regulation to control and monitor EM in animal species, it is therefore essential that notification of human AE and cystic echinococcosis (CE) cases be made mandatory in all MSs to enable effective and coherent monitoring of trends of AE and CE occurrence in humans. A re-evaluation of the case definition for 'echinococcosis' in the current EU decision 2012/506/EU, differentiating alveolar from cystic echinococcosis, will be crucial to collect real epidemiological and clinical data to manage and trace back these infections.

Regarding the available drugs for EM, due to its favorable pharmacokinetic properties and activity against both immature and mature stages, praziquantel is the substance of choice for the treatment of EM in definitive hosts, including travelling or imported dogs. In addition to treatment efficacy, the timing of treatment is crucial. Results of model simulations indicate that: (i) the risk of introduction / transmission / establishment, expressed as a function of the number of eggs deposited in a free area, can be reduced by treating dogs before or after entering the free area where no findings of the parasite have been recorded; (ii) treating dogs earlier than 24 hours, before entering a free area, allows the risk of reinfection before moving. Therefore, the risk of introduction / transmission / establishment is the lowest when treating one day prior to crossing the border; (iii) the shorter the visit of a dog living in an endemic area to a free area, the more effective it is to treat the dog before it enters this area compared to treating the dog after it has entered; (iv) the shorter the visit of a dog living in a free area to an endemic area, the greater is the advantage of delaying treatment until the dog has returned to the free area. Reconsideration of the definition of the optimal treatment window (presently up to 120 hours before entry), when moving dogs from infected to non-infected countries, might be worthwhile to reduce the risk of reinfection. A general rule is to treat as close as possible to entry into a free country.

Different methodologies are available for the detection of EM. The SCT is a post mortem approach at necropsy considered as the reference standard for the detection of EM. The lower limit of detection of the SCT is high. Usually the worm burden is low and this results in a diagnostic sensitivity of less than 100%, particularly if used in a period close to the introduction. Furthermore, the SCT is a time consuming approach. DNA-based methodologies for the detection of EM genetic material in faeces or intestinal contents may have a diagnostic sensitivity comparable to SCT. Intrinsic limitations of DNA-based methodologies such as inhibitors, costs, small volume of sample to analyse, timing and sensitivity were recently overcome. However, lack of standardization of diagnostic methods detecting EM probably causes variation in sensitivity and specificity between labs. In addition, studies on the diagnostic tests for detection of EM in animals are very heterogenic, which complicates drawing any consistent conclusions from them. A study should be undertaken to estimate the probability of each relevant test to detect infection, given that the animal is truly infected (according to the definition of test sensitivity), using an adequate sample of specimens from endemic areas where the entire range of different infection stages and intensities are represented. Such a study should follow the OIE Terrestrial Manual, Chapter 1.1.5 (OIE, 2013), and could be coordinated by the EURL for Parasites. Until better documentation is available, 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, the suggested value to be used for future surveys is 78%.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

The Commission adopted Commission Delegated Regulation (EU) No 1152/2011 of 14 July 2011 supplementing Regulation (EC) No 998/2003 of the European Parliament and of the Council as regards preventive health measures for the control of *Echinococcus multilocularis* infection in dogs.[1]

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.

The 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 date of its entry into force, i.e. by December 2016, in the light of scientific developments regarding EM infection in animals and to submit the results of the review to the European Parliament and to the Council. The review shall in particular assess the proportionality and the scientific justification of the preventive health measures.

By 31 May 2015, Finland, Ireland, Malta and the United Kingdom will have submitted documentation supporting the evidence of the absence of EM for three consecutive surveillance periods in accordance with Regulation (EU) No 1152/2011.

By the same date and in the context of the scientific and technical assistance conferred by the Commission to EFSA in May 2012, EFSA will have issued three scientific assessments of the submitted reports analysing the sampling strategy, the data collected and the detection methods used in the surveillance programmes in view of verifying compliance with the requirements laid down in Regulation (EU) No 1152/2011.

In order to meet the aforementioned deadline, updated scientific evidence from EFSA is required in order to support the Commission in preparing the review of Regulation (EU) No 1152/2011.

1.1.1. Terms of Reference

In view of the above, and in accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asks EFSA:

1. To describe *Echinococcus multilocularis* infection in animals in the European Union and adjacent countries and in particular:
    a) the geographical distribution and prevalence of *Echinococcus multilocularis* infection in the main infected domestic and wildlife species involved in the *Echinococcus multilocularis* lifecycle;
    b) the importance and role of the different host species in the life cycle of the parasite;
    c) the risk factors for and the probability of introduction and establishment of *Echinococcus multilocularis* in areas where it has never been recorded, through the movement of infected domestic and wildlife species involved in the *Echinococcus multilocularis* lifecycle;
2. To assess the current situation in the European Union and adjacent countries regarding:
    a) the monitoring and surveillance programmes of *Echinococcus multilocularis* infection in definitive and intermediate hosts, and the probability of detection if *Echinococcus multilocularis* is introduced into areas where it is has never been recorded;

[1] OJ L 296, 15.11.2011, p. 6.
[2] EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2015. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2013. EFSA Journal 2015;13(1):3991, 162 pp. doi:10.2903/j.efsa.2015.3991
[3] The views and opinions of the authors expressed herein do not necessarily state or reflect those of the ECDC. The accuracy of

b) the programmes for the eradication of *Echinococcus multilocularis* in wildlife host species;

3. To describe the current situation in the European Union and adjacent countries regarding:
   a) the risk factors associated with human alveolar echinococcosis;
   b) the impact of *Echinococcus multilocularis* infection in animals on public health;
4. To describe the efficacy of available *Echinococcus multilocularis* drugs and the effectiveness of the current species-specific treatment protocols to protect domestic species against the parasite;
5. To assess the laboratory techniques for the detection of Echinococcus multilocularis in live and dead animals, in terms of sensitivity, specificity, predictive values and practicability (i.e. rapidity, large scale use, ease of use).

1.2. Interpretation of the Terms of Reference

This scientific opinion describes several aspects related to EM infections in animals, in particular in Member States (MSs) and in countries sharing a land border with MSs when information is available.

In order to improve the readability of the present opinion, the term 'free' was used to indicate areas 'where *Echinococcus multilocularis* has never been found and/or detected'. Similarly, the term 'freedom' was used to indicate 'absence of infection'. By no means, for the time being, the absence of positive samples provided by the countries listed under Commission Delegated Regulation (EU) No 1152/2011 can be interpreted as actual complete absence of the parasite.

The current epidemiological situation of EM in Europe is presented, in particular for red foxes since there is a lack of data for the other definitive hosts (DH) and intermediate hosts (IH) species (TOR1a).

The role of different DH and IH in the life cycle of EM is described to identify the most important species to target for monitoring, surveillance and eradication programmes (TOR1b).

The current knowledge on risk factors for EM introduction, transmission and establishment in free areas is described. In addition, a conceptual framework is provided to compare by means of realistic scenarios the probability of EM introduction, transmission and establishment via movement of domestic animals and foxes (TOR1c).

The monitoring and surveillance programmes on EM in the European Union and adjacent countries are described and the difference between assessing absence of infection and early detection is explained (TOR2a).

A description of programmes for the control and eradication of EM is only provided for foxes, since data on other species are scarce (TOR2b).

A description of reported human alveolar echinococcosis (AE) cases in Europe is provided, although the true numbers are not known. The association of risk factors reported in the scientific literature is assessed, differentiating those that are relevant, globally or in a particular area (TOR3a).

An overview of published data on the burden of AE, the economic cost in humans and the economic cost in animals is included to give an impression of the impact of EM infections on public health (TOR3b). A more detailed economic analysis was considered beyond the scope of this mandate.

Treatment protocols are mainly determined by the selection of the anthelmintic drug and the timing of its administration. Praziquantel is currently the most-used and most effective anthelmintic for the treatment of EM infections. The efficacy of praziquantel in dogs is described based on available literature and the relative effect of treatment protocols to prevent EM transmission and establishment are modelled for dogs from endemic areas visiting free areas (and vice versa). In addition, some scenarios are presented to quantify how much the degree of treatment compliance has to increase to compensate for the increased probability of EM introduction when expanding the treatment window from 1 day to 5 days before a dog enters a free area (TOR4).

The most frequently used laboratory techniques for the detection of EM are reviewed and guidance is provided to substantiate test sensitivity estimates (TOR5). Predictive values of the tests are not evaluated, since these are dependent on prevalence, which varies considerably and often is unknown.

2. Data and Methodologies

2.1. Data

Eight systematic literature reviews on questions related to the TORs of this mandate were available to the working group (Casulli et al., 2015). The European Summary Report (Efsa and ECDC, 2013)[2] and the data provided by the European Centre for Disease Prevention and Control (ECDC) extracted from The European Surveillance System (TESSy)[3] were consulted to obtain information on the EM cases in animals and alveolar echinococcosis (AE) cases in humans reported until 2013. In addition, EFSA's Scientific Network for Risk Assessment in Animal Health and Welfare has been contacted requesting submission of non-published prevalence data from the period 2012–2014, data on local host populations, the notification status of EM cases in animals, the number of human AE cases since 2000, surveillance activities and data on dog movements (see Appendix E).

2.2. Methodologies

A descriptive summary of the available scientific evidence and uncertainties is provided for all TORs. This is based on the systematic literature reviews carried out under the EFSA Grant Project GP/EFSA/AHAW/2012/01 (*Echinococcus multilocularis* infection in animals; Casulli et al., 2015) and on review of additional scientific papers (mainly published after the systematic review) and the data sources mentioned in section 2.1.

A conceptual scenario-tree model has been generated to estimate the probabilities of EM introduction, transmission and establishment via movement of domestic animals and foxes (TOR1c, see Appendix B). Some examples regarding absence of infection assessment have been produced using a Bayesian approach (TOR2a) (Appendix D). A deterministic mathematical model has been used to calculate the average number of eggs excreted in a country where no findings of the parasite have been recorded by a dog exposed in an endemic area and taken into a country where no findings of the parasite have been recorded (TOR4, see Appendix F). This model has been used to analyze different treatment protocols and changing the treatment timing and considering different types of movements of domestic dogs.

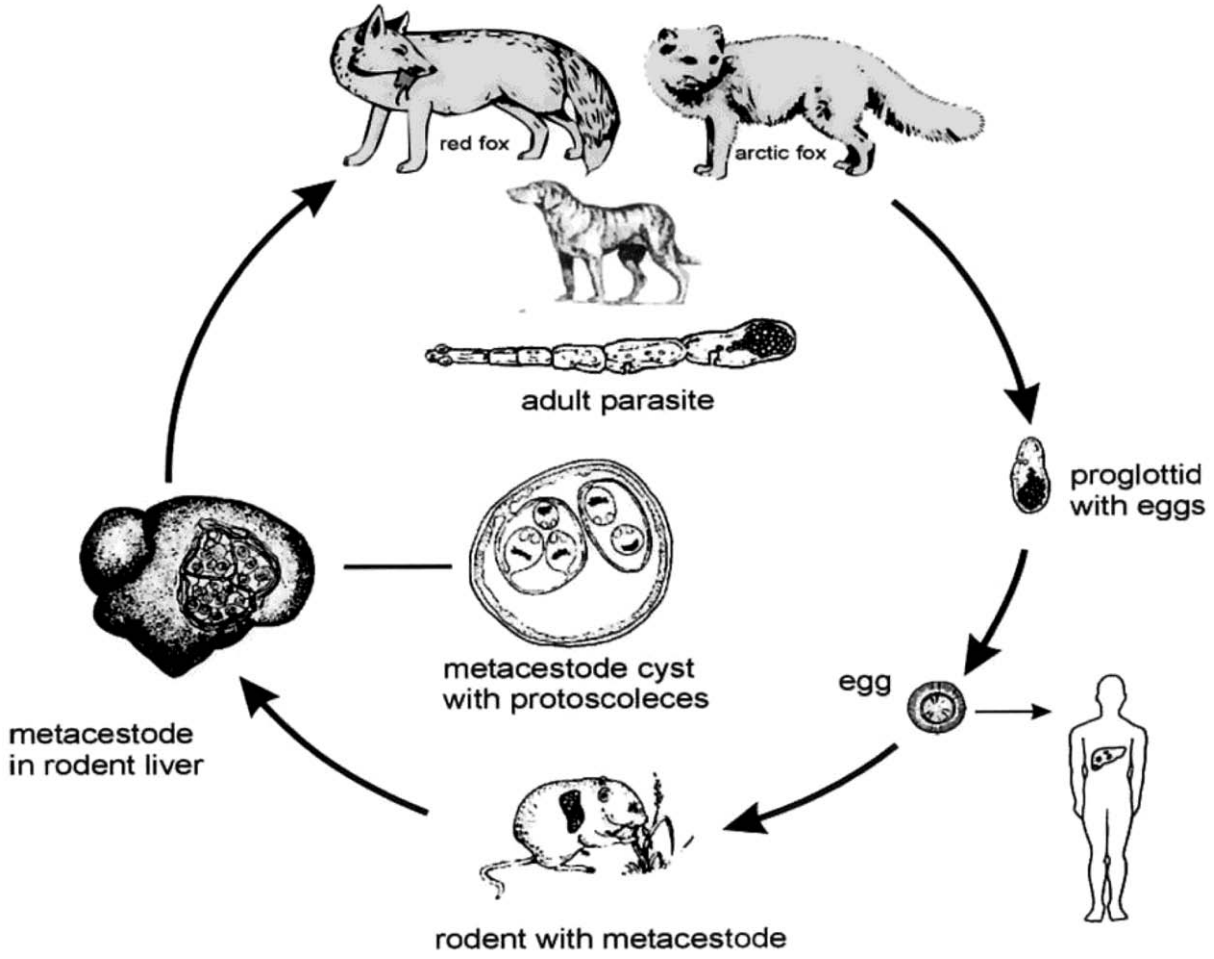
3. Assessment

3.1. Importance and role of the different host species in the life cycle of the parasite (linked to TOR1b)

Human alveolar echinococcosis (AE) is caused by the larval stage of the fox tapeworm *Echinococcus multilocularis*. It is amongst the world's most dangerous zoonoses. Naturally the parasite transmits between foxes or dogs and small mammals whilst humans are aberrant intermediate hosts (Figure 1). In rodents, the larval mass proliferates rapidly by exogenous budding of germinative tissue and produces an alveolar-like pattern of microvesicles filled with protoscolices. In humans, the larval mass resembles a malignancy in appearance and behavior, because it proliferates indefinitely by exogenous budding and invades the surrounding tissues (Moro et al., 2008). Transmission of AE to humans is by consumption of parasite eggs which are excreted in the faeces of foxes and, increasingly, dogs. Human infection can be through direct contact with the definitive host or indirectly through contamination of food or possibly water with parasite eggs (Torgerson et al., 2010).

[2] EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2015. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2013. EFSA Journal 2015;13(1):3991, 162 pp. doi:10.2903/j.efsa.2015.3991

[3] The views and opinions of the authors expressed herein do not necessarily state or reflect those of the ECDC. The accuracy of the authors' statistical analysis and the findings they report are not the responsibility of ECDC. ECDC is not responsible for conclusions or opinions drawn from the data provided. ECDC is not responsible for the correctness of the data and for data management, data merging and data collation after provision of the data. ECDC shall not be held liable for improper or incorrect use of the data.



Source: Torgerson et al., 2013.

Figure 1: *Echinococcus multilocularis* life cycle (Torgerson et al., 2010)

3.1.1. Definitive hosts

Definition

Definitive hosts are animals, which contain the adult (strobilar) stage of the cestode in the small intestine after being infected via ingestion of metacestodes from intermediate hosts. Eggs produced by the adult worms pass into the environment via faeces and are the infection source for intermediate hosts (including accidental hosts like humans). The presence of adult worms is transient (approximately one month of prepatency followed by one to several months of patency) and does not cause disease in the definitive hosts (Figure 1).

Wild carnivores

Red fox: The red fox is the principal DH in temperate parts of Europe, Asia and (probably) North America. The assessment of the importance of the red fox is based on its high susceptibility to infection with EM, the high worm burden detected in foxes, the (usually) high population densities, and the high prevalence of infection with EM compared with other potential DHs (Table 1 and Table 2; see also Appendix A, tables A1, A3 and A4). For these reasons, the red fox is considered the primary target species for surveillance. There are no areas in Europe where EM has been found in other DHs while absent from the sympatrical red fox population.

Red fox density is highly variable. Even within one country (UK) it can range between one fox per 40 km² and 30 foxes per km², depending on abundance of food. Social group density may vary between 0.2–5 families per km² in the suburbs and a single family per 10 km² in barren uplands. Fox density in

mountainous rural areas of Switzerland is 3/km², in northern boreal forests and Arctic tundra is 0.1/km², and in Southern Ontario, Canada is 1/km² (Macdonald and Reynolds, 2008).

In Sweden, an endemic country adjacent to a MS where no findings of the parasite have been recorded, the population density has been estimated in two areas, Grimsö (southern-middle part of Sweden, a southern boreal zone) and Revinge (most southern part of Sweden, nemoral zone). At Revinge the population density was estimated to be 0.8 foxes/km^2. In Grimsö the population density was estimated to be between 0.2 and 0.4. Between Grimsö and Revinge, in the boreo-neomoral zone which, apart from the most southern and western parts, covers most of south Sweden, the prevalence was estimated to be between 0.4 and 0.8 foxes/km^2. North of Grimsö, the population density was considered to decrease in north-western direction as the zones change from southern boreal, to middle boreal, north boreal and alpine zones (H. Wahlström, personal communication based on Schantz, 1981 and Lindström, 1982; Doctoral Thesis)

Dispersal of juvenile foxes starts at approximately 6 months of age. Distance moved is negatively correlated with population density, i.e. foxes in sparsely populated areas with low food resources are likely to disperse over longer distances. Even in the case of foxes from the same area, dispersal distances vary drastically between sexes and among individuals: most do not move far and only few disperse over long distances. In a review covering 24 studies from Europe and North America, juvenile foxes showed mean dispersal distances of 2.8–43.5 km (males) and 1.8–38.6 km (females), while maximum dispersal was 14–346 km (males) and 4–256 km (females) (Trewhella et al., 1988).

Arctic fox: In Europe, the Arctic fox is only relevant on Svalbard, where it maintains the EM lifecycle together with one vole species as intermediate host, and in parts of the tundra region of European Russia (Nemetsia), with lemmings and Arctic voles as intermediate hosts (Peklo, 2014). Elsewhere, Arctic foxes are reported as DHs in Arctic region of North America and Asia. There are no comparative data on susceptibility, but reported prevalence values can be high. (Table A1, Appendix A).

Other wild canids (raccoon dog, golden jackal, wolf): Where they occur in Europe, these species can act as DHs in conjunction with the red fox. There is no evidence that any of these species can maintain the lifecycle in the absence of red foxes. Raccoon dogs (*Nyctereutes procyonoides*) are currently only frequent in the eastern part of Europe (from eastern Germany onwards), with introductions having been reported from Austria; Belarus; Czech Republic; Estonia; Finland; France; Germany; Hungary; Kazakhstan; Latvia; Lithuania; Moldova; Netherlands; Norway; Poland; Romania; Slovakia; Sweden; Switzerland; Ukraine (IUCN, 2008). There is only one area (Brandenburg, Germany) where prevalence values in red foxes and raccoon dogs have been compared based on large sample sizes and where prevalences of the two species were at a similar level (Schwarz et al., 2011; see Table A4 and Appendix A). Given that this also applies for other areas, raccoon dogs could be considered an additional target for surveillance. However, its hibernation and defecation behaviour (in 'latrines') is considered to render the raccoon dog epidemiologically less important for transmission to voles, although it is highly susceptible under laboratory conditions. The Golden Jackal (*Canis aureus*) is widespread in North and north-east Africa, occurring from Senegal on the west coast of Africa to Egypt in the east, in a range that includes Morocco, Algeria, and Libya in the north to Nigeria, Chad and Tanzania in the south. They also occur in the Arabian Peninsula and have expanded their range into Europe, where they have a patchy distribution, being resident in the Balkans and, since recent times, in Hungary and south-western Ukraine (IUCN, 2008). There are no data on the susceptibility of golden jackals. European wolves have only been found infected in Latvia and Slovakia, but data from North America indicate that wolves can be frequent hosts and may play a more important part in the lifecycle than previously assumed (Schurer et al., 2014). There are no data on susceptibility of wolves, but it is considered to be similar to domestic dogs, which are highly susceptible (Table 1 and Table 2; see also Appendix A, Tables A1 and A3).

Table 1: Evidence regarding definitive host species for *E. multilocularis* in Europe (see Appendix A, Table A1 for references)

Host species	Type of evidence
Red fox (*Vulpes vulpes*)	Natural and experimental infection
Arctic fox (*Vulpes lagopus*)	Natural infection
Raccoon dog (*Nyctereutes procyonoides*)	Natural and experimental infection
Wolf (*Canis lupus*)	Natural infection
Golden jackal (*Canis aureus*)	Natural infection
Domestic dog (*Canis lupus familiaris*)	Natural and experimental infection
Wild cat (*Felis s. silvestris*)	Natural infection
Domestic cat (*Felis silvestrus catus*)	Natural and experimental infection

Table 2: Current *E. multilocularis* status of EU MS and adjacent countries

A: Endemic countries	Evidence for presence (recorded host species with number of references)
Austria	DH: red fox (6)
Belarus	DH: fox (2) IH: voles (1), muskrat (1), coypu (1), other rodents (1), shrews (1)
Belgium	DH: red fox (8) IH: voles (1), muskrat (2)
Bulgaria	IH: voles (2)
Czech Republic	DH: red fox (3), dog (1), cat (1) IH: voles (1)
Denmark	DH: red fox (3), cat (1)
Estonia	DH: red fox (1), raccoon dog (1)
France	DH: red fox (17), dog (1), cat (2) IH: voles (13), muskrat (2), coypu (1), other rodents (2)
Germany	DH: red fox (52), raccoon dog (3), dog (2), cat (5) IH: voles (3), muskrat (9), coypu (2)
Hungary	DH: red fox (4), golden jackal (1)
Italy	DH: red fox (5)
Latvia	DH: red fox (1), raccoon dog (1), wolf (1)
Liechtenstein	DH: red fox (1)
Lithuania	DH: red fox (2), raccoon dog (1), dog (1) IH: muskrat (1), pig (1)
Luxembourg	DH: red fox (1) IH: muskrat (1)
Netherlands	DH: red fox (6), cat (1) IH: muskrat (1)
Norway (Svalbard only)	DH: arctic fox (1) IH: voles (1)
Poland	DH: red fox (14), raccoon dog (1) IH: pig (2), wild boar (1)
Romania	DH: red fox (1) IH: voles (1)
Russia	DH: red fox (1), arctic fox (1) IH: lemming (1)
Slovakia	DH: red fox (11), raccoon dog (1), wolf (1), dog (2)
Slovenia	DH: red fox (1) IH: *Apodemus* (1)
Sweden	DH: red fox (2)
Switzerland	DH: red fox (14), dog (2), cat (1) IH: voles (10), pig (2)
Turkey	Numerous human cases, mainly from Eastern Anatolia; old unverifiable record from one fox and (probably misdiagnosed) cases in cattle
Ukraine	DH: red fox (2)

B: Free countries	Evidence for absence (number of references, examined host species)
Finland	5 (red fox, raccoon dog, voles)
Ireland	4 (red fox)
Malta	2 (dog)
Norway (mainland)	8 (red fox)
United Kingdom	5 (red fox, dog, cat)
C: Countries with uncertain endemicity status	**Reason for uncertainty**
Albania	No data
Bosnia-Herzegovina	Old, unverifiable records from cattle (probably misdiagnosed)
Croatia	Border to endemic country (one negative survey of 85 foxes)
Cyprus	Insufficient data (28 dogs tested negative)
Greece	Few unverified human cases
Iceland	No data
Macedonia (FYR)	One human case reported to European Registry (under country 'Greece')
Moldavia	Border to endemic countries; old record from *Mus musculus*
Montenegro	No data
Portugal	No data
Serbia	Border to endemic countries; one negative survey of 1000 foxes); one case of infected beaver, probably introduced
Spain	Insufficient data (1969 foxes tested negative)

Presence in humans is only listed for Turkey (strong, but almost exclusive evidence of presence) and countries of doubtful endemicity status, due to uncertainty on the origin of infection.
DH: definitive hosts, IH: intermediate hosts (see Appendix A, Table A3 for references).

Domestic carnivores

Dog, cat: Dogs are highly susceptible in experimental infection-studies, but the prevalence of EM in the general dog population is very low (e.g. 0.2% in Germany (Dyachenko et al., 2008)). This is likely due to a lower exposure to infectious intermediate hosts (e.g. by feeding on voles) and probably also the result of deworming treatments to which domestic dogs are subjectNo systematic assessment has considered the quantitative contribution of dogs to the infection of intermediate host populations or the effect of differences in infection probability between dogs from different backgrounds (pets, sheep dogs, hunting dogs, etc.). Unrestrained dogs exposed to potentially infected rodents were found to be frequently infected in Switzerland (8.1% according to Gottstein et al., 2001), and even the rarely infected group of pet dogs may contribute considerably to the contamination of the urban and suburban environment due to their large numbers compared with red foxes (Deplazes et al., 2011). There is no evidence that dogs can maintain the lifecycle in the absence of red foxes. Even though their contribution to the lifecycle is limited, human case-control studies suggest that dogs may be an important source of infection in humans (Kern et al., 2004 – see also Section 3.7.). In addition, infected dogs that are companion animals can potentially reach any free area, e.g. when travelling with their owners, and may therefore be important for parasite dispersal (Davidson et al., 2012). Cats show a low susceptibility to experimental infection, but there are numerous records of naturally infected animals from several countries. Their contribution to the lifecycle is probably small, but additional assessment is warranted. (Table A1, Appendix A)

3.1.2. Intermediate hosts

Definition

Intermediate hosts are animals containing the larval (metacestode) stage of the cestode in internal organs, usually the liver. Protoscolices forming in the metacestodes develop into adult worms once ingested by a definitive host (after preying on the intermediate host). In competent intermediate hosts, metacestodes probably continue to grpw for the lifetime of the host (similarly to a tumour), eventually leading to the host's death due to replacement of organ tissue (alveolar echinococcosis). The time needed for the metacestode to reach fertility (characterised by the presence of protoscolices) is 2 to 3 months in some competent rodent species. After 4 months and later, many thousands of protoscolices can be present in metacestodes developing from one or few established oncospheres. Some (unsuitable) host species are known to limit the metacestode growth or even kill

the metacestodes through immune processes, preventing the development of protoscolices in the metacestode, and therefore can not transmit the parasite to a definitive host. Intermediate hosts can be infected only through the accidental ingestion of cestode eggs from the environment, not via ingestion of metacestodes from other intermediate hosts.

Competent and epidemiologically relevant intermediate hosts

Voles: In Europe, various species of the genera *Microtus, Arvicola, Myodes* and *Lemmus* are confirmed as suitable hosts based on field studies and/or experimental infections. However, the relative importance of individual species for the maintenance of the lifecycle, also depends on additional parameters such as population densities and predation rate by definitive hosts. There is evidence that the common vole, *Microtus arvalis*, is most important for the parasite in some areas, e.g. the Ardennes region of North-Eastern France (Guislain et al., 2008), and the southern limit of the parasite in Switzerland coincides with presence and absence of this species (Guerra et al., 2014). In other areas, water voles *Arvicola* spp. may maintain transmission, e.g. in Hungary and in urban areas of Central Europe. The transmission role of the aquatic form of the water vole may partly account for the association of EM presence with the vicinity of surface water e.g. in Germany and Hungary (Staubach et al., 2001; Tolnai et al., 2013) (Table A2, Appendix A).

Other small mammals: various murid rodents (*Mus musculus, Apodemus* spp, *Rattus norvegicus*), hares and shrews have been found naturally infected, but these seem to be sporadic events and the importance of these rodents appears negligible (probably as a function of partial resistance to the parasite and/or low attractiveness as fox prey). **Muskrats** (*Ondatra zibethicus*), **nutria** (*Myocastor coypus*) **and beaver** (*Castor fiber*) are suitable hosts, but are likely to be infrequent prey for foxes due to their large size and habitat specificity; they may, however, have a role in dispersal of the parasite. There are two incidences when beavers intentionally translocated (for re-stocking or re-introduction) from an endemic area (Southern Germany) to free areas (UK and Serbia) being found infected (Barlow et al., 2011; Cirovic et al., 2012). Muskrats, as common animals in wetlands, frequently show high prevalences compared to other intermediate hosts, and may have a potential as target species in addition to a definitive host species for surveillance in circumstances where carcasses are readily available. (Table A2, Appendix A).

The relative importance of different rodent and other small mammal species for maintenance of the lifecycle differs according to geographical areas, the type of environment, and other parameters (e.g. species, population densitiy and predation behaviour of DHs). Prevalence in small rodents is highly variable on small scales and generally much lower than in DHs (Guislain et al., 2008, Guerra et al., 2014, Staubach et al., 2001; Tolnai et al., 2013. Large rodents (e.g. muskrats) can show high prevalence, but are only present in special environments. This extreme variability does not make any of those potential IH particularly suitable for surveillance purposes.

Accidental or refractory intermediate hosts:

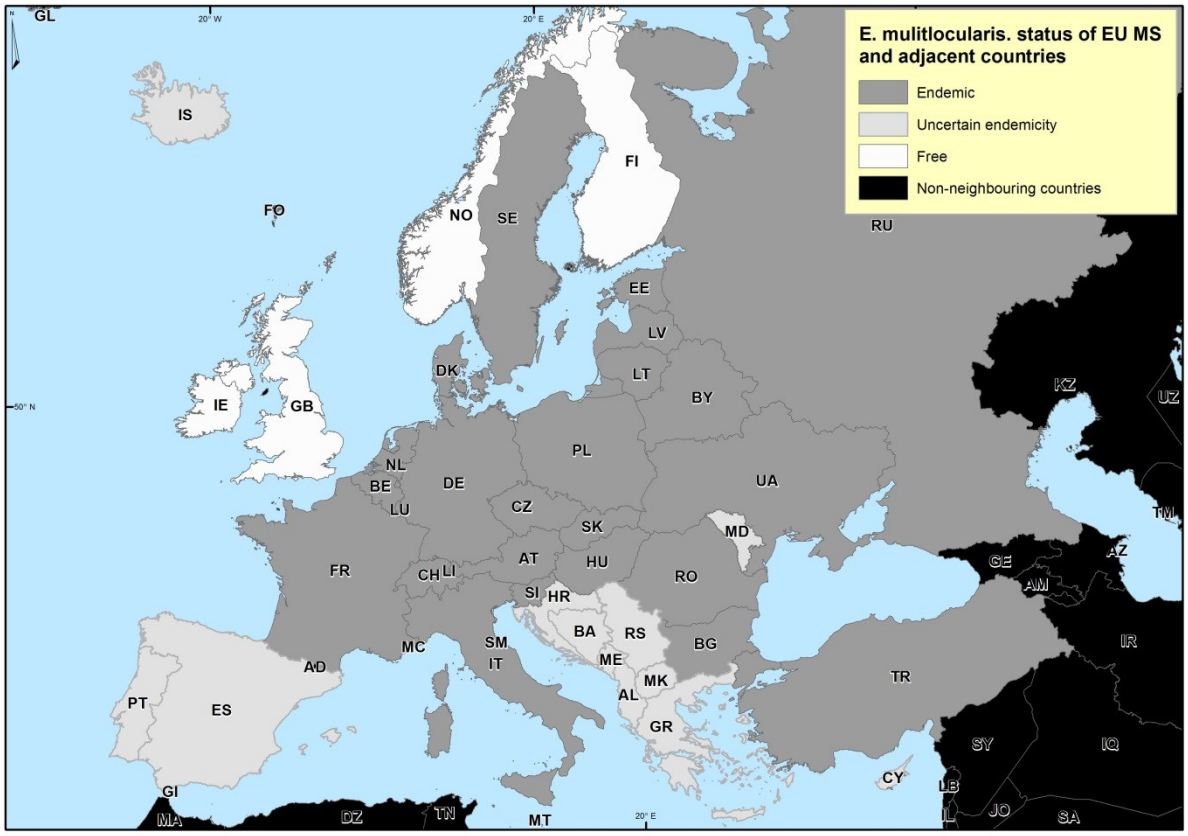
Ungulates: Wild boar and domestic pigs can be infected but do not develop disease and play no role in transmission; calcified, died out lesions (verified by PCR) are frequently seen in the livers of wild boar and domestic pigs kept outdoors in highly endemic areas. It is difficult for untrained persons to differentiate such lesions from other, more common, lesions, such as 'white spots', and therefore surveillance of suspected lesions will have to use PCR, which is likely to be too expensive to be applied in a monitoring programme. Furthermore, the expected prevalence in these species is very low, in particular in domestic pigs kept indoors. For these reasons wild boar and domestic pigs are not considered to be a relevant species in a monitoring programme. Extensive liver lesions appearing as AE have been reported from domestic ruminants, but, upon molecular examination (PCR), turned out to be unusual growth forms of CE (Heath et al., 2005; Adriano Casulli, ISS Rome, personal communication, 25 August 2015). Therefore, any finding of AE in such animals needs molecular confirmation. Experimental infections of large herbivores were invariably abortive, resulting in small, calcified liver lesions (Ohbayashi et al., 1971). (Table A2, Appendix A)

Zoo animals: A number of unrelated mammal species from outside the parasite's geographical range (e.g. wallaby, hyrax) were found accidentally infected. Interestingly, some non-human primates, both monkeys and apes, are highly susceptible to AE (Deplazes et al., 2001), and losses of such animals kept in European and Japanese zoos are not infrequent. As no primate species occurs naturally in the

range of EM, with the possible exception of Western China, this susceptibility has no consequence for transmission. (Table A2, Appendix A)

Dogs: Lethal hepatic AE in domestic dogs is not uncommon and shows the susceptibility of dogs to infection with the metacestode stage of the parasite, in addition to their role as definitive hosts. So far, this seems to be a feature of domestic dogs only; no records of AE are known from wild canids (e.g. foxes). (Table A2, Appendix A)

3.2. Geographical distribution and prevalence of *Echinococcus multilocularis* infection (linked to TOR1a)



Map based on Table 3 in Appendix A.
Free: MS or AC (Norway) listed in Annex I of Commission Delegated Regulation (EU) No 1152/2011 of 14 July 2011; Uncertain endemicity: freedom not documented but no case reported.

Figure 2: *Echinococcus multilocularis* status of EU Member States and adjacent countries

Since the 1980s, EM has been recorded in animals from 17 countries previously thought to be free (Davidson et al., 2012). The endemicity status of these countries is based on animal records. Human cases are difficult to interpret because the geographical origin of infection is unknown, and because of differences in notification requirements throughout the EU and adjacent countries. Whether these new EM records are due to range expansion or reflect an increased surveillance effort will be difficult to prove, since there is a general lack of (negative) baseline data from previous periods in many of these 'newly endemic' countries. However, there are convincing data that EM prevalence and abundance of host species increased dramatically in some countries during the 1990s and since (Romig et al., 2006a; Gottstein et al., 2015). Range increase is likely, but it cannot be excluded that the parasite had remained undetected due to low prevalence or presence in small transmission foci within the 'newly endemic' countries, which may have expanded in the wake of population increases of red foxes (Davidson et al., 2012).

In many countries, the distribution of EM is not homogeneous, showing areas with high and low prevalence levels (Table 4). These differences in prevalence levels have been linked to various factors,

most frequently the use and structure of landscape, which influences species range and abundance of rodents as intermediate hosts (Romig et al., 2006b).

Detailed information on each country with reference to the data sources is available in Table A3 and A4 of Appendix A.

3.2.1. Norway, Sweden and Finland

EM on the Norwegian arctic island of Svalbard was reported for the first time in 2001 after the anthropogenic introduction of a species of vole (*Microtus levis*) from Eastern Europe during the twentieth century (Henttonen et al., 2001). The parasite population using Arctic foxes as definitive hosts is not closely related to those on the European mainland (Knapp et al., 2012). EM has not been detected in mainland Norway (Wahlström et al., 2011a; 2015a). Furthermore no human cases of AE have been reported. Any finding of EM in animals or echinococcosis in humans is notifiable (Wahlström et al 2015a). In countries where no findings of the parasite have been recorded it can be suspected that the awareness of this disease and therefore also the sensitivity of the surveillance system is low. However, in Norway a total of 19 echinococcosis cases, considered to be CE, were notified in the period 2006-2013 and seroconversion but no lesions have been reported in two arctic fox field researchers from Svalbard, indicating that the surveillance detects cases of echinbococcosis (Wahlström et al 2015a). Mainland Norway has never recorded EM findings and was added to the list of countries complying with the conditions laid down in Regulation (EU) No 1152/2011 by Decision of the EEA Joint Committee No 103/2012 of 15 June 2012 amending Annex I (Veterinary and phytosanitary matters) to the EEA Agreement.

However, in 2012, the Norwegian Scientific Committee for Food Safety has concluded that it was likely that *EM* would be detected in mainland Norway during the next decade (VKM, 2012). They also highlighted that with the current surveillance program it was unlikely that *E. multilocularis* would be detected upon its first introduction. Theoretically, nearly 1,000 foxes could have become infected before the first case was detected since the surveillance program was designed to detect a prevalence level of <1% in an estimated red fox population size of 70,000–120,000. It was also highlighted that the paucity of border control checks is a concern with regard to the risk of importing infected dogs from the EU into mainland Norway (Høgåsen et al., 2012). It was considered that there was a moderate probability of importing *EM* with rehomed stray dogs from Eastern Europe. This was further highlighted by Hamnes et al. (2013) who found, at examination of a large number of the rehomed stray dogs, that they had not been given the correct anthelmintic treatment prior to and post import (Wahlström et al 2015b).

Sweden was considered free until a few years ago (Osterman et al, 2011; Wahlström et al 2011a). However, EM since 2011 has been detected in 4 counties in south and central Sweden since 2011 (Wahlström et al., 2015). Whether this is the result of anthropogenic introduction (e.g. via dogs), or the parasite has previously escaped detection due to rare occurrence, is not known.

There are no reports of EM in animals from Finland despite considerable monitoring efforts (Wahlström et al 2011a, 2015a; EFSA, 2015). A risk assessment performed in 2001 (Maijala et al., 2001) highlighted the importance of preventing introduction of EM , as the conditions for the spread of the parasite appear favorable, with both suitable definitive and intermediate hosts. It was concluded that there was a considerable risk of introduction with wildlife, but the risk was difficult to assess as information about the EM situation in North-Western Russia was lacking. Furthermore, the risk of introduction with infected pets was regarded as real. To prevent introduction, deworming of imported pets against cestodes was recommended. The control of fox and raccoon dog populations was considered as the best means to control EM spread in Finland. However, this was considered very difficult to perform (Wahlström et al 2015a).

The risk of introduction by wild life from Sweden is considered negligible. The reasoning behind this is that only a few kilometres of the 555 km long Swedish-Finnish border is a land border, thereby interfereing with migration of wild life. Furthermore the closest case of EM in Sweden occurred about 1,000 km from the Finnish border. Extensive surveillance has been performed in the nothern parts of Sweden (bordering Finland) without any finding of EM.

EM in animals and echinococcosis in humans is notifiable in Finland. During 2000–2014 (until 22 September), 22 cases of echinococcosis were reported. None of the reported cases was considered

autochthonous and most of them were diagnosed as caused by *Echinococcus granulosus* G1. No AE cases have been reported (Wahlström et al, 2015b).

In conclusion, there is no indication that EM or AE is present in Finland today. The risk of introduction by wildlife from Sweden is considered negligible. The risk of introduction by wiild life from Russia is considered to be low, however due to lack of surveillance data on wildlife in the Russian area adjacent to Finland, this risk is not possible to estimate. However, there is a real risk of introduction by imported dogs that are insufficiently dewormed.

3.2.2. Central area (France to the Baltic States and Poland, Denmark to Italy and Romania)

Until the 1990s, only a 'core' area consisting of Eastern France, Southern Germany and parts of Switzerland and Austria were known to be endemic. Now the parasite has been shown to occupy most – if not all – of Central Europe, in the north from the Normandy region through the Netherlands, Denmark and Poland to Estonia, in the south to Southern Switzerland, northern Italy, Hungary and Romania, and in the east at least to Western Ukraine and Southern Belarus. Estimated prevalences within this region vary: particularly high estimates are reported from the historic 'core' area, but also from mountainous landscapes ranging from the Ardennes through central Germany into the Czech Republic, and in the High Tatra region of Poland and Slovakia. Range extension may have occurred in some parts, e.g. in Belgium and the Netherlands (Vervaeke et al., 2006), but a previously undetected presence in the presumed 'new' areas cannot be excluded. The increase in the prevalence of infected animals over time is likely to have taken place in most areas since the 1990s in the wake of growing fox populations. Such increases are best documented for Eastern France, parts of Germany, Poland and parts of the Baltic States, but are often anecdotal for other areas and are difficult to verify due to lack of previous baseline data (Romig et al., 2006a; Davidson et al., 2012; Gottstein et al., 2015). There is a clear trend towards the establishment of urban and peri-urban EM transmission in central Europe (Deplazes et al., 2004). (Tables A3 and A4, Appendix A)

3.2.3. Spain/Portugal/France

There are no records of animal infection or human cases. Only negative data in one fox survey in Spain are available (Table A3, Appendix A). Information is also lacking for the neighbouring regions of Western and Southern France.

3.2.4. Italy

So far, EM has been found consistently only in isolated foci of the northern provinces of Trentino Alto Adige, but not yet in regions further south and west. However, the surveys had a limited scope. Molecular data indicate that these north Italian foci might be ancient endemic foci rather than an introduction from neighbouring highly endemic Tyrol in Austria, which is supported by old records of human cases from the area (Casulli et al., 2009). (Tables A3 and A4, Appendix A)

3.2.5. Balkan Peninsula (former Yugoslavia, Albania, Greece and Bulgaria)

The most southern recent records of infected foxes come from Slovenia, southern Hungary and Central Romania. However, further south in Croatia and Serbia only a few fox surveys have been done. These did not identify infected animals. There are convincing reports of metacestodes in rodents in Southern Bulgaria. Several older reports of human cases in these countries are difficult to assess. Ecological conditions appear favourable for the parasite in many parts of this region, so further surveys are warranted. (Tables A3 and A4, Appendix A)

3.2.6. Eastern Europe (Belarus, Ukraine and Russia)

As presently confirmed, the endemic area stretches into Western Ukraine and Southern Belarus. Whether there is a connection to the endemic parts of Russia is unclear due to lack of surveys in the European part of that country. Accessible data indicate that, within the Russian Federation, EM and human AE are frequent in parts of Siberia and the Far East (Martynenko et al., 1988; Bessonov, 2002). There are no records of human AE cases from the north-western parts of European Russia, and records from animals are restricted to a highly endemic focus in the tundra zone of the far North

(Nenetsia Autonomous Okrug) (Peklo, 2014). It cannot be excluded that the parasite is absent from Karelia and the Kola Peninsula, but its presence is highly likely in Kaliningrad (located between high endemic areas of Poland and Lithuania) and the region bordering Estonia to the East. Data from Northwestern Russia are needed to be able to estimate an introduction probability into Finland (Tables A3 and A4, Appendix A)

3.3. Risk factors for and the probability of introduction, transmission and establishment of *E. multilocularis* (linked to TOR1c)

3.3.1. Risk factors

Risk factors must be known to assess the probability of introduction, transmission and establishment of EM in areas where no findings of the parasite have been recorded. They may also serve as the basis for the implementation of risk-based surveillance. In fact, very few science-based estimates of relative risk values have been reported so far. An example is given by Ziadinov et al. (2008) and Antolova et al. (2009). These authors refer to the effect of '*exposure to intermediate host*' and report an odds ratio (OR) of 4.28 for use of dogs for hunting vs. dogs not used for hunting, and an OR = 0.39 for dogs being tied up at all times vs. being allowed to roam all or some of the time. A total of 50 EM-positive out of 466 dogs were included in this study (Ziadinov et al., 2008). An OR of 6.36 for shepherd-use dogs vs. all other dog categories in the study, an OR of 7.05 for being fed with raw viscera of sheep, pigs and occasionally cattle vs. not or unknown feeding of raw viscera, and an OR of 6.09 for catching rodents vs. not or unknown catching of rodents were identified in another study. All of these latter OR-values, however, have very wide confidence intervals due to only 8 EM-positive cases out of the 289 dogs in the entire study (Antolova et al., 2009). In addition, a careful evaluation of potential confounding factors should be considered.

Several other potential risk factors are difficult to identify unambiguously due to lack of appropriate data or studies, such as the age of infected definitive hosts, climate and climatic changes, geographic location, DH population dynamics and their interaction with IH.

Probability of introduction

Despite a good theoretical understanding of the ways of introduction of EM into areas where no findings of the parasite have been recorded, there are gaps in the knowledge on which in practice are the most important infection routes.

Furthermore, the true disease status of an area is often not known, for example, if surveillance has not been carried out or if the results are not available.

Regarding the compliance with de-worming requirements for dogs before entry into areas where no findings of the parasite have been recorded, the available data are basically limited to the UK, where border compliance checks are implemented and records of movements/entries are kept (Roberts, 2015. See also Appendix B).

The knowledge on the movement of wildlife DH, including red foxes, across borders is scarce (see also Section 3.1.1).

Furthermore, it is unlikely to detect a recent introduction of EM due to its slow spread and resulting very low prevalence, see Section 3.4.3.

In principle, EM can be introduced by moving definitive hosts with a pre-patent or patent infection, infected intermediate hosts that carry fertile larval stages (metacestodes) or infectious parasitic stages, and plants or other items contaminated with eggs into areas where no findings of the parasite have been recorded. Lack of compliance with existing regulations on the treatment of dogs with a drug effective against EM before entry of the dog into a country where no findings of the parasite have been recorded also represents a potential risk factor for the introduction of EM.

EM introduction into Svalbard has been reported (Hentonnen et al., 2001; Knapp et al., 2012), but it is not clear, if the introduction of the parasite has taken place into other European countries that had been definitely free before. In 2011, Sweden first reported the detection of parasite in foxes after intensive surveillance had not detected EM in the country for many years (Osterman Lind et al., 2011). It has been argued by some authors that the parasite was detected in areas, both in Denmark

and Sweden, into which the dog nematode *Angyostrongylus vasorum* has also been introduced, presumably by infected dogs. Nevertheless, it cannot be ruled out completely that the parasite had been present before at a very low prevalence level in foxes or in small foci and has remained undetected for a long period of time despite the surveillance system in place. Most likely, the actual prevalence was lower than the target design prevalence of 1% prevalence. In some situations, EM was detected by more sensitive surveillance systems (Tackmann et al., 1998; Wahlström et al., 2012).

Probability of transmission and establishment

Introduction of the parasite into areas where no findings of the parasite have been recorded is necessary, but not sufficient, for the establishment of its life cycle. The latter requires transmission from definitive hosts to intermediate hosts and back to definitive hosts to close the life cycle of the parasite. Establishment is generally considered to be the 'Perpetuation, for the foreseeable future, of a pest (organism or disease) within an area after entry'. It is obvious that appropriate definitive and intermediate hosts must exist to support the life cycle, but the knowledge on the potential role of environmental factors for the persistence of the life cycle is scarce.

Hence, the probability of the infection becoming established will also vary from one area to another. In broad terms, it will vary depending on the exposure of intermediate hosts to a contaminated environment; the ingestion of infected intermediate hosts (mainly voles) by definitive hosts; and survival of the parasite to patent infection in the definitive host. This is determined by the presence of intermediate hosts and definitive hosts, geographic and environmental characteristics, presence of competitors to reduce likelihood of establishment and the likelihood of repeated introductions. It is conceivable that dogs that have eaten potentially infected intermediate hosts (i.e. small mammals) have a higher probability of being infected with the parasite as compared to pet dogs that feed on canned or other heat-treated food. For an area, where no suitable wild canid hosts and no highly suitable intermediate hosts (i.e. voles) exist, such as Malta, transmission and establishment is considered not to be possible. Therefore, it is unlikely that a dog resident in such an area, which never travelled to an endemic area, or was properly treated before or after leaving an endemic area, will be infected, unless there was anthropogenic introduction of infected small mammals on the island, as seen with Svalbard in 1999-2000 (Henttonen et al. 2001).

In a Systematic Review on the risk factors for introduction, transmission and establishment of EM in free areas through movements of domestic and wildlife species involved in the EM lifecycle (Casulli et al., 2015), the only eligible publication of Stieger et al. (2002) analysed the high prevalence of EM reported from foxes in the city of Zurich, Switzerland. This work shows that differences in the prevalence, habitats and ecology of definitive and intermediate hosts may influence the risk of transmission and establishment of the parasite in urban and peri-urban areas. From a total of 604 tested putative fox faecal samples, 156 (25.8%) were positive in a copro-antigen enzyme-linked immunosorbent assay ELISA with a distinct increase in the proportion of positive samples from the urban to the peri-urban zone. Furthermore, samples collected in the border zone had significantly more copro-antigen-positive results during winter. The prevalence of the parasite in rodent intermediate hosts was 9.1% (81/889) for *Arvicola terrestris* (with 3.5% of the animals harbouring between 14 and 244,400 protoscolices) and 2.4% (2/83) for *Clethrionomys glareolus*. EM-infected *A. terrestris* was found in 9 of 10 trapping sites in the border zone. The high infection pressure in the periphery of urban areas might pose a risk for infection with EM for domestic carnivores as well as for urban humans inhabitants. It should be noted, however, that the work was conducted in a surrounding area with a high prevalence of EM infection in foxes. Whether or not this is comparable to the situation in countries where no findings of the parasite have been recorded, is difficult to assess.

In addition, these issues were addressed in greater detail in a systematic review of EM infections in domestic and wild animals (Otero-Abad and Torgerson, 2013). This review article lists studies that assessed potential associations between EM infection in foxes and environmental factors such as seasonal and spatial variations of the prevalence, altitude, average annual maximum temperature, precipitation, geographic areas and land-use. The authors also extracted studies that identified statistically significant determinants of infection of foxes with the parasite such as higher intensity of infection in juvenile foxes, at least under high endemic conditions.

The role of different species as DH or IH is described in Section 3.1.2. Reports exist on the probability of introduction of EM by infected dogs or intermediate hosts, but there is no information regarding the transmission and establishment of the infection after a given introduction. As a consequence, there

are no data in the literature allowing a quantification of the risk for any of the species. In addition, there are no studies performed which systematically analysed the risk factors for animals to become infected with EM.

Vegetables, mushrooms, berries, fruit or plants may become contaminated with eggs of EM on the ground, e.g. through contact with fecal material of infected definitive hosts. Introduction of the parasite into areas where the parasite has never been recorded through raw contaminated food or plants and the subsequent establishment of the life cycle is in principle possible. However, there are no data in the literature allowing a quantification of the probability and the identification of further potential risk factors.

3.3.2. Conceptual model

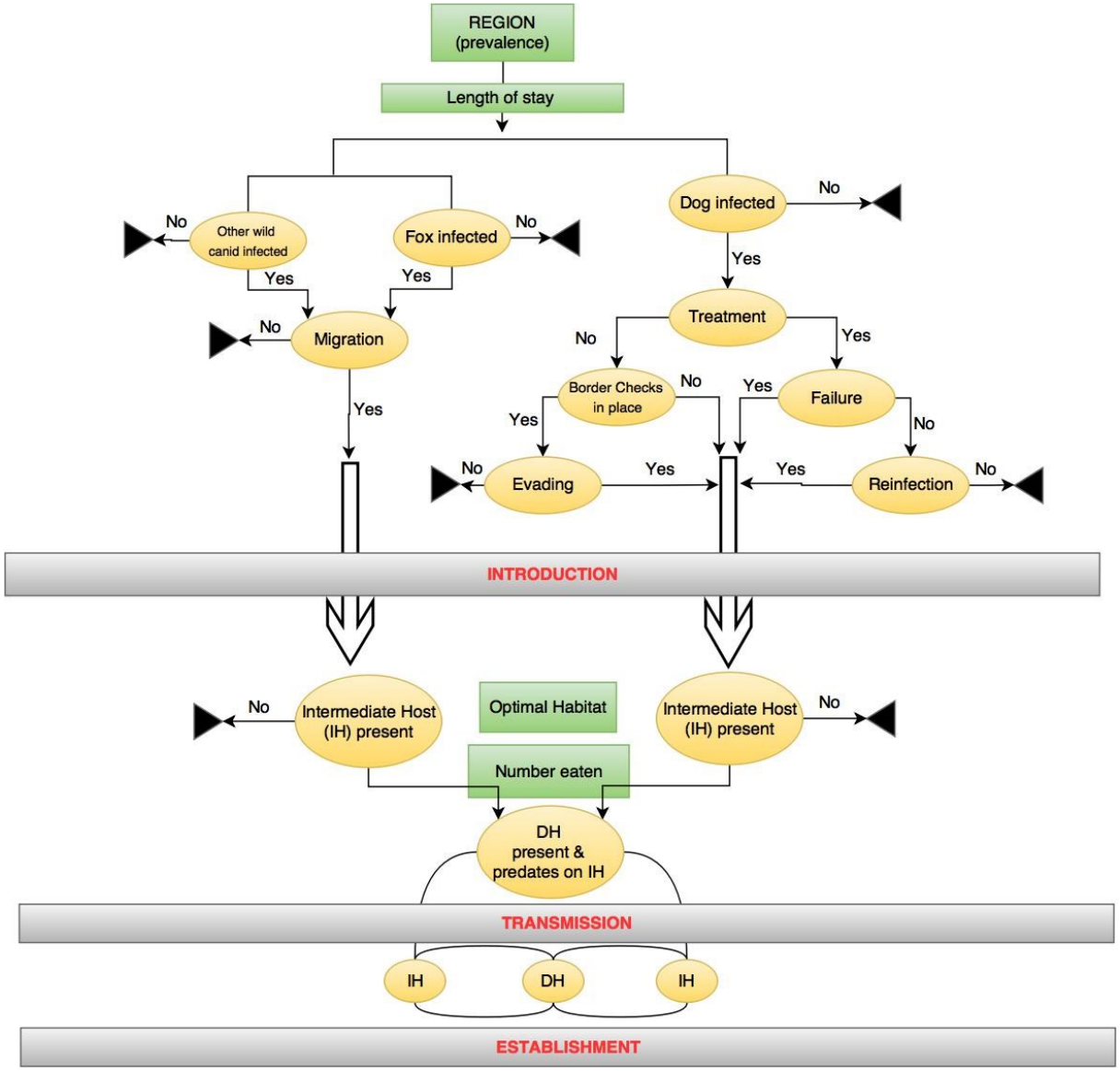
A conceptual model, represented in a scenario tree (Figure 3), has been developed to include and describe all potential pathways through which EM can be introduced from an endemic area into a free area by wild DH or by dogs. The scenario tree consists of the following nodes:

- *Area*: the country / region from where the infected animal originates, characterised by a given prevalence;
- *Animal*: three different alternatives are included: travelling dogs, foxes and other wild DH (racoon dogs, wolves);
- *Infection*: the probability that the animal is infected, depending on the lenght of stay in the area;
- *Introduction*: the probability that the parasite is introduced into the free country
- *Transmission*: the probability of the life cycle initiated in the free country;
- *Establishment:* probability of perpetuation (for the foreseeable future) of the EM lifecycle within an area after entry. It is the final step and cannot be modelled due to the uncertainty as expressed in earlier sections, on the factors affecting it.

For dogs moving to a free country, introduction is conditional on the following nodes:

- *Treatment compliance*: if the dog is treated correctly (24-120 hours before entry)
- *Treatment failure*: probability that a treatment fails;
- *Probability of re-infection*: probability of re-infection after an effective treatment and before entry;
- *Border checks*: presence of border compliance checks
- *Evading:* probability of evading the border compliance checks in place

The scenario tree shows that there are three pathways whereby a travelling dog may enter a free country with different probabilities of being infected depending on the prevalence of the country of origin and the time spent in that country. A dog may be not treated, or treatment was not effective (due to drug inefficacy or dog vomiting drug soon after ingestion), or the dog is re-infected between treatment and entry. Each pathway leads to **introduction**. A second step is where exposure of intermediate hosts and the native definitive hosts are infected in a **transmission** cycle. The final step is **establishment**, where the life cycle is perpetuated for the foreseeable future in the originally free country.



The black triangles indicate a stop in the flow.

Figure 3: Conceptual model on the probability of introduction, transmission and establishment of *Echinococcus multilocularis* in a free country

3.3.3. A probabilistic model to quantitatively estimate the probability of introduction and establishment

Based on the conceptual model described in the previous section, a probabilistic model has been developed. Provided that all input data are gathered, the model can give a quantitative estimate of the probability of introduction and establishment in a free MS.

The routes of introduction considered in this modelling exercise are the domestic dogs and the red foxes.

The time frame considered is 1 year.

The probabilistic model is fully described in Appendix B and may allow estimating:

- The probability of introduction, defined as the probability that at least one infected DH (i.e. any DH harbouring at least one live parasite, irrespective of its stage of development) will successfully move from an endemic area into a free area.

There is information in the scientific literature about the probability of transmission of EM, however these data are relevant only for geographical areas that are suitable for the parasite (i.e. suitable for the DH, for the IH, for the survival of the eggs, etc.). Therefore, a detailed map of a given area reporting that information should be available in order to allow a proper quantitative assessment. However, those characteristics can vary at very short distances, which makes it very difficult, if not impossible, to draw such maps. Because that information is not available for any area in Europe, the quantitative assessment of the probability of establishment, defined as the probability that an introduction will lead to perpetuation, for the foreseeable future, of EM within a previously free area, was not possible.

In general, it should be highlighted, that as complete data is not available at present, it is not possible for the model results to represent any particular MS. To draw conclusions for individual MSs, data for those MSs has to be available and the model must be run separately for each MS.

For this reason, the model was used to better understand the relationship between the input (the parameters in the model) and the output (the probability of introduction) by carrying out a 'sensitivity analysis' (Saltelli et al., 2008). Three main aspects were investigated: i) the impact of the border compliance checks (in place / not in place) and; ii) the impact of the red fox, relative to domestic dogs, on the probability of introduction; iii) the impact of the degree of treatment compliance on the probability of introduction by dogs.

3.3.3.1 Parameterisation

The parameters in the model where estimated based on available scientific literature or official national data. When no data were available, expert estimates were used considering that the parameterisation is not MS specific. Different extreme scenarios were explored instead, covering the range of plausible estimations of the relevant parameters. The 16 explored scenarios are the outcome of the combination of four parameters: (i) the prevalence of infection in the dog population of the endemic country (0.002 and 0.01); (ii) the prevalence of infection in the fox population of the endemic country (0.0001 and 0.16); (iii) the probability of a dog being in compliance with the treatment requirements given that it is not checked at the border (because it evades or because the border compliance checks are not in place (0.4 and 0.8)); (iv) the reduction factor of the probability of being infected, as a function of the time spent in an endemic country (0.16 and 1). Table 3 reports the list of the relevant parameters, including the values used and their source.

The results are reported in the following sections.

Table 3: Summary of the parameters used in the modelling exercise

Notation	Short description	Values (mean if stocastic)	Source
N_{foxes}	Number of foxes moving into a free area	0 - 50	Independent variable
N_{dogs}	Number of dogs moving into a free area	0 – 30,000	Independent variable
ρ_{FOX_i}	Prevalence of infected foxes in the i^{th} country of origin	0.0001	T Romig[(a)] and H Wahlström[(b)], personal communication
		0.16 (stochastic)	Karamon et al. (2014)
ρ_{DOG_i}	Prevalence of infected domestic dogs in the ith country of origin	0.002 (stochastic)	Dyachenko et al., (2008)
		0.01	(Worst case scenario) T Romig[(a)], personal communication
P_{ev}	Where border compliance checks are in place, this is the probability that a dog evades the checks.	0.09 (stocastic)	H Roberts[(c)], personal communication

Notation	Short description	Values (mean if stocastic)	Source
P_{ke}	Given that the dog evaded the border compliance check in place (P_{ev}), this is the probability that this dog is still in compliance	0.4 0.8	H Roberts[(c)], personal communication
P_{kne}	Given that the dog did not evade the border compliance check in place ($1 - P_{ev}$), this is the probability that this dog is in compliance	0.99 (stocastic)	H Roberts[(c)], personal communication
P_{kNBC}	Where no compliance checks are in place, this is the probability that a dog is in compliance.	0.4 0.8	H Wahlström[(b)], personal communication
P_{fail}	Probability that a deworming treatment was not effective (not all worms killed)	0.08 (stocastic)	Table A4
P_{day1}	Probability that the treatment has been administered within 24 hours before the dog enters a free country	0.2	H Roberts[(c)], personal communication
P_{reinf}	Probability that a dog gets re-infected following an effective deworming treatment administered between 24 and 120 hours prior to entry	0.03	R Bødker[(d)], personal communication (section 3.5)
F_{inf}	Reduction factor of the probability of being infected (ρ_{DOG_i}) as a function of the time spent in an endemic country	0.16 (2 weeks in an endemic area) 1 (living in an endemic area)	R Bødker[(d)], personal communication (section 3.5)

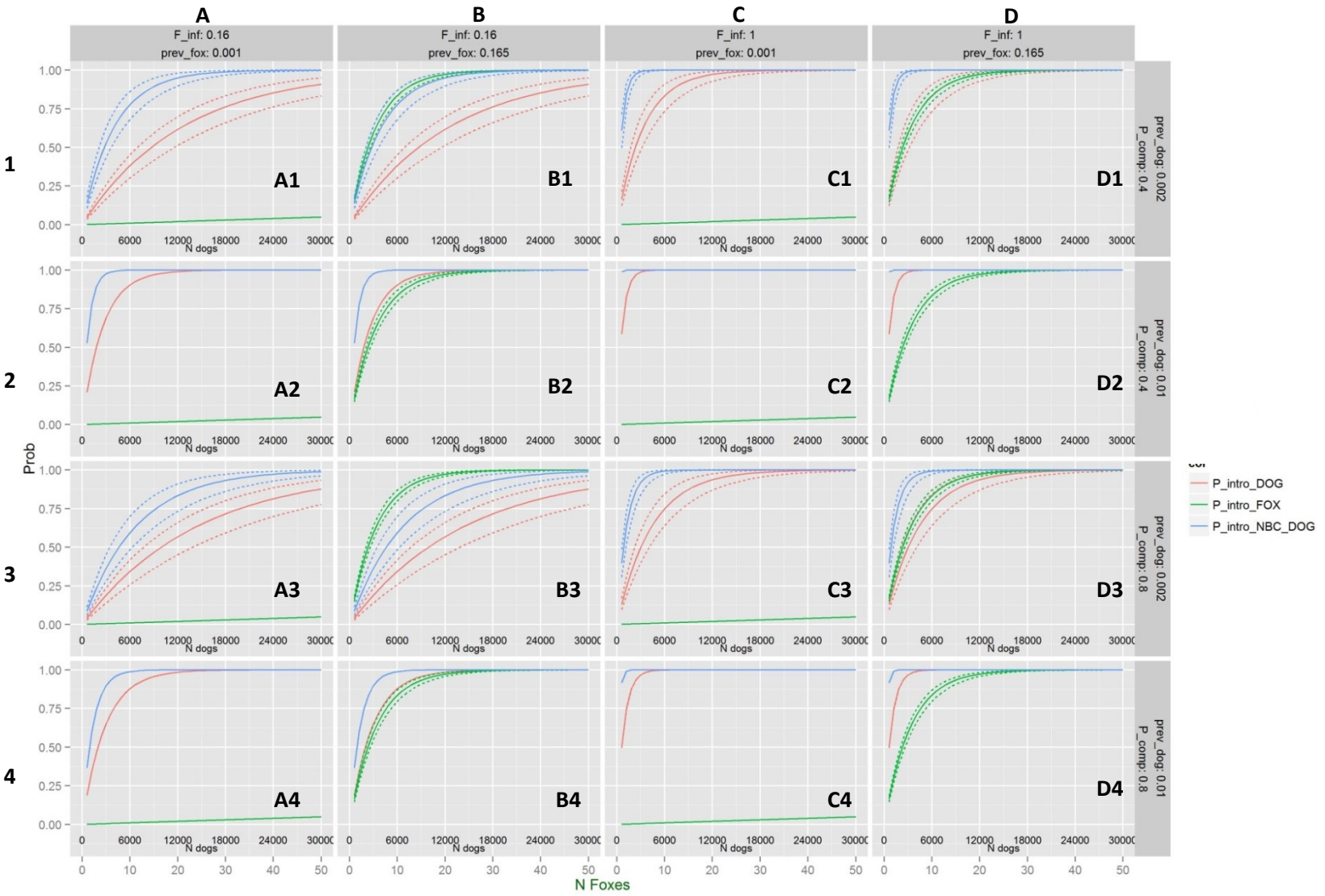
(a): Universität Hohenheim, Parasitology.
(b): National Veterinary Institute, Sweden, Uppsala.
(c): International Disease Monitoring and Risk Analysis, APHA, Defra.
(d): National Veterinary Institute. Section for Epidemiology. Technical University of Denmark. Bülowsvej.

3.3.4. The probability of introduction of *E. multilocularis* in free areas: the model results

The model results are shown in Figure 4. The lines in the Trellis plot show the probability of introduction dependent on the number of animals (dogs / foxes) moving into the free country. The red and the blue lines refer to dogs (border compliance checks in place / no border compliance checks in place), while the green lines refer to foxes. The different scenarios are:

Columns A and B refer to dogs living in a free country, travelling in an endemic country for two weeks and then coming back home (Finf=0.16), while columns C and D refer to dogs living in an endemic country and then entering a free country (Finf=1).

Columns A and C consider a low-prevalence fox scenario ($\rho_{FOX_i} = 0.001$), while columns B and D consider a high-prevalence fox scenario (worst case, $\rho_{FOX_i} = 0.16$).



X-axis: number of dogs/foxes; Y-axis: probability of introduction. Red line: dogs & border checks in place. Blue line: dogs & no border chacks in place. Green line: foxes. The black x-axis in each small plot indicates the number of dogs (blue and red lines). The green x-axis at the bottom of the figure refers to foxes (green line). Dashed lines=95% confidence bounds (where relevant).

Figure 4: Trellis plot with 16 different scenarios

Where no border compliance checks are in place (blue line), two scenarios of compliance ($\boldsymbol{P_{kNBC}}$ and $\boldsymbol{P_{kEV}}$) are considered: rows 1 and 2 refer to a probability of compliance equal to 40%, while rows 3 and 4 refer to 80%.

Rows 1 and 3 consider a low-prevalence dog scenario ($\rho_{DOG} = 0.002$), while rows 2 and 4 consider a high-prevalence dog scenario (worst case; $\rho_{DOG} = 0.01$).

The presence of border compliance checks always reduces the probability of introduction (Figure 4; comparison between red and blue line for all 16 scenarios).

The number of animals required to introduce the parasite with a certain probability is always lower for foxes compared to dogs. The only exceptions are when the prevalence in foxes is very low and the moving dogs are resident in the endemic area (column C). For the particular probability of at least 75% (value arbitrary chosen) the exact number of animals are read from Figure 4 and reported in Appendix B, Table B11.

In the following, the impact of the parameters of the model on the probability of introduction is explored. This is achieved by comparing alternative scenarios in terms of number of animals that have to move into a free country in order to reach at least, e.g., 75% probability of introduction:

- When **border compliance checks are in place**, the number of domestic dogs that need to be moved into the the free country is 1.75 to 4 times higher.
- For a country adjacent to an endemic area (**prevalence of foxes equal to 16%**) introduction would require 75 to 1200 times more dogs moved, compared to foxes, in case no border compliance check is in place. While 150 to 2550 times more dogs moved are required, compared to foxes, in case border compliance checks are in place,
- For a free country adjacent to an area with a **very low prevalence in foxes (0.001%)** the moving dogs have a prominent role (1.16 to 2.31 more foxes required, compared to dogs) only if they are all resident in an endemic area.
- The **degree of compliance in dogs that are not checked for compliance** (because no border checks are in place or because they evaded) plays a less important role on the probability of introduction of EM, compared to other parameters. An increase from 40% to 80% of the degree of compliance requires 1 to 1.5 more dogs to be moved, if the free country has border checks in place. The number of moving dogs is 1 to 2 times higher if the free country has no border checks in place.

Finally, it has to be highlighted that despite the implementation of appropriate mitigation measures, it is inevitable that infected dogs enter free countries. However, other factors in addition to introduction are important in establishing EM in free countries.

3.3.5. Probability of transmission and establishment in light of the model results

The model results, supporting previous reports, show that introduction will take place in spite of existing barriers with a probability close to 100%. This outcome appears to be inconsistent with the field observation, that e.g. the UK and Ireland have not detected EM yet in spite of the large number of dogs arriving each year and the number of samples collected each year to fulfil the relevant legislation. It is, however, important to take account of the fact that transmission and establishment are prerequisites to detection, since the introductions of EM by dogs in and by themselves might not be detectable in the fox population. The latter yields the tested samples, and the dog-borne infections may not be able to spread to the foxes, e.g. due to environmental, geographical or demographic barriers in the transmission cycles, barriers which are poorly recognized and understood. It should be realized, however, that often in countries with high-risk EM-areas, other adjacent areas are found to have low prevalence for unknown reasons (see Table A4).

The hypothesised environmental effects on the transmission and establishment may also contribute to the apparent gradient of EM prevalence within Europe between central and northern countries. The low-risk areas (Sweden) borders the yet free areas of Norway and Finland, so the prevailing factors may not be conducive to EM spread.

3.4. Monitoring and surveillance programmes of *E. multilocularis* infection in EU and adjacent countries(linked to TOR2a)

3.4.1. Mandatory surveillance for EM in countries where no findings of the parasite have been recorded

Four EU MS claiming freedom from EM currently implement a surveillance programme in accordance with Commission Delegated Regulation (EU) No 1152/2011 on preventive health measures for the control of EM infection in dogs. These are the whole territories of Finland, Ireland, Malta and the UK (Part A of Annex I). One EEA State, mainland Norway (Svalbard excluded), has also claimed freedom from EM and implements a surveillance programme in line with Regulation (EU) No 1152/2011.[4] No country or area at present has taken advantage of the use of Part B of Annex I, thereto allowing countries with a very low prevalence of disease/ infection with an eradication programme to continue to require pre-entry treatment of dogs for a limited time period of five years. Indeed, there are no eradication programmes for EM in any EU Member State or other affected country. For those Member States claiming freedom from EM (EC 1152/2011), EM infection in animals is compulsorily notifiable under national law.

Annex II to Regulation (EU) No 1152/2011 stipulates the requirements for the pathogen-specific surveillance programmes in order for the Member States to be maintained on the list set up in Part A of Annex I to that Regulation. The programme shall use appropriate sampling, either risk-based or representative, that ensures detection of EM if present in any part of the Member State at the design prevalence of not more than 1% at confidence level of at least 95%, using ongoing collection of samples from definitive hosts during 12-month surveillance periods.

EFSA has developed a scientific report and a technical report in 2012 (EFSA, 2012a,b) aiming at defining the principles and procedures established therein. Those principles have been applied in the assessment of each of the national surveillance reports submitted to the Commission for annual reporting.

Firstly, the quality of the surveillance reports of the four Member States and Norway is assessed by checking the description of the surveillance system for completeness against the relevant elements. For each relevant element, data are provided in the surveillance report (See Tables 4 and 5).

Secondly, the raw data on individual samples submitted by the five countries via the EFSA Data Collection Framework (DCF) are analysed. For the purpose, the software R[5] is used to compute descriptive statistics. In the context of EM surveillance, the epidemiological unit is defined as the individual definitive host animal.

In the context of the scientific and technical assistance conferred to EFSA by the Commission in May 2012 and by the EFTA Surveillance Authority in 2014, the programmes for the four MSs and Norway are reviewed by EFSA on an annual basis. The outcomes of this review ('Assessment of *Echinococcus multilocularis* surveillance reports submitted yearly in the context of Commission Regulation (EU) No 1152/2011) are published each year in October by EFSA (EFSA 2013, 2014, 2015a and 2015b). In these five countries, no positive case of EM has been detected by the surveillance programmes in the period 2012–2014. However, the required design prevalence of 1% with 95% confidence was not achieved by the surveillance programmes of Norway (in 2013) and Malta (in 2014) due to insufficient sample size.

The current EM surveillance programme is output-based, providing flexibility about the level of sampling required, the use of risk-based or representative strategies and the combination of tests (see Section 3.9) which can be used to achieve the required confidence.[6] However, there is a lack of specific data to obtain sensitivity estimates at country level of the diagnostic tests (see Section 3.9),

[4] Decision of the EEA Joint Committee No 103/2012 of 15 June 2012.
[5] R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/
[6] Annex II of Commission Delegated Regulation 1152/2011

which means that comparing results between countries, between species, or from one year to the next when different tests are used, is difficult.

Other sampling issues exist around the host characteristics and the timing of the sampling. The objective is to obtain a representative sample, but in practice, the final sample collected by the countries, may be biased by several factors. As an example, hunted and trapped animals make up convenience samples, which may not be considered representative samples. Hunting is often carried out in areas where the animals are considered pests and therefore the animals are selected and may not compose a representative sample. For welfare reasons there is a moratorium in most countries on hunting at certain times of the year, so the samples are not evenly spread throughout the year. Young animals may harbour higher parasite burdens than adults (although there is little or no evidence of acquired immunity (Millner-Gulland et al, 2004)), but the age of the animals when tested is not known/recorded. Estimates of the population size for each country use different methodology or old estimates, so these values should be taken with caution.

The reason for the temporal distribution of sampling was the aim to avoid culling adult female foxes with fox cubs dependant on their dam fox for food. It is reported that collection of samples during the winter months only would 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). The impact of the sampling distribution over time on the interpretation of the outcome has been assessed (EFSA, 2013), suggesting that concentrating the samples in the second half of the sampling period, in a Freedom from Disease framework, could be more effective when a quantitative evaluation has not been performed on this subject.

Table 4: 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 twelve-months sampling period. It may go from January to December, but this is not a restriction: the reporting period can include twelve 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	**Sampling activity over time**	Number of samples collected each month within the theoretical sampling period
4	**Number of samples**	Total number of samples collected during the theoretical sampling period
5	**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).
6	**Laboratory test completion**	Comparison between the year when the samples are collected and the year when the test was completed
7	**Host**	Target population size (N); additional information on the host species
8	**Sampling Strategy and Design**	As reported (e.g. representative sample – risk based sample)
9	**Sampling point**	Activity adopted for the sample collection (e.g. hunting, veterinary activity, …)
10	**Sampling Area**	Number of NUTS 3 regions covered by the sampling, number of samples per NUTS 3 region, number of samples per 1000 Km^2
11	**ASe**	Area Sensitivity: level of confidence when stating that the actual prevalence is below the threshold foreseen in the relevant legislation (0.01). The area sensitivity was calculated using the RiBESS tool (EFSA, 2012b)

Table 5: Summary of current *Echinococcus multilocularis* surveillance in EU Member States and Norway under Commission Regulation (EU) No 1152/2011 (see also Appendix D)

Country	Single Epidemiological unit	Estimates of the definitive host population sampled	Diagnostic test used	Risk Based
Finland	Yes	Red Fox (150,000) and Raccoon Dog (230,000)	SCT and MC-PCR (Isaksson et al., 2014)	No
Ireland	Yes	Red Fox (150,000)	SCT	No
Malta	Yes	Domestic Dog [hunting (10,000), stray (2,000), rural (4,500)]	Sieving/Flotation followed by Copro-DNA detection with PCR	Yes
Norway[(a)]	Yes	Red Fox (70,000)	MC-PCR	No
UK	No – two geographical units	Red Fox in GB (250,000)	Copro-DNA detection with PCR (GB)	No
		Red Fox in NI (14,000)	SCT (NI)	

(a): Mainland.

At present, the sampling strategy does not take account of results from previous surveillance activities (i.e. the prior probability of infection absence); rather each year is treated independently. This differs from the common epidemiological concept of 'documenting freedom from disease', which makes use of accumulating negative test results over a period of several years, while discounting the weight of the evidence by correcting for an estimated annual probability of introduction (Martin et al. 2007).

- In Finland, two definitive wild host species are sampled, the red fox and the raccoon dog. The red fox has a higher population density in the southwest, while the raccoon dog has a higher density in the Southeast (particularly along the Russian border).
- On the British Isles the red fox is the only wild DH. The UK is split into two epidemiologically and geographically separate areas (Northern Ireland and Great Britain). The Republic of Ireland is considered to be a separate epidemiological area. However, the Republic of Ireland and Northern Ireland share a land border, which does not prevent fox migration from one area to the other.
- Although Malta reports risk-based surveillance, the weights used for the risk factors have not been documented nor validated. Science-based estimates of relative risks, which would support implementing risk-based surveillance, have not been reported so far.
- The domestic dog is reported as the only canid present in Malta, where there are no wild canid host species. The most suitable intermediate host species (arvicolid rodents) are not reported (Savona-Ventura, 2001).
- Norway has several definitive wild host species: red foxes, wolves, arctic foxes and raccoon dogs. The current legislation considers the mainland of Norway, hence, excluding Svalbard where EM has been detected in the Arctic fox and the sibling vole (Fuglei et al., 2008).

3.4.2. Surveillance and monitoring in EU countries where findings of the parasite have been recorded

The detection of *Echinococcus spp*. in animals is notifiable in some MS, but there is frequently no requirement for notification of human cases (Table E1, Appendix E). There is no EU requirement for the monitoring or surveillance of EM in countries where findings have been reported in the EU. Certain countries have been carrying out targeted surveillance to answer specific issues such as geographic expansion, effectiveness of control strategies, changes in pathogenicity. For examples, see Appendix D.

Notification of cases is important in order to verify whether EM is present in a country where findings of the parasite have been recorded and if so, in which host species. However, infection with EM in animals being asymptomatic, official statistics have a limited use to estimate prevalences as the data collected will most likely be biased and infection will be under-reported. To ensure a consistent prevalence estimate, repeated, randomised surveys on the healthy DH population have to be done.

There is considerable spatial and temporal heterogeneity in EM distribution within a country and across Europe (see Section 3.2). This is compounded by the nature of infection, such as the variability in pathogen prevalence in infected rodents or contaminated soil, having an indirect lifecycle in two different species of wildlife, both with variable distribution, which contributes to parasite clustering or aggregation. In addition, the genetic susceptibility or acquired immunity in susceptible populations has an unknown impact on the EM distribution. Therefore, the results of local or regional surveys cannot be extrapolated to a whole country.

3.4.3. Absence of infection and early detection of introduction

The principle used to substantiate absence of infection, (i.e. the probability that qualifies a statement on the presence/absence of a given disease/infection from a population (Cameron and Baldock, 1998), is to estimate the so called surveillance system sensitivity (SSe). In probabilistic terms, the System Sensitivity is the probability to obtain at least one positive test, given that the infection is present at or above a given prevalence (the design prevalence, DP) in the target population. The test sensitivity and the DP are used in estimating the number of samples needed to reach the required level of confidence in infection absence (provided no EM-positive samples have been detected). This leads to an increased efficiency and flexibility when compared to input-based surveys. Appendix D contains a detailed description of the estimation procedures.

In contrast, the Probability of Freedom (P_{free}) is the probability that the infection is absent (i.e. DP < 1%) given that all samples tested negative. This corresponds to the Negative Predictive Value of the Surveillance System. P_{free} can be estimated incorporating evidence of infection absence from previous surveillance activities using Bayes' theorem (Cannon, 2002). The procedure prescribed in the EM regulation does not take into account prior information on absence of infection based on the outcome of previous surveillance activities. For the purpose of demonstrating absence of infection, the inclusion of the Probability of Freedom concept in the regulation and its implementation in the surveillance activities may allow a reduction of the sample size.

The current regulation prescribes the use of a design prevalence of < 1%. The design prevalence is a theoretical value, which allows the estimation of the confidence in being below 1% prevalence of EM given the number of tests done, all with a negative result. A sufficient number of samples examined with the estimated test sensitivity for the laboratory in question are needed to reach at least 95% confidence. The need for design prevalence has been described as follows:

'In populations with high levels of disease it is easy to detect infected animals, so the sensitivity of the surveillance system is high. When prevalence is low, disease is harder to find and sensitivity of a given approach is lower. It is therefore necessary to set a standard hypothetical prevalence of disease (design prevalence) against which to measure surveillance sensitivity' (Cameron, 2012).

If a single positive animal is detected, then official infection absence can no longer be conferred on that area. Accordingly, at least 300 animals need to be tested from an overall population of at least several thousand with a test of known sensitivity (Cannon and Roe, 1982). If all sampled animals test negative, the primary condition in the Regulation for being listed and maintained in Annex I thereto is fulfilled. The approach is further elaborated in Appendix D.

The legally required design prevalence of < 1% (95% confidence) to substantiate infection absence, however, is unlikely to detect the introduction of infection within a short time. The time to first detection is likely to vary considerably across different countries.

In fact, if the rate of spread of the infection is low, as for EM, there would be a considerable time lag between the introduction and the first detection, as it will take time to reach a proportion of infected animals greater than the design prevalence (i.e. the Limit of Detection of the surveillance system).

In general, however, it should be expected that countries claiming absence of disease/infection can also substantiate their ability to detect an existing infection shortly after it developed. Documenting infection absence without an adequate early detection might expose the proclaimed absence-of-infection status to criticism, because it is *de facto* mainly historical given the relative inability to detect a very low prevalence of infection caused by a recent introduction.

Early detection requires a lower design prevalence than documenting freedom (Cameron, 2012), although the parameter substantiating the confidence in early detection in case of a new introduction

is the same as that used to substantiate absence of infection, i.e. the surveillance system sensitivity. A suitable surveillance system should possess the following characteristics: i) be continuous (to allow for early detection of an infection introduced at any time), ii) have comprehensive coverage of the population, and iii) be sensitive with a very low design prevalence (Cameron, 2012).

To illustrate the two last points, assume that in an EM-free country there is a population of red foxes estimated at 150,000 individuals. Using the design prevalence applied in the infection absence estimation, i.e. 1%, up to 1,500 foxes may be infected, but the country could nevertheless document absence with 95% confidence, if approx. 400 foxes tested negative out of the population of 150,000 (assuming a test sensitivity of 0.75). Waiting for 1,500 infected foxes to be present in the country before EM is detected would be considered far from 'early' detection. It may take many years for EM to achieve the assumed 1% population prevalence, so it might be more suitable to assume e.g. 0.1% (i.e. 150 infected foxes) as reasonable design prevalence for early detection. The confidence in detecting the infection at a design prevalence of 0.1% with the sample of 400 foxes is however only 26%. Achieving a 95% confidence to detect an infection presence at 0.1% requires an annual sample of 4,000 foxes for testing, which may be neither practically nor economically feasible.

As shown above, if the prevalence is very low then more animals would need to be tested to detect infected animals. For a design prevalence of < 0.1%, at least 3,000 samples are required, provided there is 100% test sensitivity. The case in Sweden helps understanding this issue: after nine years (2000 to 2009) of testing fox samples in a surveillance programme with 1% design prevalence, a single fox tested positive in 2010. Further testing of foxes using a design prevalence of 0.1% detected two more infected foxes distributed over a wider geographical area, which made it unlikely that the incursion had taken place recently (Wahlström et al., 2011). Denmark had reported EM in 2000 in foxes in the Copenhagen area, but did not carry out regular surveillance until the Swedish cases were reported in 2011. Between 2011 and 2013 a total of 676 wild carnivores were sampled in Denmark and four tested positive. All were clustered near the German border, and based on these preliminary data a national prevalence estimate of 0.7%, and a local estimate of 31% (95% CI: 7–55) were obtained (Enemark et al., 2013).

Another option might be to perform risk-based surveillance. This approach is based on the identification of subpopulations with different relative risk. The subpopulations can be defined by individual characteristics (e.g. hunting dog vs. domestic dog) or by geographical provenience (close to the borders adjacent to an endemic country vs. close to the sea). If the relative risk of such sub-populations could be documented, the number of samples required to obtain a specified probability of infection absence can be lowered/decreased compared to a representative sample (EFSA, 2012b).

In summary, the inclusion of the concept of the Bayesian Probability of Freedom or the implementation of a risk based approach, supported by properly documented risk factors, may allow a reduction of the sample size.

3.5. Efficacy of available Echinococcus multilocularis-deworming drugs and the effectiveness of the current species-specific treatment protocols to protect domestic species against the parasite (linked to TOR4)

3.5.1. Drug efficacy

Praziquantel is currently the most-used and most effective anthelmintic for the treatment of EM infections (EFSA, 2015). Praziquantel is a pyrazinisoquinoline derivative acting at the level of cell membrane permeability of cestodes and schistosomes, resulting in the killing of the parasites. Praziquantel is active against both immature and mature stages of EM in the intestine (Rommel et al., 1976; Thomas and Gönnert, 1978; Sakamoto, 1977; Andersen et al., 1981; Kazacos et al., 1993 and 1994; Schroeder et al., 2009). For use in dogs, veterinary medical products containing praziquantel are available for oral or parenteral administration (intramuscular, subcutaneous). For the treatment of EM the approved minimum recommended dose is 5mg/kg bw for single oral administration and 5.7 mg/kg bw for single intramuscular or subcutaneous administration. After oral administration in dogs, praziquantel is rapidly and nearly completely (80–100%) absorbed from the gut, reaching maximum serum levels between 30 to 120 minutes after administration. Praziquantel is well distributed in all tissues with higher concentrations in the liver and duodenum. The substance is rapidly metabolized in the liver. Due to a pronounced first pass effect, the level of un-metabolized praziquantel in the blood after oral administration is lower compared to the blood levels found after parenteral administration. Praziquantel is re-secreted in its active form into the small intestine, thereby achieving relatively large quantities and, thus, is capable to reach even the juvenile stages of EM which are hidden in the intestinal crypts. The half-life of praziquantel is three hours in the dog. Praziquantel is mainly excreted via the kidneys. The efficacy of the anthelmintic treatment becomes less when the dose given is lower, in particular below 5 mg per kg body weight, and results may also be influenced by the infective dose used (EFSA, 2015).

Six studies using the recommended treatment dose for oral and parenteral administration of Praziquantel were identified in the literature review and, additionally, data from two published abstract were used. Treatment efficacy was based on the quantification of worm load after praziquantel treatment. Praziquantel at a single oral dose of 5mg/kg bw resulted in five of the studies in a 100% reduction of worm count while the efficacy was nearly 100% (99.96 to 99.99%) in the remaining three studies. The studies comprised 76 dogs of which 70 were completely cleared and six had remaining worms after treatment and hence the average treatment efficacy at dog level was 92.1%. On average the worm burden was reduced by 99.95% in the 76 treated dogs (see Table A5 in Appendix A).

Epsiprantel is a benzazepin analogue to praziquantel and currently marketed in veterinary medical products containing fixed combinations of epsiprantel and pyrantel embonate. Information in the scientific literature on the activity of Epsiprantel against EM is scarce and the exact mode of action is not known. However, due to its structural similarity to praziquantel it is assumed that epsiprantel has the same endpoint(s) against cestodes (i.e. increases the membranes′ permeability for calcium ions, resulting in disintegration of the tegumentum). In contrast to praziquantel, epsiprantel is poorly absorbed from the intestine, hence its efficacy is limited to lumen dwelling adult stages of EM. The minimum recommended single oral dose against EM and *E. granulosus* is 5.5 mg epsiprantel per kg bw in dogs. In a controlled study on dogs experimentally infected with protoscoleces of EM, a single oral doses of 4.9 to 5.8 mg/kg bw (mean: 5.3 mg/kg) resulted in a worm count reduction of over 99%, however, residual worm burdens of up to 10 to 1480 worms persisted in 4 out of 8 (see Table 6 in Appendix A).

Most of the available studies were carried out on pre-patent infections that may not be fully representative for the patent stage. In addition, while systematic scientific trials can achieve a near 100% reduction in intestinal worm load after treating dogs with 5 mg praziquantel per kilo bodyweight, the reduction following treatment by owners and local veterinarians may be lower. The owner of a dog regurgitating the drug on the way back from the veterinarian may ignore the incident and not have the dog retreated. Such potential reduction in treatment efficacy cannot be quantified. Therefore, a conservative estimate of 0.4% has been used in this scientific opinion for treatment failure based on an experimental trial (Eckert et al 2001).

3.5.2. Quantifying the relative effectiveness of treatment protocols to prevent establishment

In addition to treatment efficacy, the timing of the treatment is important. Worms only live for approximately 90 days and the maximum worm load in a dog is therefore reached after 90 days. A reinfection period after treatment of even a few days will be important as reinfection takes place with 1.1% of the maximum worm load per day (1/90). To prevent reinfection, dogs should be treated as close as possible to entering the country where no findings of the parasite have been recorded. However the narrower the treatment window the more difficult it is for dog owners to plan the treatment, therefore a very narrow treatment window may decrease compliance. Current legislation requires a treatment in a 24–120 hour period before crossing a border to a country where no findings of the parasite have been recorded. Prior to Regulation (EU) No 1152/2011, the treatment window required under national rules (in accordance with Article 16 of Regulation (EC) No 998/2003) for deworming treatments was 24–48 hours for Malta, the UK and Ireland, 1–10 days for Sweden, and not more than 30 days before entry for Finland. The new 24–120 hour window has increased the risk of reinfection but may on the other hand also have increased compliance. It is therefore relevant to investigate to what extend the increased risk of reinfection may have been been compensated for by a potential increase in the number of dogs in compliance. In the sections below, a model will be used to quantify the impact of various treatment windows and different compliance levels on the EM introduction/transmission/establishment probability. Modelling the impact of treatment in the following sections has been restricted to praziquantel.

3.5.3. A mathematical model for optimizing treatment protocols

This section presents the structure and assumptions for the developed mathematical model. The model is here used to quantify the relative effect of treatment protocols to prevent establishment. The model defines risk of establishment as being a function of the number of eggs deposited in the free country rather than a function of the number of infected dogs entering the free area. Scenarios are described for dogs from endemic areas visiting free areas (Section 3.5.4), dogs from free areas visiting endemic areas and returning to a free area (Section 3.5.5), and the relation between treatment window and compliance by the owners (Section 3.5.6). These different scenarios are crucial to understand the intimate relationship between the treatment timing and the probability of reinfection. The model is fully tailored to infected dogs, assuming they are the only ones representing a risk of introduction.

A qualitative import risk assessment model has previously been presented by EFSA (2007), estimating the annual risk of importing infected dogs from an endemic area to a free country when taking into account the number of dogs imported, the probability of infection in the countries of origin, treatment efficacy and reinfection probability after treatment. This approach identified a relatively high probability of reinfection with large treatment windows because the drug is only effective 24 hours after oral intake. Praziquantel administrated 10 and 30 days prior to entering a free country therefore left 9 and 29 days, respectively, for the dogs to be re-infected in endemic areas. This is important because the lifespan of the worms is only 90 days and the maximum prevalence is therefore reached in a dog population after 90 days exposure in an endemic area. A reinfection period of e.g. 9 days will thus allow for 10% of the maximum prevalence to be reached in the period between treatment and crossing the border. In the worst case, the previous Swedish and the Finnish protocols only reduced the probability of importing an infected dog with 90% and 68% respectively. The rules for prophylactic treatment are now harmonized in all free countries, and allow for treatment up to 5 days prior to entry in a free country.

In the previous EFSA risk assessment (2007), risk was defined as the probability of introducing a dog with an infection. However, EM may not be so contagious that a single infected animal crossing the border necessarily results in the successful establishment of the parasite. This is because a worm will produce a large number of eggs in its lifetime. But on average only very few of these eggs will manage to complete the development cycle and result in a new adult tapeworm. The real concern associated with import and travel of companion animals is the probability of establishment of EM in a free area rather than the probability of importing an infected dog. Therefore, an alternative deterministic mathematical model has been developed to calculate the average number of eggs excreted in a free country by a dog exposed in an endemic area. The model quantifies the risk as the

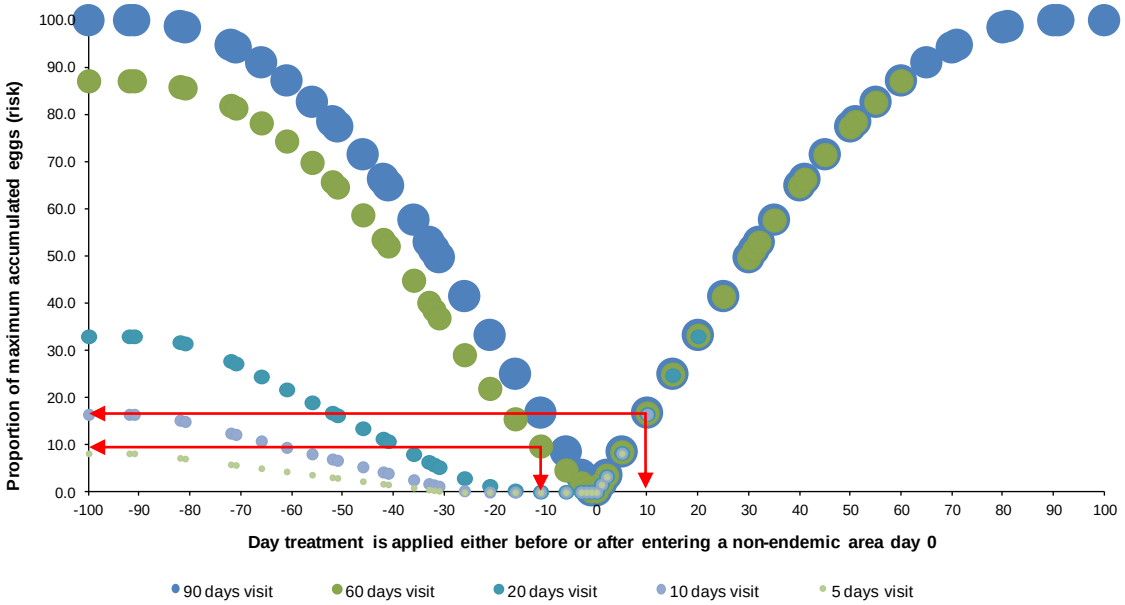
cumulative number of eggs excreted by a dog in the free country. It is assumed that the risk of establishing the parasite in a free area is linearly proportional to the number of eggs excreted in this area. In order to calculate the number of eggs excreted, the model calculates the probability that a dog is infected in the endemic area of exposure, and also the number of worms the dog may be infected with, as well as the duration of each of these worms' remaining lifespan, together with the number of days the dog will spend in the free country. The model also takes into account that the worms undergo a 30 days immature stage before developing into a mature egg producing stage. The model is based on a number of simplifying assumptions e.g. that the daily egg production per worm is constant, that all eggs are excreted within 24 hours after treatment and that treatment efficacy of e.g. 95% can be modeled as if 95% of worms are killed and the remaining 5% worms are completely unaffected. These assumptions can be violated in real life, e.g. eggs' excretion has been reported to last up to 60 hours after treatment (Kazacos et al, 1994). However, a possible violation of one or more of these assumptions is not expected to affect the overall conclusions. In addition, the aim of the deterministic model is not to quantify the risk exactly but rather to identify and rank key risk mechanisms related to the size of treatment window, treatment before vs. after crossing the borders and the relation between treatment window and owners compliance and how these vary with different travel scenarios. A more detailed description of the model is provided in Appendix G and the following sections describe some selected scenarios. The model allows for treatment of dogs not only before moving to a free area, but also after entering the free area as this will also reduce the number of excreted eggs in the free area.

The model is used to quantify the relative treatment effect (i.e. compared to a non-treated dog) for dogs from endemic areas imported to or visiting free areas (Section 3.5.3), for dogs from free areas visiting endemic areas and then returning to their free areas (Section 3.5.4) and to quantify the potential relationship between treatment regulations and the owners' compliance with these (Section 3.5.5).

3.5.4. Treatment of dogs from endemic areas visiting free areas

In this section, the effect of the timing of the deworming treatment and the time spent in an endemic area on the risk of introduction/transmission/establishment is assessed for dogs from endemic areas visiting free areas for up to 90 days.

A visit of 90 days has the same effect on the risk of establishment as permanent import since the lifespan of the worms is 90 days, hence, the maximum worm load is reached after 90 days. Treatment efficacy is assumed to be 99.6%. The number of eggs deposited in the free area in the different scenarios is given as percentage of the maximum egg excretion, i.e. in case a dog, living in an endemic area, is moved into a free area for more than 90 days without having been treated (worst case). In this way the calculations become independent of the exposure level in the endemic area, and present the relative effect of treatment given on different days compared with no treatment.



The number of eggs are given as percentages of the number of eggs deposited if treatment is not applied and the dog is fully exposed and stays in a free area for 90 days, thus that the results are presented as the relative impact of the treatment strategies and travel history. The five sets of data points show that the relative risk depends both on the duration of the visit and on which day the treatment is administered. Multiple scenarios are presented for the treatment day, ranging from 100 days before (day -100) up to 100 days after (day 100) entering the free area. Interpretation of the figure is explained in more detail in Appendix G.

Figure 5: Number of eggs deposited in a free country by dogs exposed for more than 90 days and then visiting free areas for 90, 60, 20, 10 or 5 days

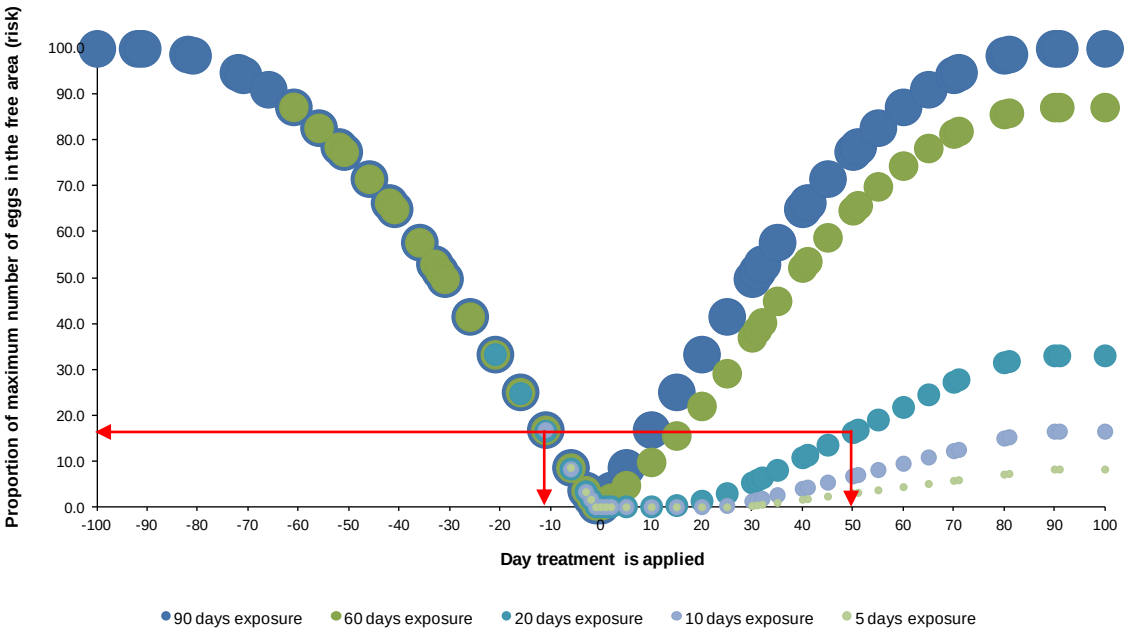
The shorter the visit to the free area the lower the probability and the more effective it is to treat dogs before they enter the free area compared to treating dogs after they enter the free area. For instance, treating a dog 11 days (day-11) prior to visiting a free area for a 60 day period will reduce the introduction risk to 9.8%, while postponing risk to nine days after entry at day 9 will only reduce risk to 16.8% (Figure 5, red arrows). This suggests that in this type of scenario (dog living in an endemic country and visiting an area where no findings of the parasite have been recorded) the timing of the treatment greatly influences the risk and it is best to treat dogs before they enter the free area. More precisely, the number of deposited eggs in the free area can be reduced by treating before or when entering the free area. The number of eggs excreted in the free area is lowest when treating one day prior to crossing the border (day -1).

Short visits will always result in fewer eggs being deposited in the area where no findings of the parasite have been recorded. It should be noted that 20-days visits (or shorter) are so short that any re-infecting worms will remain in the pre-patent stage during the entire visit and eggs will only be deposited in case of treatment failure.

The model shows that short visits to a free country by potentially infected dogs carry a much lower risk of establishment compared to longer visits or permanent import of dogs from endemic areas.

3.5.5. Treatment of dogs from free areas visiting endemic areas before returning to a free area

In this section, the relative effect of deworming treatment is assessed for dogs from free areas visiting endemic areas for up to 90 days before returning to a free area (e.g. dogs from the UK visiting risk areas for a limited period and then returning home). A visit of 90 days in the endemic area is equivalent to permanent import since the lifespan of the worms is 90 days, hence, the maximum worm load is reached after 90 days. A fixed 100% compliance and 99.6% treatment effectiveness are assumed in this section.



The number of eggs are given as percentages of the number of eggs deposited if treatment is not applied and the dog is fully exposed and stays in a free area for 90 days, thus that the results are presented as the relative impact of the treatment strategies and travel history. The five set of data points show that the relative risk of establishment by dogs returning to a free country after being exposed for various periods depends both on the duration of exposure and on which day treatment is applied. Multiple scenarios are presented for the treatment day, ranging from 100 days before (day -100) until 100 days after (day 100) entering the free area. Interpretation of the figure is explained in more detail in Appendix G.

Figure 6: Number of eggs deposited in a free country by dogs exposed for 90, 60, 20, 10 or 5 days before moving to a free area for more than 90 days

The shorter the exposure period in the endemic area the greater the advantage of delaying treatment until dogs are back in the free area (Figure 6). For instance, treating a dog 11 days (day-11) prior to returning to a free area after being exposed in an endemic area for 20 days will only reduce the number of eggs excreted in the free area (the risk) to 16.6% compared to import of untreated dogs, because this protocol will leave ten days for the dogs to be re-infected before returning. On the other hand, in order to reach the same level of reduction, the dog can be treated up to 50 days (day +50) after it comes back home (Figure 6, red arrows). From another perspective, delaying the treatment to e.g. day 10 after returning to the free area will reduce the risk to 0.1% (for a dog exposed for 20 days). In fact, worms in a dog exposed for 20 days cannot mature before treatment is given (i.e. after 10 days from returning), and hence the only risk (0.1%) is resulting from treatment failure. Also in this case, i.e. considering a dog living in a country where no findings of the parasite have been recorded, visiting an endemic area and then returning home, the timing of the treatment greatly influences the risk. However, the model suggests that the incubation period (the immature non-egg-laying stage) makes it often safe to delay treatment until returning to the free home country and also treatment in the free area prevents re-infection. It is thus much safer to delay treatment until return to the area where no findings of the parasite have been recorded, particularly after short visits to an endemic area (less than 30 days).

Treatment of dogs one day prior to entering the area where no findings of the parasite have been recorded always results in the lowest proportion of maximum excreted eggs in the free area. Treating before returning to a free area on the other hand always carries a risk of re-infection between treatment and entry into the free area. A treatment administered before and after entry to a non endemic area would be the most effective strategy. When first treatment is done prior to day -1 in the endemic area and second treatment is given in the free area and within 30 days after first treatment drug faliure is the only risk of introduction as the first treatment would kill any worms aquirred before

and the second treatment would kill any reinfecting worms acuirred after first treatment and before they produce eggs.

3.5.6. Compliance and treatment window

As shown above in Figure 5 and Figure 6, it is optimal to apply treatment one day prior to entering a free area. However this is logistically difficult to administer for dog owners traveling between countries. Recently a treatment window requiring deworming up to 120 hours prior to entry in a free country was implemented in Europe (Regulation (EU) No. 1152/2011). This carries a risk of reinfection of the treated dogs, but does on the other hand make it easier for the dog owners to arrange for the dogs to be treated in the visiting endemic country, and hence, has the potential to increase compliance and may thus compensate for the increased risk of re-infection. Compliance is here defined as the proportion of dogs entering a free country that are being treated according to the regulations.

In this section, an attempt is made to quantify how much compliance has to increase to compensate for the increased reinfection risk when expanding the treatment window from 24 hours before entry to 48 hours, 72 hours, 96 hours, 120 hours and 144 hours before entry. Similar to above (Sections 3.5.4 and 3.5.5), long-term exposed dogs living in endemic areas visiting free areas for short periodshave to be distinguished from dogs living in free areas visiting endemic areas for short durations of exposure before returning to the free area. The calculations are explained in last part of Appendix F.

As the compliance levels are not known for each EU MS, with the exception of the UK, three different suboptimal compliance levels of 95%, 80% and 50% were explored at the optimal treatment time (day -1). The increase in compliance required to keep the risk at the same level as treatment on day -1 was calculated, given a treatment administered at day -2, day -3, day -4, day -5 or day -6. As the re-infection risk increases as treatment is given earlier it was calculated how much compliance needed to increase in order to keep the risk stable. This is the break-even point, where risk is being defined as the number of eggs excreted in a free area.

Table 6: Level of compliance needed (%) to counteract increase in re-infection risk as treatment window is increased from day -1 for long-term exposed dogs staying in the free area for more than 90 days (dogs)

Day -1 (reference)	Day -2	Day -3	Day -4	Day -5	Day -6
50%	51%	52%	53%	54%	54%
80%	82%	83%	85%	86%	88%
95%	97%	99%	Not possible	Not possible	Not possible

The table shows how much compliance needs to increase to counteract the increased probability that a dog is reinfected after treatment (when treatment is given day -2 to day -6 instead of the optimal treatment day -1), in order to prevent the 'risk of establishment' from increasing. The actual risk of establishment is not shown in the table.

If dogs are introduced from endemic areas and the compliance is 80% when a treatment window of just one day (day -1) is prescribed, the re-infection risk can be held stable (Table 1) as long as compliance increases with a wider treatment time window. An increase in compliance up to 86% is necessary to fully compensate for the increased risk of re-infection due to the extension of the treatment window (from day -1 to day -5). If the compliance increases from 80% on day -1 to more than 86% on day -5 then the risk of establishment will actually decrease, resulting in a treatment strategy that is both more flexible for owners and carries lower risk of spreading the infection. However, if the level of compliance is 95% (or higher) at treatment day -1, increasing the treatment window to earlier than three days prior to import cannot be fully compensated by an increased compliance even if compliance reaches 100% (Table 6). In this case the treatment strategy will still be more flexible for the owners and may increase compliance, but even 100% compliance will not be able to prevent the risk from increasing.

Table 7: Level of compliance needed (%) to counteract increase in re-infection risk as treatment window is increased from day -1 for long-term exposed dogs visiting a free area for 60 days

Day -1	Day -2	Day -3	Day -4	Day -5	Day -6
50%	51%	52%	52%	53%	54%
80%	81%	82%	83%	84%	85%
95%	96%	97%	97%	98%	Not possible

If the dogs are staying in the endemic area for more than 90 days (as are imported dogs) but only visiting a free country for 60 days then increasing a treatment window with a 95% compliance at day -1 to a treatment window of five days can be compensated if the compliance increases to 98% (Table 7). And if the visit is as short as 20 days (Table 8) or even just five days (Table 9) the treatment window can be increased to 6 days without increasing risk even if compliance does not increase. The reason for this is that no re-infecting worms after treatment will be able to mature and produce eggs before the dog again leaves the free area since the pre-patent period is 30 days. However if compliance increases as the treatment window is allowed to expand this will reduce number of eggs excreted in the free country and thus reduce risk of establishment.

Table 8: Compliance needed to prevent increase in re-infection risk as treatment window is increased from day -1 for long term exposed dogs that stay in the free area for 20 days (visit)

Day -1	Day -2	Day -3	Day -4	Day -5	Day -6
50%	50%	50%	50%	50%	50%
80%	80%	80%	80%	80%	80%
95%	95%	95%	95%	95%	95%

Table 9: Level of compliance needed (%) to counteract increase in re-infection risk as treatment window is increased from day -1 for long term exposed dogs visiting an area where no findings of the parasite have been recorded for just 5 days

Day -1	Day -2	Day -3	Day -4	Day -5	Day -6
50%	50%	50%	50%	50%	50%
80%	80%	80%	80%	80%	80%
95%	95%	95%	95%	95%	95%

Table 10: Level of compliance needed (%) to counteract increase in re-infection risk as treatment window is increased from day -1 for dogs exposed for 20 days before returning to a free area

Day -1	Day -2	Day -3	Day -4	Day -5	Day -6
50%	52%	55%	59%	63%	67%
80%	85%	89%	93%	99%	Not possible
95%	>99%	Not possible	Not possible	Not possible	Not possible

Table 11: Level of compliance needed (%) to counteract increase in re-infection risk as treatment window is increased from day -1 for dogs exposed for just 5 days before returning to a free area

Day -1	Day -2	Day -3	Day -4	Day -5	Day -6
50%	63%	84%	Not possible	Not possible	Not possible
80%	>99%	Not possible	Not possible	Not possible	Not possible
95%	Not possible	Not possible	Not possible	Not possible	Not possible

If dogs from a free area are exposed for a limited period in an endemic area before they return to a free area and stay there permanently (more than 90 days), then it is difficult to compensate for the increased risk of re-infection associated with treating the dogs earlier than one day before returning to the free area. Only a very low initial compliance of 80% at day -1 can be compensated by treating within two days before entry (day -2) if the compliance increases to 99% (see Table 11). This is because the risk of egg excretion in the free area is a result of reinfection between treatment and the time of returning to the free area (e.g. treatment day -2 leaves one day for reinfection while treatment days -3 doubles this reinfection period to two days). This doubling of reinfection risk is not possible to compensate with increased compliance unless the compliance at day -1 is very low.

The level of compliance with the requirement to treat dogs entering the four Member States and Norway is uncertain as not always recorded. It is assumed that the knowledge of the owners about border compliance checks are in place in a given country increases the degree of compliance of dog, while knowing that no border compliance checks are in place is likely to decrease the compliance. While the UK data suggests that an increased treatment window from 24 hours to 120 hours has increased the *number* of dogs in compliance, as the overall number of travelling pets has also increased, this has had less effect on the *proportion* of animals in compliance. The above calculations demonstrate that it is possible that an increased compliance as a result of allowing earlier treatment may counteract the increased risk caused by re-infection between treatment and entering a free area, but it is only likely to be effective for dogs living in endemic areas and visiting free areas. The shorter time a dog from an endemic area visits the free area the more likely the risk is to break even because of increased compliance. If the visit is so short that the dog living in an endemic country will return within 30 days after being treated, then re-infection does not increase risk and therefore any improvement in compliance will actually reduce the overall risk of eggs being deposited in the free area.

An important finding is that dogs living in free countries and visiting endemic areas for shorter periods before returning to their free countries constitutes a risk of egg excretion in their home countries that will require large increases in compliance to counteract. For shorts visits in endemic areas, e.g. 5 days, it is unlikely that even dramatic improvements in compliance will be enough to counteract the increased risk resulting from an increase in treatment window from two days to five days. However, it must be remembered that the absolute risk from a short visit in an endemic area is lower than the absolute risk from a long visit, regardless of the treatment window allowed.

While an increased treatment window may increase treatment compliance and thus reduce risk or at least result in the same risk for dogs from endemic areas visiting a country where no findings of the parasite have been recorded, this is not likely to be the case for dogs from countries where no findings of the parasite have been recorded returning from visits in endemic areas. For dogs returning to counties where no findings of the parasite have been recorded very large improvements in compliance is needed to prevent the risk from increasing when increasing the treatment window. Unfortunately, most dogs crossing the border tothe UK appear to belong to the latter group while we do not have data for the Malta, Ireland or the Nordic countries. But the negative effect of increased treatment windows is much bigger for dogs returning to a free country than are the positive effect for dogs from endemic areas visiting a free country, and since the absolute risk during short visits is also higher in dogs returning to free areas compared to dogs visiting free areas (when treatment is done before crossing the border). Therefore, when the treatment protocol was harmonized in the new legislation, the overall effect of increasing the treatment window in the case of the UK, Ireland and Malta from two days to five days increased the probability of introduction of the parasite into these countries. Whereas for Finland, the window was tightened and therefore the risk of introduction was reduced.

Large treatment windows were previously in place in, e.g. Finland, extending up to 30 days prior to entry. Anecdotal evidence suggests the long treatment window facilitated compliance as it was easier for dog owners to plan the treatment. However, in some cases it lead the owners of dogs residing in free countries who were intending to visit endemic countries and then return to the free country to treat the dog before visiting the endemic area. While such a protocol from the owner's side may appear to be technically legal it has no protective affect at all because treatment take place before the dog is exposed. In this case it would therefore be much more beneficial if owners were allowed to delay treatment until returning from the trip to an endemic area. This would also make it easier for owners to plan the deworming and thus comply and it would kill potential infections acquired on the

trip. Indeed, the mathematical model developed here demonstrates that even if the compliance may be unchanged when delaying treatment, the risk of introducing the parasite will still be lower when deworming takes place in the free home country.

3.6. Programmes for the eradication of *E. multilocularis* (linked to TOR2b)

This section is limited to considering the control and eradication of EM in foxes as the main definitive host (see Section 3.1.1) and because data on other species are scarce.

Several studies have shown that anthelmintic treatment of foxes with praziquantel decreases the prevalence of EM both in urban and rural areas (Table 7, Appendix A). The decrease in prevalence has been shown to be larger in the **centre of the baiting area** compared to the marginal areas, a difference probably caused by migrating foxes (Schelling et al., 1997). Comte et al. (2013) evaluated the effect of baiting in two medium size cities (< 100,000 inh.), where intervention was not done in the surrounding areas. The prevalence decreased in one city but not in the other, and the authors suggest that frequency of baiting should be adapted to the local situation (Comte et al., 2013).

As seasonal variations of EM prevalence occur, studies lacking control areas or only having historic control areas cannot determine how much of a change in prevalence is caused by the control and how much is due to seasonal variations (Schelling et al., 1997; Tackmann et al., 2001). Furthermore, changes due to baiting may sometimes be smaller compared to changes over time (Tsoumada et al., 2002). In one study where the prevalence was estimated 12 times during a four year period prior to baiting, the prevalence estimates varied between 12.6% to 36.6% (Tackmann et al., 2001). In another study, the prevalence in the control area varied during the study period from 33.3 to 60% (Antolova et al., 2006).

Different baiting strategies have been used, the **baiting area** has varied from a few km^2 (Antolova et al., 2006; Hegglin et al., 2003 and 2007) to 5000 km^2 (Tackmann et al., 2001) (Table 7, Appendix A). Hegglin and co-workers showed that with intensive baiting over a long period (3.5 years) even in small areas (1 km^2) the parasite was probably eliminated and recolonization took several years (Hegglin et al., 2003). Romig et al. (2007) highlighted the need to combine arial distribution of bait in rural areas with distribution by hand in more urban areas to get adequate coverage.

Baiting **frequencies** have usually been every four or six weeks, in some studies baiting intensity has decreased over time (Table 7, Appendix A). Inoue et al. highlighted the importance of frequent baiting (monthly) at least during periods (summer, autumn) where transmission is considered high (Inoue et al., 2007). König et al (2008) and Hegglin et al (2008) also highlighted the need for frequent baiting (monthly). Romig and colleagues (2007) showed that baiting with six week intervals reduced the prevalence, when increasing the baiting interval to 3 months (after 21 months) the prevalence stabilised and increasing it to 6 months resulted in an increase in prevalence.

Baiting **intensity** has usually been 50 baits/ km^2, however in certain studies lower baiting density has been used, especially in those where baits are delivered by hand at fox dens (Table 7, Appendix A). It has been shown that it is possible to decrease prevalence in small, highly endemic areas if intensive baiting is done (Hegglin et al., 2003). The authors concluded that baiting densities in urban habitats should exceed 20 baits/ km^2 and also that manual distribution of baits at selected sites will improve uptake of baits by foxes. König et al. (2008) concluded that a baiting intensity of at least 30 baits/ km^2 is needed and recommended at least 40/km^2 to have a reserve. As the fox population has increased since rabies eradication, a higher intensity than during the rabies campaign (15/km^2) is needed (König et al., 2008). Romig et al. concluded that one of the reasons for not being able to interrupt the life cycle in a baiting study was the low baiting intensity (20 baits/ km^2) (Romig et al., 2007).

The **duration** of baiting in the studies varied from seven months to four years. In one study it was concluded that baiting needs to be done for more than one year (Tsukada et al., 2002), which was supported by a study in Switzerland, where during the first year of baiting, the prevalence in foxes decreased significantly, but no difference was detected in the *A. terrestris* prevalence. However, the prevalence in *A. terrestris* in bait areas during the second year of baiting was significantly lower reflecting a lower infection pressure for intermediate hosts (Hegglin et al., 2003). König et al. (2008) showed that the prevalence in foxes could be reduced to close to zero in less than one year by intensive baiting (every four weeks initially and then every sixth week) and combining arial distribution

with distribution by hand at strategic places identified in previous studies. However, Hegglin et al. (2008) highlighted that, if local disappearance of the parasite is to be obtained, intensive baiting should be continued even after the prevalence has decreased drastically.

Most studies used a dose of 50mg praziquantel which is considered sufficient, as a dose of 5mg/kg bodyweight has been shown to have a 100% deworming effect (Rommel et al., 1976).

The importance of **bait uptake** was highlighted in a study by Antolova et al., (2006), where baiting failed to decrease the prevalence which was considered to be due to a high population density of wild boars interfering with bait uptake by foxes. The bait disappearance rate for the red fox has been shown to vary from 48% to nearly 100% (Marks and Bloomfield, 1999; Thoma, 2008; Hegglin et al., 2004) cited by Janko and König (2011). Ecological parameters of the fox as well as bait competitors have great influence on bait disappearance (Janko and König, 2011). To ensure that baiting strategies are efficient and cost effective it is important to determine bait consumption of red foxes and competitor species (Janko and König, 2011).

As baiting is quite **expensive**, there is also a need to identify how to distribute baits in a more cost efficient way (Schelling et al., 1997). König et al. (2008) highlighted the importance of involving hunters for bait distribution by hand in urban and suburban areas and also for evaluation of the results by collection of foxes. Kamiya et al. (2007) concluded that in all campaigns in Japan the involvement of local volunteers is important to obtaining a sustainable system to control the parasite. Hegglin et al. (2003) recommended that public health authorities should focus on urban areas intensively used for recreational activities where the parasite is highly endemic to decrease the potential risk of alveolar echinococcosis, a conclusion supported by Antalova et al. (2006) and König et al. (2008).

Kamiya et al. summarised information on **fox hunting** and potential control of EM. In Eastern Hokkaido (Japan), hunting was increased in 1970 but despite that, the parasite is still present although at a low prevalence. A nation-wide hunting ban on foxes in the United Kingdom was reported not to have measurable impact on the fox population density in selected areas (Baker et al., 2002 cited by Kamiya et al., 2007). Kamiya et al. (2007) concluded that fox hunting by culling or trapping does not have an effect on the number of foxes and therefore no effect on controlling EM. Hegglin et al. summarized that although under strict conditions the fox population can be reduced in extended areas (Heydon et al., 2000 cited by Hegglin et al., 2015), it is well accepted that regulating fox populations on a larger scale is difficult to achieve (Gentle et al., 2007 cited by Hegglin et al., 2015). Culling can increase the proportion of sub-adult foxes which disperse over large distances and also may harbour higher worm burdens (Morishima et al., 1999 and Hofer et al., 2000). This is in accordance with a French fox hunting study where the proportion of sub-adult foxes and also the prevalence of EM increased to a higher level compared to the control area (Comte, 2014).

Eradication of EM in the European wildlife could theoretically be achieved by means of baites in small areas where foxes are present, but the intervention needs to be perpetuated to maintain the status. In large areas, long term control - but not elimination - of the parasite may be possible by baiting. Increased fox hunting/trapping is not considered to be effective in controlling the parasite. Control by baiting requires more knowledge about how and where to control the parasite in a cost efficient way. Distribution of baits needs to cover both rural and urban areas, baiting frequency and bait density need to be high (approx. 50 baits/ km^2), duration of baiting needs to be long (several years), areas where control is cost efficient need to be identified (urban/suburban), baiting uptake needs to be considered. More research, including modelling, is needed for long term sustainable control of the parasite: Can baiting frequency be reduced if the time/season of baiting is adapted to the life cycle of the foxes? Can baiting intensity be reduced by strategic placement of the baits? Can costs of baiting be reduced by involvement of local hunters/residents?

3.7. Risk factors associated with human alveolar echinococcosis (linked to TOR3a)

3.7.1. Cases of AE in EU

Surveillance of human AE

Alveolar echinococcosis is a parasitic zoonotic disease characterized by an asymptomatic incubation period in humans of around 5–15 years and the slow development of a primary tumour-like lesion which is usually located in the liver. Clinical signs usually include weight loss, abdominal pain, general malaise and signs of hepatic failure. Larval metastases may spread either to organs adjacent to the liver or distant locations following dissemination of the parasite via the blood and lymphatic system (http://www.who.int/mediacentre/factsheets/fs377/en)

The true number of AE cases in Europe is unknown. This is primarily due to lack of notification requirement at the species level in several Member States (Table E1 in Appendix E). Moreover, case definitions of 'Echinococcosis' in Europe, according to Commission Decision 2012/506/EU, do not make distinction between alveolar and cystic echinococcosis and consequently between Echinococcus multilocularis and granulosus, respectively. In some MS notification requirement is at the genus level, however, as cases of CE usually far outnumber the cases of AE, the number of 'Echinococcus' cases in humans will primarily reflect the number of CE cases. This lack of notification requirement at the species level has been highlighted previously (EFSA opinion, 2006). In addition underreporting also occurs due to poor knowledge of clinical symptoms and incorrect clinical management (Romig et al., 1999b; Jorgensen et al., 2008; Stojkovic et al., 2015).

EurEchinoReg network was initiated in 1998 for the assessment of human alveolar echinococcosis across European borders. Five-hundred fifty-nine patients were voluntarily enrolled in the European Register (autochthonous cases diagnosed between 1982 and 2000). The majority of the cases originated from rural areas from Eastern France to Western Austria (Kern et al., 2003).

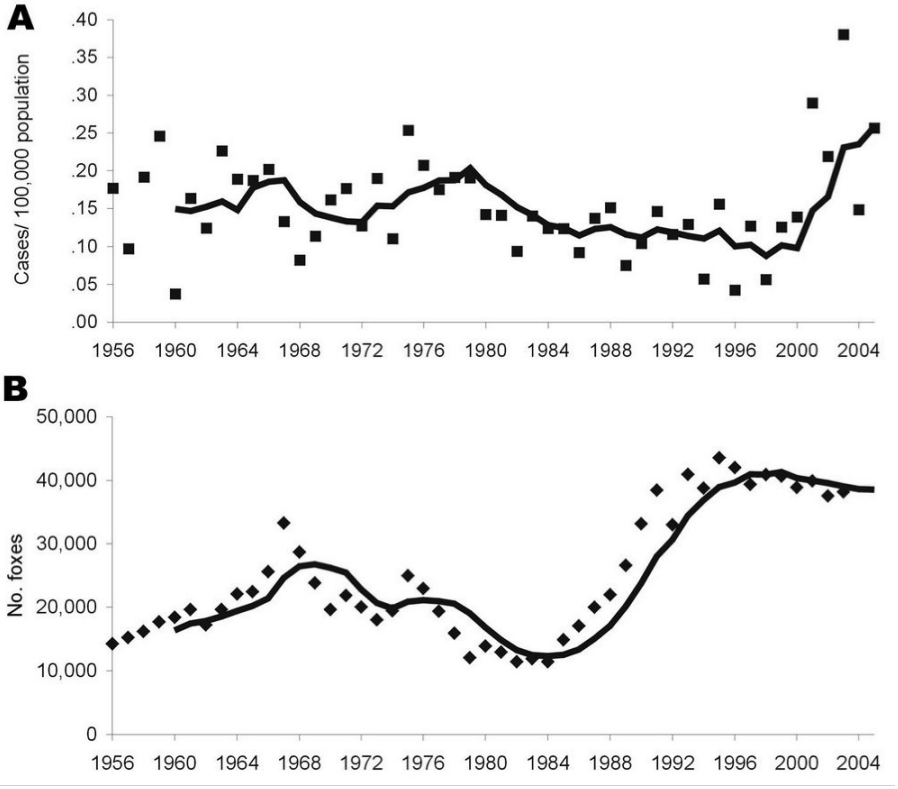
According to Kern and colleagues, by December 2000, 73.0% of the patients were alive (n=408), 21.3% had died (n=119), and 5.7% were lost to follow-up (N=32). Unfortunately such European register on AE was not available after 2000, while a European CE register was recently created in 2015 (http://www.heracles-fp7.eu/erce.html; Tamarozzi et al., 2015). In France, a country previously known to register half of all European patients, a national register is still maintained in which AE was diagnosed in 509 patients from the period of 1982-2011 (Said-Ali et al., 2013).

Some interesting features are coming from the endemic country of Germany where AE reporting became mandatory from 2001. In fact, Jorgersen and colleagues (2008) used a 3-source capture–recapture analysis to generate an estimate of the true number of AE cases in Germany from 2003 through 2005 and to assess the sensitivity of national surveillance. Results demonstrated that the national surveillance system failed to detect 67% of AE cases in Germany over 3 years (Jorgensen at al., 2008). Moreover, Nothdurft and colleagues (1995) conducted one of the few retrospective cross-sectional studies in Europe to investigate the epidemiology of echinococcosis in Bavaria, Southern Germany. A standardized questionnaire was sent to all hospitals in Bavaria and in a second step, a team of reviewers was sent to all relevant hospitals for active case finding in hospital statistics and medical records. A total of 216 patients with echinococcosis were detected, of whom 58 had alveolar echinococcosis. According to these data, the prevalence in Bavaria was calculated to be 0.5 per 100,000 inhabitants with peak values in the counties of Swabia (2.4) and Upper Bavaria (0.6). The annual mean incidence of newly diagnosed cases amounted to 0.03 per 100,000. The distribution of prevalence in man was closely correlated to the prevalence in foxes throughout Bavaria ($p < 0.05$).

Moreover, EM was recently detected in animal hosts from Estonia (Moks et al., 2005), Latvia (Bagrade et al., 2008) and Lithuania (Mažeika et al., 2003; Bružinskaitė-Schmidhalter et al., 2012) (see Section 3.2). Twenty-nine cases of AE were reported from Latvia during the period 1996-2010 (Tulin et al., 2012) and 80 AE cases from 1997-2006 (Bružinskaitė et al., 2007), both from single hospitals.

An increased incidence was also recently noted from countries recognized as historically highly endemic. For instance in Switzerland where Schweiger and colleagues (2007) retrospectively analysed AE cases from three centres with a total of 494 cases recorded during last 50 years. Annual incidence per 100,000 population increased from 0.12–0.15 during 1956–1992 and a mean of 0.10 during

1993–2000 to a mean of 0.26 during 2001–2005 (Schweiger et al., 2007). One possible explanation for increased human cases in Switzerland, is the increasing fox population after implementation of rabies control with 15 years shift due to the incubation period of the EM infections in man (Schweiger et al., 2007; Figure 7).



Source: Schweiger et al. (2007)

Figure 7: Data points with moving 5-year average for annual incidence of human AE in Switzerland (A) and annual number of foxes hunted per year in Switzerland (B), used as a fox population density marker

In line with the above trend, in Austria the annual incidence were reported as 2.4 and 2.8 cases/100,000 population during 1991–2000 and 2001–2010, respectively. Hence, the registration of 13 new AE patients in 2011 was unexpected for Schneider and colleagues (2013). The authors argued that the increasing fox populations and past AE underreporting might have caused such increase in reported cases.

In addition, several factors were pointed out as reason of the increase of EM/AE in Europe, such as: the landscape change composition and use; urbanization of foxes; changing human attitudes and behaviour toward foxes; globalization; wildlife (re)introduction; effect of climate change on the environment and survival of eggs and DH/IH populations survival (synthesized in Davidson et al., 2012; Atkinson et al., 2013; Gottstein et al., 2015).

These recent findings/expanding of the parasite in several new areas such as the Baltic regions, Denmark, Netherlands, Poland, Romania, Slovakia, Slovenia and the unexpected increase of human AE incidence in endemic countries such as in Austria, France and Switzerland are suggesting the disease is spreading and increasing in Europe (see Table E2 in Appendix E on available scant data on AE). Improved awareness and better diagnostic tools may have also contributed to increase in records of this parasite in animals and humans. Nevertheless, over the past 20 years, intensive epidemiological research seems to confirm EM/AE expansion in European countries (Gottstein et al., 2015).

An appropriate surveillance/reporting scheme for AE at the EU level is not currently implemented and it should be considered necessary. Cases of both cystic and alveolar echinococcosis, caused by

E. granulosus and EM respectively, are reported jointly to ECDC as echinococcosis as the EU level case definition does not differentiate between the two clinical forms of the disease. They can be differentiated in the data reported to ECDC by the reported species, but this is not currently implemented. Also the notification of human echinococcosis is not mandatory in all MSs. Mandatory submission of echinococcosis data at species level should be considered, as necessary for understanding the epidemiology of different diseases as AE and CE. In fact, not only the true number of patients with AE is unknown in Europe, (because notification is not mandatory at EU level), but even when notification is provided at national level (for instance in Germany), the system failed to detect 67% of AE cases over 3 years (Jorgensen et al., 2008). An appropriate surveillance scheme, data models at the EU level and mandatory notification are necessary to overcome current quality problems of the data provided by Member States to EFSA and ECDC and reported in The EU summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks (EFSA and ECDC, 2013). As an example, 794 'echinococcosis' cases in Europe have been reported in 2013. This does not correspond to 1,379 CE records per year reported by Hospital Discharge Records (HDRs) in Italy during the period of 2001–2013 (Brundu et al., 2014; Tamarozzi et al., 2015). Hence, a reporting scheme differenciating AE from CE and autochthonous from imported human cases would be important to trace back infections.

Implementation of such mandatory surveillance and an improved reporting scheme at EU level is crucial to collect real epidemiological and clinical data for the management of this disease, overcoming currently problems on data quality and quantity.

3.7.2. General risk factors

The following risk factors of potential global relevance were identified in a Systematic Review (Casulli et al; 2015): dog ownership, playing with dogs, female gender, age above 20 years and occupation (livestock herding) (Table E4 in Appendix E). It must be emphasized that only risk factors that were identified in studies that were eligible according to the inclusion factors of the Systematic Review could be included. It is also important to note that some of the risk factors could be influenced by the socio-cultural situation in particular affected areas such as China, as several studies included in the analysis had been conducted there. The available data suggest that the dog can be relevant as a risk factor for human infection, although the dimension of the risk is influenced by the exposure of dogs to infected intermediate hosts, which have to be eaten by a dog to infect it, and socio-cultural conditions determining the exposure of humans to faeces of infected dogs and materials contaminated with such faeces.

Five out of seven case-control studies included in the Systematic Review (Casulli et al; 2015) were conducted in the EU, one in China and one in North America. Dog ownership, cat ownership, having a kitchen garden, occupation (farming), haymaking in meadows not adjacent to water, going to forests for vocational reasons, chewing grass and handling foxes were identified as potential risk factors, whereas particular Human leukocyte antigen (HLA) types turned out to be protective against AE (Table E4 in Appendix E). When the analysis was restricted to case-control studies performed in Europe, dog ownership, cat ownership, living in a rural area, having a kitchen garden, occupation (farming), haymaking in meadows not adjacent to water, going to forests for vocational reasons, chewing grass and handling foxes were identified as potential risk factors, whereas particular HLA types turned out to be protective against AE (Table E4 in Appendix E).

For some of the reported risk factors, a biological interpretation of the size and direction of the estimated impact on the risk of infection is lacking. It should be emphasized that some of these potential risk factors may represent confounders (e.g. age, gender and even dog ownership) or other types of biases. In principle, a pooled analysis of European data may be possible if the raw data from the respective studies were made available by the owners of the information. Such a joint analysis might attempt to separate true risk factors from potential confounders, e.g. by stratification or appropriate multivariate analysis.

3.7.3. Risk factors relevant in particular areas

Risk factors in the EU

In Europe, dog ownership, cat ownership, living in a rural area, having a kitchen garden, occupation (farming), haymaking in meadows not adjacent to water, going to forests for vocational reasons, chewing grass and handling foxes were identified as potential risk factors, whereas particular HLA types turned out to be protective against AE (Table E11 in Appendix E).

Risk factors outside the EU

Although the socio-cultural situation in affected areas outside Europe, in particular in China, may have a major impact on dog-related risk factors, it cannot be ruled out that dog ownership and playing with dogs might also be or become relevant as potential risk factors in Europe.

3.7.4. Knowledge gaps

The number of human AE cases in the MS is not known as there is no requirement for notification at species level at EU level.

As mentioned before, for human AE, a substantial level of underreporting must be anticipated (Jorgensen et al., 2008; Schneider et al., 2013). The incidence of the disease may be three-fold higher as shown by German national surveillance data (Jorgensen et al., 2008).

Although it is clear that humans get infected with EM by oral uptake of tapeworm eggs, the relative importance of some definitive hosts (fox, dog, raccoon dog) for human infection is not known. It can be assumed, however, that cats play a minor role because they often fail to develop patent infections or shed only a small number of eggs.

The proportion of humans developing AE relative to the exposed population is unclear. It has been claimed that a substantial proportion of exposed people does not develop alveolar echinococcosis (Gottstein et al., 2014).

The relative importance of the various risk factors for human infections is unknown. It is unclear, whether the importance of different risk factors varies among different areas. The unknown, presumably very long incubation period (several years) for human AE and the fact that oral uptake of tapeworm eggs is the predominant route of infection for humans makes it extremely difficult to study risk factors because neither the exposure to potentially contaminated food nor the presence in endemic areas can be established or excluded for such long periods of time.

3.8. Impact of *E. multilocularis* infection in animals on public health (linked to TOR3b)

Burden of AE

In untreated cohorts, the fatality exceeded 90% within 10 years (Ammann et al., 1996). The introduction of benzimidazoles such as albendazole during the seventies considerably improved the prognosis (Wilson et al., 1992). Long-term follow-up of 117 patients showed that the 5 year survival increased to 88% with this improved management (Bresson-Hadni et al., 2000). In a study from Switzerland, it was noted that for an average 54-year-old patient diagnosed with AE in 1970 the life expectancy was estimated to be reduced by 18.2 years for men and 21.3 years for women, respectively. By 2005, this was reduced to approximately 3.5 and 2.6 years, respectively (Torgerson et al., 2008).

A global view on AE burden based on disability weights for hepatic carcinoma and estimated age and gender specific incidence, were used to calculate the AE disability-adjusted life years (DALYs) estimated in a median of 666,434 DALYs per annum (CIs 331,000–1.3 million) (Torgerson et al., 2010). Approximately 18,235 (CIs 11,900–28,200) new cases of AE per annum are estimated globally with 16,629 (91%) occurring in China and 1,606 outside China. The annual incidence of confirmed cases was also estimate as 0.02–1.4 per 100,000 persons in Central Europe (WHO, 2001). A more recent estimation is reporting an average incidence of 0.03 to 0.30/100,000 inhabitants/year in endemic countries such as Austria, France, Germany and Switzerland, with some observed nested

clusters reaching an incidence from 4.7 to 8.1 cases/year/100,000 (Gottstein et al., 2015). The estimated median annual number of AE cases from the EU countries in 2010 was 130 (Torgerson et al., 2010), with the highest count for Germany (N=61), followed by France (N=21), and the Baltic states Estonia, Latvia and Lithuania with 9 cases each. The estimated annual number of cases from Switzerland was 20 (Torgerson et al., 2010). Increased incidence rates of human AE have been detected during the last 20 years (see Section 3.7.1). A more recent estimation is reporting around 170-200 human AE cases per year in central and Eastern Europe (Conraths and Deplazes, 2015).

The recent EM findings in several new areas such as Latvia, Lithuania, Estonia, Sweden and Denmark as well as the unexpected increase of human AE incidence in endemic countries such as Switzerland and Austria suggest a spread and increase of the parasite in Europe.

Economic cost in humans:

Benzimidazoles cost for a patient per year was estimated between US$5,500 to 17,800 (Reuter et al., 1998). Total treatment cost per case per life was estimated at US$ 300,000 (Romig et al., 1999b). More recently, in Switzerland, the total median treatment cost for each case of AE was estimated at €103,312 (CIs €90,230–€118,146). In addition, each AE patient lost an average income of € 78,485 (€45,454–€125,614). The median cost per case, when saved pension costs are deducted due to premature mortality, was estimated at €108,762 (€48,302–€178,568), with a total cost per year of €2.0 million (€0.9–€3.5 million). If saved pension costs are not deducted, then the cost per case rises to €182,594 (€144,818–€231,448), with total costs of €3.5 million (€2.5–€4.9 million) (Torgerson et al., 2008).

In the UK, the estimated cost associated with AE potential introduction was composed by 'loss of earnings + cost of hospitalizations + cost of long-term care + cost of fatalities'. Lowest case scenario (using the lowest incidence levels to assess the possible impact on the UK) was £2,177,965. Worst case scenario (using the highest incidence levels to assess the possible impact on the UK) was £33,310,056.

All these economical estimations remain uncertain and extrapolation of costs at European level is difficult, mainly for two reasons: different health care costs among MS and different time to detection which deeply impact on disease stage and consequently on prognosis.

As suggested by Gottstein and colleagues (2015), if early detection and clinical management do not make progress beyond current practices, European health systems could probably face costs in the range of one or more billion euro to care for the number of AE patients expected in the next two decades.

Economic cost in animals:

Since the life cycle of EM takes place largely in wild animals, deworming dogs with praziquantel is the only cost for animals. It should be considered as an indirect cost that can only be applied in those infected MS where the drug is not used for routine de-worming for other parasites.

A model on cost/benefit analysis for deworming cats and dogs for EM was performed in Sweden accounting for veterinary fees. The cost of regular deworming of dogs and outdoor cats in risk areas during a year was estimated at 5.6 million euro for dogs and 4.2 for cats. The model assumed a regular deworming every 28 days with the recommended dose (5mg / kg body weight) of Droncit ® vet (packing tablets 2 x 50 mg) and assumed 7.5% of the dogs and 1.5%of cats in Sweden will be dewormed regularly in accordance with the recommendations. It was also estimated an average weight of 19 kg for dogs and 5 kg for cats.

3.9. Laboratory techniques for the detection of *E. multilocularis* (linked to TOR5)

3.9.1. Overview of the laboratory techniques for detection of *E. multilocularis*

The required properties of tests for EM diagnosis should be to measure the actual infection status with high sensitivity and specificity, able to perform *intra vitam* and post mortem examination of animals, suitable for mass-screening, enable DNA quantification, safe for laboratory personnel and cost effective. A comprehensive view on the descriptions and limitations of the *intra vitam* /*post mortem*

methods used for the detection of *E. multilocularis* in DH and IH is reported in Appendix G, namely sedimentation and counting technique (SCT), segmental sedimentation and counting technique (SSCT), intestinal scraping technique (IST), shaking in a vessel technique (SVT), copro-antigen ELISA, and DNA-based tests.

Two main approaches are used for the diagnosis of EM in foxes:

- The SCT is a post mortem approach at necropsy considered as the reference standard for the detection of EM. It focuses on the identification of EM worms in the intestines using classical parasitological methods. The lower limit of detection of the SCT is high (i.e. a high worm burden is needed for the test to give a positive response). Usually, and particularly in non endemic / free areas, the worm burden is low which reduces the probability of a positive diagnosis in an infected animal. This results in a diagnostic sensitivity of less than 100%, particularly if used in a period close to the introduction. Furthermore, the SCT is a time consuming approach
- DNA-based methodologies for the detection of EM genetic material in faeces or intestinal contents. It is an *intra vitam* or *post mortem* approach providing indirect evidence on the presence of the parasite. Intrinsic limitations of DNA-based methodologies such as inhibitors, costs, small volume of sample to analyse, timing and sensitivity were recently overcome. It might be that newly developed DNA extraction methods such as DNA fishing (MC-PCR) and implemented real-time PCR methods are equally or more sensitive than SCT, particularly in samples with a low worm burden (Øines et al., 2014; Isaksson et al., 2014, Helene Wahlström, Personal communication, 2015).

The diagnostic methods have increasing sensitivity with increasing worm burden (Karamon et al., 2010), but are only applicable on dead animals. In addition, these tests are very labour intensive. The diagnosis of EM in faecal samples from living foxes and other definitive hosts in general is dependent on the stage of infection, i.e. pre-patent or patent infections, and may be hampered by intermittent shedding of eggs. Sieving/flotation can detect eggs, however using microscopy on faecal samples is difficult because the eggs cannot be differentiated from other taeniid eggs and sieving therefore has to be followed by PCR. The copro-antigen ELISA shows good sensitivity in high endemic areas, where infected animals are characterized by high worm burden, but it is not highly sensitive in animals with low worm burdens. Most PCR-tests have a lower sensitivity compared to the SCT. However, a recent study showed, by means of latent class analysis, that the sensitivity of the MC-PCR is comparable to SCT (Wahlström H, 2015).

The predictive values of the different tests depend on the prevalence of EM in the particular ~~study~~ target population. SCT is considered the reference standard, but even this test has limitations in terms of sensitivity as indicated above.

The available studies of the performance of the diagnostic tests for detection of EM in live or dead animals are very heterogenic, which complicates drawing any conclusions from them, also because the different tests aim at different targets (i.e. adult worms / eggs). A systematic review (Casulli et al, 2015) demonstrated that there is a gap in evaluating diagnostic tests with scarce information on the diagnostic sensitivity, even for those tests considered as the most relevant to detect the parasite in definitive wildlife populations. In addition, a lack of standardization of the different diagnostic methods to detect EM may cause variation in diagnostic sensitivity and specificity between labs, which may adopt steps that are slightly deviating from the procedures described in the scientific literature.

Table 12: Summary of the test diagnostic sensitivity values as reported by the MS in the framework of the surveillance implemented according to Commission Delegated Regulation (EU) No 1152/2011 of 14 July 2011 compared to the values reported in Conraths et al., 2015

MS	Test used		Test sensitivity as reviewed by	
	Reported method (Sensitivity)	**Cited Ref by MS**	**Casulli et al. (2015)**	**Conraths et al (2015)**
Finland	MC-PCR (fishing Real time PCR targetting *12S rRNA* gene (78% by internal validation)	Isaksson et al., 2014	88%–95.7%	88% (compared to Isaksson et al, 2014 on RT-PCR)
Ireland	Sedimentation and Counting Technique, tecnica parassitologica classica (Se 98%)	Eckert, 2003	98%	83.8% (Eckert et al. 2001)
Malta	Sieving/flotation of faecal samples for copro-egg detection and Multiplex-PCR targetting 12S rRNA and *nad1* genes (Se 94%)	Mathis et al. 1996	88%–95.7%	50% (Trachsel et al., 2007)
UK	sieving/flotation of faecal samples for copro-egg detection and modified PCR Cest1-Cest2 targetting *nad1* mithocondrial gene (proposed Se 85%)	Mathis et al. 1996; Dinkel et al., 1998	88%–95.7%	89% (compared with IST)
Norway	MC-PCR (fishing Real time PCR targetting *co1*mithocondrial gene) (Se 63%)	Øines et al., 2014	88%–95.7%	88% (Isaksson et al., 2014)

Table 12 summarises and describes the uncertainty around the diagnostic sensitivity of the different tests used by the laboratories involved in the surveillance programmes adopted by the MSs listed under the Commission Delegated Regulation (EU) No 1152/2011. It can be seen that there is a high degree of discrepancy between the values reported by the MSs and the ones reported in the reviews performed by Casulli and by Conraths (Casulli et al. 2015; Conraths et al., 2015). Morover, even when the reported method is similar (e.g. Finland and Norway) the proposed values are very different. Finland performed an internal validation of the test used, concluding differently from Isaksson (Isaksson et al., 2014) on the test sensitivity. This confirms the above statement on the important role of the internal protocol when estimating the diagnostic sensitivity and on the need for standardisation.

In conclusion, studies on the diagnostic tests for detection of EM in animals are very heterogenic, which complicates drawing any conclusions from them. Conditions should be established to enable estimation of the test sensitivity of the individual laboratories participating in EM surveillance programmes, in order to achieve better estimates of the true confidence of absence of infection. This should preferable be done in line with the guidelines from OIE (OIE, 2013), which also require a case definition, and coordinated by the EURLP.

3.9.2. Guidance to substantiate test sensitivity estimates

Regulation (EU) No 1152/2011 provides that all Member States listed in Annex I thereof, i.e. the countries where no findings of the parasite have been recorded, shall implement a pathogen-specific surveillance programme. Test sensitivity is one of the parameters required to calculate the number of samples to be taken in a surveillance (EFSA, 2012b). The countries involved use different tests to detect EM (see Section 3.4.1) and their protocols often deviate from those used in publications reporting test sensitivity estimates. As there is no diagnostic method available with 100% sensitivity, it is difficult to evaluate the diagnostic sensitivity of the different tests. Furthermore, it is preferred that test characteristics are evaluated on the population on which the test shall be used. However, this raises another difficulty since there are no positive samples in countries where no findings of the parasite have been recorded.

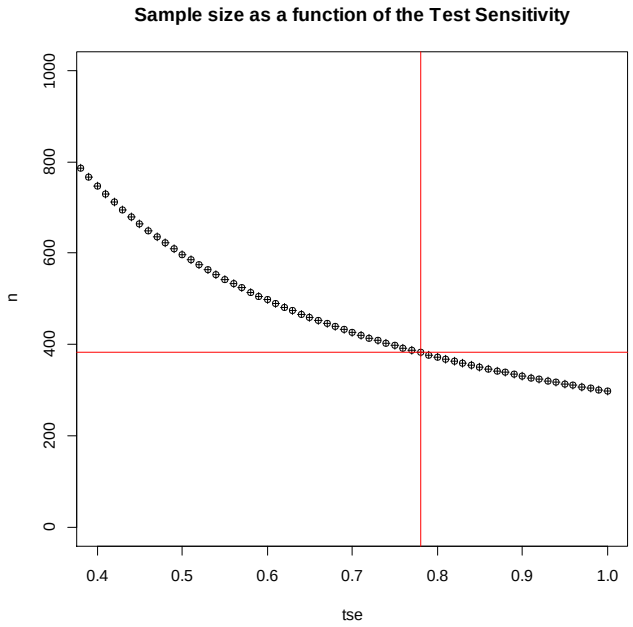


Figure 8: Sample size needed to detect the infection, should this be above the maximum allowed prevalence of 1%, with a 95% confidence, as a function of the test sensitivity

A study should be undertaken to estimate the probability of each relevant test to detect infection, given that the animal is truly infected (according to the definition of test sensitivity), using an adequate sample of specimens from endemic areas where the entire range of different infection stages and intensities are represented. Such a study should follow the OIE Terrestrial Manual, Chapter 1.1.5 (OIE, 2013), and could be coordinated by the EU Reference Laboratory (EURL) for Parasites.

Until better documentation is available, the diagnostic sensitivity should be set conservatively to the lowest value from the ones listed in Table 12, excluding the lowest 20th percentile. In this case, the suggested value to be used for future surveys is 78%. The required sample size for each country will be equal to 383 (Figure 7), the latter allowing the detection of an infected animal with a 95% confidence, should the prevalence in the DH be above the maximum allowed prevalence of 1%.

4. Conclusions

TOR 1: To describe *E. multilocularis* infection in animals in the EU and AC and in particular:

a) the geographical distribution and prevalence of *E. multilocularis* infection in the main infected domestic and wildlife species involved in the *E. multilocularis* lifecycle:

- Until the 1990s, only a 'core' area consisting of Eastern France, southern Germany and parts of Switzerland and Austria were known to be endemic.
- Since the 1980s, EM-infections in animals were recorded in 17 countries in Central Eastern Europe where no cases were reported before.
- The observed prevalence of EM-infected animals as well as the abundance of host species has increased in the Baltic regions, Denmark, the Netherlands, Poland, Romania, Slovakia, Slovenia during the 1990s and later.
- The distribution of EM is not homogeneous, showing areas with high and low prevalence levels in foxes, ranging from values close to 0% (e.g. Denmark, northeast Germany, Sweden), to values close to 50% (e.g. part of France, southern Germany, part of Switzerland).
- These differences in prevalence levels in foxes, among the countries where EM has been reported, have been linked most frequently to the use and structure of landscape, which influences the species range and abundance of rodents as intermediate hosts and to the microclimatic conditions necessary for the transmission and establishment of the parasite.
- Accessible data indicate that, within the Russian Federation, EM and human AE are frequent in parts of Siberia and the Far East.

b) the importance and role of the different host species in the life cycle of the parasite:

Wild carnivores

- Due to its high population densities, high susceptibility to EM infection, high worm burden in infected animals, and higher infection prevalence compared to other potential definitive hosts, the red fox is considered to be the principal definitive host in temperate parts of Europe, Asia and (probably) North America.
- The prevalence of infected animals in raccoon dog populations in Eastern Europe and in Eastern Germany has been shown to reach levels similar to those observed in red foxes.
- Raccoon dog, golden jackal and wolf can act as definitive host, but there is no evidence that they can maintain the lifecycle in the absence of red foxes.

Domestic carnivores

- There is no evidence that dogs and cats can maintain the life cycle of EM in the absence of red foxes.
- The prevalence of EM in the general dog population is very low.
- No systematic assessment has been done anywhere on the quantitative contribution of dogs to the infection of intermediate host populations.
- Living and working with dogs might be or become relevant as potential risk factors for AE in Europe.
- Cats show low susceptibility to experimental infection.
- Natural infection of cats has been recorded in several countries.
- Current knowledge suggests that the contribution of cats to the EM lifecycle is low.

Competent, epidemiologically relevant intermediate hosts

- In Europe, various vole species of the genera *Microtus*, *Arvicola*, *Myodes* and *Lemmus* are confirmed as suitable intermediate hosts based on field studies and/or experimental infections.
- The common vole, *Microtus arvalis*, is the most important intermediate host in areas such as Northeast France (Ardennes) and Switzerland, while the water voles *Arvicola* spp. may maintain transmission in Hungary and urban areas of Central Europe.
- Muskrats (*Ondatra zibethicus*), nutria/coypu (*Myocastor coypus*) and beaver (*Castor fiber*) are suitable intermediate hosts, but are likely to be infrequent prey for foxes due to their large size and habitat specificity.
- The relative importance of different rodents and other small mammal species for maintenance of the lifecycle differs according to geographical areas, the type of environment, prevalence of infection and other parameters. This extreme variability does not make any of those potential IH particularly suitable for surveillance purposes.

c) the risk factors for and the probability of introduction and establishment of *E. multilocularis* in areas where it is has never been recorded, through the movement of infected domestic and wildlife species involved in the *E. multilocularis* lifecycle;

Introduction

- Movement of definitive hosts with a pre-patent or patent infection (i.e. infected domestic and wildlife species involved in the *E. multilocularis* lifecycle) is an important introduction pathway.
- In principle, EM can also be introduced by infected intermediate hosts that carry fertile larval stages (metacestodes) or infectious parasitic stages, or by other items, e.g. plants, contaminated with eggs into free areas.
- It is difficult to distinguish introduction of EM from its first detection (i.e. established, but not detected) if no adequate surveillance had been in place in areas deemed to be free.
- Based on the introduction model used in this opinion, in order to reach a probability of introduction of at least 75%:
  - The **presence of the border compliance checks** increases the number of dogs that need to pass the border by 1.75 to 4 times.
  - If no border compliance check is in place for a country adjacent to an endemic area (**prevalence in foxes equal to 16%**) introduction would require 75 to 1200 times more dogs than foxes crossing the border. If border compliance checks are in place, 150 to 2550 times more dogs than foxes crossing the border.
  - For a free country adjacent to an area with a **very low prevalence in foxes (0.001%)** the crossborder movement of dogs has a prominent role (1.16 to 2.31 fewer dogs coming from an endemic area relative to migrating foxes).
  - The **degree of non-compliance to treatment among dogs that are not checked at the border** (because no border checks are in place or because the border checks have been evaded) plays a less important role on the probability of introduction of EM, compared to other parameters.
  - Despite the implementation of appropriate mitigation measures, it is inevitable that infected dogs enter free countries. However, other factors in addition to introduction are important in establishing EM in free countries.
- In this opinion, the model results do not represent any particular MS.

Establishment

- For the transmission and establishment of the life cycle, appropriate definitive and intermediate hosts must be present.

- Environmental factors influence the persistence of the lifecycle; therefore the probability of EM becoming established will vary from one area to another. However, the knowledge on the geographical distribution of the environmental factors for the persistence of the life cycle is scarce.
- In areas where no suitable autochthonous wild canid hosts and no highly suitable intermediate hosts exist, e.g. Malta, establishment of the EM cycle is considered close to impossible.

TOR2: To assess the current situation in the EU and AC regarding:

a) the monitoring and surveillance programmes of *E. multilocularis* infection in definitive and intermediate hosts, and the probability of detection if *E. multilocularis* is introduced into areas where it is has never been recorded;

- Only the countries that are listed under Commission Delegated Regulation (EU) No 1152/2011 are obliged to implement surveillance activities.
- The diagnostic sensitivity of the tests used in these EM surveillance programmes is not supported by robust scientific evidence and the tests are not validated according to OIE standards.
- Obtaining a representative sample from host populations is hampered by the impossibility of implementing a representative random sample in wildlife and the scarcity of knowledge on the distribution of red fox populations at regional level.
- Political borders do not necessarily match with the epidemiologically relevant units as they do not provide a barrier for wildlife EM hosts.
- In order to allow early detection of EM infection, a very low design prevalence of e.g. 0.1% is required, as it may take many years for EM to reach a prevalence of 1% in the population. However, such a low design prevalence may make surveys for early detection impracticable due to the large sample size required.
- Provided that the risk factors are properly documented, the implementation of a risk based approach may allow a reduction of the sample size.
- For the purpose of demonstrating absence of infection, the inclusion of the concept of the Bayesian Probability of Freedom in the regulation, may allow a reduction of the sample size.
- The notification of the detection of the parasite is mandatory only in a few MS and there is no requirement for monitoring or surveillance of EM in EU countries where findings of the parasite have been recorded.
- The detection of the parasite is currently not notifiable in non-free MS. Occurrence may be reported at genus or species level. However, *E. multilocularis* and *E. granulosus*, although they belong to the same genus, have different lifecycles and cause completely different pathologies in humans. *Echinococcus* notifications should always be done at species level to enable an understanding of the actual trend and geographical distribution of these infections.
- Considering the spatial and temporal heterogeneity in the EM distribution within a country, the results of local or regional surveys cannot be extrapolated to a whole country.

b) the programmes for the eradication of *E. multilocularis* in wildlife host species;

- Eradication of EM in the European wildlife could theoretically be achieved by means of baits in small areas where foxes are present, but the intervention needs to be perpetuated to maintain the status.
- In large areas, long term control - but not elimination - of the parasite may be possible by baiting.

- Control by baiting requires more knowledge about how and where to control the parasite in a cost efficient way.
- Increased fox hunting/trapping is not considered to be effective in controlling the parasite.

TOR3: To describe the current situation in the EU and AC regarding:

a) the risk factors associated with human AE;

- The following potential risk factors have been identified in Europe: Dog ownership, cat downership, living in a rural area, having a kitchen garden, occupation (farming), and haymaking in meadows not adjacent to water, going to forests for vocational reasons, chewing grass and handling foxes.
- Particular Human leukocyte antigen types have been found out to be protective against AE.
- The presumably very long incubation period of the human AE makes the study of risk factors extremely difficult; hence uncertainty on the risk factors is high.

b) the impact of *E. multilocularis* infection in animals on public health;

- The true number of cases of AE is not known in Europe mainly because of under-reporting.
- There has been an increase in the number of reported AE cases in new areas, such as Lithuania and Latvia, and an increase of the human AE incidence in endemic countries, such as Austria, France, Poland and Switzerland, which suggests a geographic spread and an increase of this disease/infection in Europe.
- If early detection and clinical management do not make progress beyond current practices, European health systems could probably face costs in the range of one or more billion of euros to care for the number of AE patients expected in the next two decades.

TOR4: To describe the efficacy of available *E. multilocularis* drugs and the effectiveness of the current species-specific treatment protocols to protect domestic species against the parasite;

- Due to its favorable pharmacokinetic properties and activity against both immature and mature stages, praziquantel is the substance of choice for the treatment of EM in definitive hosts, including travelling or imported dogs.
- In addition to treatment efficacy, the treatment timing is crucial. A general rule is to treat as close as possible to entry into a free country.
- Results of model simulations indicate that:
  - The risk of introduction/transmission/establishment, expressed as a function of the number of eggs deposited in a free area, can be reduced by treating moving dogs before or after entering the free area.
  - Treating dogs earlier than 24 hours before entering a free area allows the risk of reinfection before moving. Therefore, the risk introduction / transmission / establishment is the lowest when treating one day prior to crossing the border and increases when the treatment is administered more than 24 hours before crossing the borders.
  - The shorter the visit of a dog living in an endemic area to a free area, the more effective it is to treat the dog before it enters the free area compared to treating the dog after it enter the free area.
  - The shorter the visit of a dog living in a free area to an endemic area, the greater is the advantage of delaying treatment until the dog has returned to the free area.

TOR5: To assess the laboratory techniques for the detection of E. multilocularis in live and dead animals, in terms of sensitivity, specificity, predictive values and practicability (i.e. rapidity, large scale use, ease of use).

- The SCT is a post mortem approach at necropsy considered as the reference standard for the detection of EM. The lower limit of detection of the SCT is high. Usually the worm burden is low and this results in a diagnostic sensitivity of less than 100%, particularly if used in a period close to the introduction. Furthermore, the SCT is a time consuming approach
- DNA-based methodologies for the detection of EM genetic material in faeces or intestinal contents may be equally or more sensitive than SCT. Intrinsic limitations of DNA-based methodologies such as inhibitors, costs, small volume of sample to analyse, timing and sensitivity were recently overcome.
- Lack of standardization of diagnostic methods detecting EM probably causes variation in sensitivity and specificity between labs.
- Studies on the diagnostic tests for detection of EM in animals are very heterogenic, which complicates drawing any conclusions from them. Conditions should be established to enable estimation of the test sensitivity of the individual laboratories participating in EM surveillance programmes, in order to achieve better estimates of the true confidence of absence of infection.

5. Recommendations

- The public health risk associated with human cases of AE is the reason behind the EU regulation to control and monitor EM in animal species. It is therefore essential that notification of human AE (and CE) cases be made mandatory in all Member States to enable effective and coherent monitoring of trends of AE (and EC) occurrence in humans. A reconsideration of 'echinococcosis' case definition in the current Commission Decision 2012/506/EU, differentiating alveolar from cystic echinococcosis, will be crucial to collect specific epidemiological and clinical data to manage and trace back these infections.
- Routine surveillance to substantiate the absence of EM in domestic dogs is not scientifically justified in countries where no definitive wildlife hosts are present. Such countries, e.g. Malta, do not need to carry out surveillance on domestic dogs to substantiate EM-freedom. The option of making the treatment non compulsory anymore for dogs entering such country is a public health issue and relates to the risk of humans getting infected by the parasite by means of contaminated dog faeces.
- Surveillance to enable early detection of newly introduced EM in an assumed free country requires a considerable increase in the number of samples and tests compared to what is needed for substantiating absence of infection from EM at a design prevalence of 1%.
- Reconsideration of some aspects of the current legislation to optimise the surveillance activities might be relevant. In detail: (i) the identification of epidemiologically relevant units should be independent from the political borders; (ii) for the purpose of demonstrating absence of infection, the inclusion of the concept of the Bayesian Probability of Freedom in the regulation, may allow a reduction of the sample size.
- Studies to improve the knowledge on epidemiological risk factors, including geographical risk factors, should be encouraged to enable well-founded risk-based sampling in geographical subpopulations of hosts to improve the detection.
- Studies to improve the knowledge on the probability of transmission and establishment of new EM introduction in free countries should be encouraged.

- Reconsideration of the definition of the optimal treatment window (presently up to 120 hours before entry) when moving dogs from infected to non-infected countries might be worthwhile to reduce the risk of reinfection. A general rule is to treat as close as possible to entry into a free country.
- A study should be undertaken to estimate the probability of each relevant test to detect infection, given that the animal is truly infected (according to the definition of test sensitivity), using an adequate sample of specimens from endemic areas where the entire range of different infection stages and intensities are represented. Such a study should follow the OIE Terrestrial Manual, Chapter 1.1.5 (OIE, 2013), and could be coordinated by the EURL for Parasites.
- Until better documentation is available, 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 under Commission Delegated Regulation (EU) No 1152/2011. In this case, the suggested value to be used for future surveys is 78%.

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Glossary

Adjacent country	Country sharing a land border with a Member State of the European Union
Border check	Checks made by appointed staff at the entry points into the UK for travelling pets. The transport companies (airlines, ferry operators and Eurotunnel staff) are responsible for ensuring the paperwork for travelling pets is correct. In turn, the transport companies are licensed and audited by APHA to make sure they are doing a good job.
Patency	the condition of showing detectable parasite infection
Prepatent period	The interval between infection of an individual and the first ability to detect from that host a diagnostic stage of EM.
Proglottid	One of the segments of a tapeworm, containing the reproductive organs.
Probability of introduction	Probability that at least one infected definitive host, or that at least one infected and infectious intermediate host, are introduced into a free MS (or parts thereof) during a defined period
Probability of transmission	Given that introduction has occurred, it is the probability that the parasite infects an intermediate or definitive host which in turn infects a definitive or intermediate host.
Probability of establishment	Probability that an infected DH is introduced into a free area and is able to transmit the parasite to an IH and the next DH; such that it leads to completing the life cycle and into the future. A function of both the introduction and transmission probabilities, but also dependent upon other environmental factors, population demographics and likely repeated introductions.
Overall probability of establishment	Perpetuation, for the foreseeable future, of a pest (organism or disease) within an area after entry

Abbreviations

AC	adjacent country
AE	alveloar echinococcosis
BC	border compliance checks
CE	cystic echinoccosis
cELISA	copro-antigen enzyme-linked immunosorbent assay
CI	confidence Interval
CL	confidence Level
DALY	disability-adjusted life years
DH	definitive host
EM	*Echinococcus multilocularis*
EUSR	European Surveillance Report
HLA	human leukocyte antigen
IH	intermediate host
IST	intestinal scraping technique
MC-PCR	magnetic capture – polymerase chain reaction
MS	Member State of the European Union
NBC	no border compliance checks
OIE	Office International des Épizooties
SCT	sedimentation and counting technique
SSCT	segmental sedimentation and counting technique
SVT	shaking in a vessel technique

Appendix A – Overview tables for host species, geographic distribution and prevalence

Table A1: Evidence regarding definitive host species for *E. multilocularis* in Europe

Host species	Type of evidence	References (selected)
Red fox (*Vulpes vulpes*)	Natural and experimental infection	See refs. Table A4; Kapel et al., 2006
Arctic fox (*Vulpes lagopus*)	Natural infection	Peklo, 2014; Stien et al., 2010
Raccoon dog (*Nyctereutes procyonoides*)	Natural and experimental infection	Machnicka et al., 2003; Schwarz et al., 2011; Kapel et al., 2006
Wolf (*Canis lupus*)	Natural infection	Bagrade et al., 2009; Schurer et al., 2014
Golden jackal (*Canis aureus*)	Natural infection	Szell et al., 2013
Domestic dog	Natural and experimental infection	Gottstein et al., 2001; Dyachenko et al., 2008; Kapel et al., 2006
Wild cat (*Felis s. silvestris*)	Natural infection	Umhang et al., 2015
Domestic cat	Natural and experimental infection	Meyer & Svilenov, 1985; Petavy et al., 2000; Dyachenko et al., 2008; Kapel et al., 2006; Umhang et al., 2015

Table A2: Evidence regarding intermediate host species for *E. multilocularis*

Host species	Type of evidence	Host competence in hosting / transmitting EM	References (selected)
Common vole (*Microtus arvalis*)	natural and experimental infection	competent	Loos-Frank, 1987; Delattre et al., 1988; Woolsey et al., 2015a
Field vole (*Microtus agrestis*)	natural and experimental infection	competent	Delattre et al., 1988; Woolsey et al., 2015b
Common pine vole (*Microtus subterraneus*)	natural infection	competent	Delattre et al., 1988
Sibling vole (*Microtus levis*)	natural infection	competent	Stien et al., 2010
Bank voles (Myodes spp.)	natural infection	competent	Delattre et al., 1988; Reperant et al., 2009
Water voles (*Arvicola* spp.)	natural infection	competent	Hofer et al., 2000; Petavy et al., 2003
Snow vole (*Chionomys nivalis*)	natural infection	competent	Genov et al., 1980; Siko Barabasi et al., 1995
Lemming (*Lemmus lemmus)*	natural and experimental infection	competent	Peklo, 2014; Ohbayashi et al., 1971
Muskrat (*Ondatra zibethicus*)	Natural and experimental infection	competent	Romig et al., 1999a; Hanosset et al., 2008; Ohbayashi et al., 1971
Beaver (*Castor* spp.)	natural infection	competent	Janovsky et al., 2002; Cirovic et al., 2012
Nutria (*Myocastor coypu*)	natural infection	competent	Hartel et al., 2004, Umhang et al., 2013
Alpine marmot (*Marmota marmota*)	natural infection	competent	Callait, 2003
Muridae (*Apodemus* spp., *Mus* spp., *Rattus* spp.)	natural and experimental infection	partly competent	Delattre et al., 1988; Petavy et al., 1991; Ohbayashi et al., 1971
Brown hare (*Lepus europaeus*)	natural infection	competent	Chaignat et al., 2015
Shrew (Sorex sp.)	natural infection	competent	Ohbayashi et al., 1971

Host species	Type of evidence	Host competence in hosting / transmitting EM	References (selected)
Large omnivores (suids)	Domestic pig: natural and experimental infection; wild boar: natural infection	Refractory after early metacestode development	Sydler et al., 1998; Pfister & Frank, 1988; Pfister et al., 1998
Ruminants and horses	various ruminants: experimental infection; horses: natural and experimental infection	Refractory after early metacestode development	Lukashenko 1971; Ohbayashi et al., 1971; Miyauchi et al., 1984
Non-human primates	Accidental infection in captivity and experimental infection	Competent to refractory	Deplazes & Eckert, 2001; Rehmann et al., 2005; Ohbayashi et al., 1971
Domestic dogs	Natural infection	Invasive, but largely sterile growth	Deplazes & Eckert, 2001

Table A3: Current *E.multilocularis* status of EU MS and adjacent countries (present as map). Presence in humans is only reported for Turkey (strong, but almost exclusive evidence of presence) and for countries of doubtful endemicity status (defined as such due to uncertainty on the origin of infection). DH: definitve hosts, IH: intermediate hosts

A: Endemic countries	Evidence for presence (recorded host species with number of references)	References (only one reference per species)
Austria	DH: red fox (6)	See Table A4
Belarus	DH: fox (2) IH: voles (1), muskrat (1), coypu (1), other rodents (1), shrews (1)	See Table A4; Marcinkute et al., 2015
Belgium	DH: red fox (8) IH: voles (1), muskrat (2)	See Table A4; Hanosset et al., 2008
Bulgaria	IH: voles (2)	Genov et al., 1980, Genov et al., 1981
Czech Republic	DH: red fox (3), dog (1), cat (1) IH: voles (1)	See Table A4; Martinek et al., 1998; Martinek et al., 2001a; Cada et al., 1999
Denmark	DH: red fox (3), cat (1)	See Table A4; Dyachenko et al., 2008
Estonia	DH: red fox (1), raccoon dog (1)	See Table A4; Marcinkute et al., 2015
France	DH: red fox (17), dog (1), cat (2) IH: voles (13), muskrat (2), coypu (1), other rodents (2)	See Table A4; Petavy et al., 1991; Petavy et al., 2000; Delattre et al., 1988; Umhang et al., 2013; Umhang et al., 2015
Germany	DH: red fox (52), raccoon dog (3), dog (2), cat (5) IH: voles (3), muskrat (9), coypu (2)	See Table A4; Schwarz et al., 2011; Dyachenko et al., 2008; Loos-Frank, 1987; Romig et al., 1999a; Hartel et al., 2004
Hungary	DH: red fox (4), golden jackal (1)	See Table A4; Szell et al., 2013
Italy	DH: red fox (5)	See Table A4
Latvia	DH: red fox (1), raccoon dog (1), wolf (1)	See Table A4; Bagrade et al., 2008; Bagrade et al., 2009
Liechtenstein	DH: red fox (1)	See Table A4
Lithuania	DH: red fox (2), raccoon dog (1), dog (1) IH: muskrat (1), pig (1)	See Table A4; Bruzinskaite et al., 2007; Bruzinskaite et al., 2009; Bruzinskaite-Schmidhalter et al., 2012; Mazeika et al., 2009
Luxembourg	DH: red fox (1) IH: muskrat (1)	See Table A4; Nicodemus, 2012
Netherlands	DH: red fox (6), cat (1) IH: muskrat (1)	See Table A4; Dyachenko et al., 2008; Borgsteede et al., 2003
Norway (Svalbard only)	DH: arctic fox (1) IH: voles (1)	Stien et al., 2010
Poland	DH: red fox (14), raccoon dog (1)	See Table A4; Machnicka et al., 2003; EFSA,

A: Endemic countries	Evidence for presence (recorded host species with number of references)	References (only one reference per species)
	IH: pig (2), wild boar (1)	2015a
Romania	DH: red fox (1) IH: voles (1)	See Table A4; Siko Barabasi et al., 1995
Russia	DH: red fox (1), arctic fox (1) IH: lemming (1)	Peklo, 2014
Slovakia	DH: red fox (11), raccoon dog (1), wolf (1), dog (2)	See Table A4; Hurnikova et al., 2009; Martinek et al., 2001b; Antolova et al., 2009
Slovenia	DH: red fox (1) IH: *Apodemus* (1)	See Table A4; Brglez & Krystufek, 1984
Sweden	DH: red fox (2)	See Table A4
Switzerland	DH: red fox (14), dog (2), cat (1) IH: voles (10), pig (2)	See Table A4; Gottstein et al., 2001; Stieger et al., 2002; Sydler et al., 1998
Turkey	Numerous human cases, mainly from eastern Anatolia; old unverifiable record from one fox and (probably misdiagnosed) cases in cattle	Altintas, 1998; Altintas, 2003; Miman & Yazar, 2012
Ukraine	DH: red fox (2)	See Table A4
B: Free countries	**Evidence for absence (number of references, examined host species)**	**References**
Finland	5 (red fox, raccoon dog, voles)	Wahlström et al., 2011; EFSA, 2007; EFSA, 2013a; EFSA, 2014
Ireland	4 (red fox)	Murphy et al., 2012; EFSA 2011-2014
Malta	2 (dog)	EFSA, 2013b; EFSA, 2014
Norway (mainland only)	8 (red fox)	Davidson et al., 2009; Eckert et al., 1991; Wahlström et al., 2011 ; EFSA, 2013a; EFSA, 2015a ; EFSA, 2015b
United Kingdom	5 (red fox, dog, cat)	Smith et al., 2003; Learmount et al., 2012; Dyachenko et al., 2008; EFSA, 2013b; EFSA, 2014
C: Countries with uncertain endemicity status	**Reason for uncertainty**	**References**
Albania	No data	
Bosnia-Herzegovina	Old, unverifiable records from cattle (probably misdiagnosed)	Kolarova, 1999
Croatia	Border to endemic country (one negative survey of 85 foxes)	Rajkovic-Janje et al., 2002
Cyprus	Insufficient data (28 dogs tested negative)	EFSA, 2015a
Greece	Few unverified human cases	Theodoropoulos et al., 1978
Iceland	No data	
Macedonia (FYR)	One human case reported to European Registry (under country 'Greece')	Kern et al., 2003; Kern, pers. comm.
Moldavia	Border to endemic countries; old record from *Mus musculus*	Andreiko, 1961 (cited in Abuladze, 1964)
Montenegro	No data	
Portugal	No data	
Serbia	Border to endemic countries; one negative survey of 1000 foxes); one case of infected beaver, probably introduced	Cirovic et al., 2012
Spain	Insufficient data (1969 foxes tested negative)	EFSA, 2013a

Table A4: *E. multilocularis* prevalence (%) in red fox in EU MS and adjacent countries. Data are from necropsy of fox carcasses unless stated otherwise. Prevalence is expressed as the median of study results. Data are divided in two periods, before and after 1995. Fox population increases (and, where evidence exists, *E. multilocularis* prevalence increases) have started around 1990 in most countries, a development that lasted until approximately 2000. Therefore, the data are (approximately) divided into periods of low and high fox densities. When data were obviously published more than once, only the publication with the most comprehensive information (e.g. regional distribution within a country) was considered (this also applies to EUSR data).

Country	1974 to 1994		1995 to 2015	
	Prevalence (%) -Median (range)-	**Number studies (references)**	**Prevalence (%) -Median** (range)-	**Number studies (references)**
Austria				
West (Vorarlberg,, Tyrol)	**20.5** (-)	1 (EurEchinoReg, 1999)	No data	
Other regions	**2.7** (-)	1 (EurEchinoReg, 1999)	**6.4** (0.2-7.4)	3 (Duscher et al., 2005a; Duscher et al., 2005b; EurEchinoReg, 1999)
Belarus	No data		**7.4** (-)	1 (Shimalov & Shimalov 2003)
Belgium				
North and Brussels	**2.0** (-)	1 (Vervaeke et al., 2006)	**0.7** (0.0-1.8)	4 (Brochier et al., 2007; Vervaeke et al., 2003; Vervaeke et al., 2005; Van Gucht et al., 2010)
South	**33.1** (15.3-51.0)	2 (Losson et al., 1997; Brochier et al., 1992)	**20.2** (19.4-25.1)	3 (Vervaeke et al., 2006; Losson et al., 2003; Hanosset et al., 2008)
Czech Republic	No data		**11.5** (7.4-33.7)	4 (EurEchinoReg, 1999; EFSA, 2006; EFSA, 2007; EFSA, 2013a)
Denmark	No data		**0.6** (0.03-1.2)	2 (Saeed et al., 2006; Wahlström et al., 2015)
Estonia	No data		**29.4** (-)	1 (Moks et al., 2005)
France				
'old' endemic area	**21.5** (4.2-33.7)	7 (Coudert et al., 1970; Petavy et al., 1990; Bert et al., 1987; Baudouin & Aubert, 1993; Pesson & Carbiener, 1989; Petavy et al., 1991; Aubert et al., 1986)	**33.1** (16.0-53.0)	4 (Guislain et al., 2008; Raoul et al., 2001; Combes et al., 2012; EFSA, 2013a)
			29.9 (-) (urban)	1 (Robardet et al., 2008)
'new' endemic area	No data		**9.6** (-)	1 (Combes et al., 2012)
Germany				
Bavaria	**27.8** (15.1-28.0)	3 (Zeyhle et al., 1990; Vos & Schneider, 1994; Nothdurft et al., 1996)	**41.4** (40.4-55.5)	3 (König et al., 2005; Janko et al., 2011; König & Romig, 2010)
			20.2 (-) (urban)	1 (König & Romig, 2010)

Country	1974 to 1994		1995 to 2015	
	Prevalence (%) -Median (range)-	**Number studies (references)**	**Prevalence (%) -Median** (range)-	**Number studies (references)**
Baden-Württemb.	**22.9** (12.0-57.9)	9 (Ewald, 1990; Zeyhle et al., 1990; Schott & Müller, 1990; Wessbecher et al., 1994; Janka & Stoye, 1998; Fesseler, 1990; Bilger et al., 1995)	**37.0** (-) 17.3 (-) (urban)	1 (EurEchinoReg, 1999) 1 (Deplazes et al., 2004)
Berlin	**0.0** (-)	1 (Schoeffel et al., 1991)	No data	
Brandenburg	**8.3** (-)	1 (Tackmann et al., 1998)	**2.4** (-)	1 (Staubach et al., 2001)
Bremen	No data		No data	
Hamburg	**0.0** (-)	1 (Zeyhle et al., 1990)	No data	
Hesse	**29.0** (3.0-38.0)	3 (Zeyhle et al., 1990; Eskens, 1997; Ballek, 1991)	**38.2** (35.9-40.5)	2 (Immelt et al., 2009; Eskens, 1997)
Lower Saxony	**9.8** (5.8-13.8)	2 (Zeyhle et al., 1990; Berke et al., 2008)	**14.4** (-)	1 (Berke et al., 2008)
Mecklenburg-Vorp.	**0.6** (-)	1 (Kiupel, 1996)	No data	
North Rhine-West.	**7.5** (6.8-11.1)	3 (Zeyhle et al., 1990; Ballek, 1991; Takla, 1996)	**23.6** (16.1-36.8)	3 (Hartel et al., 2004; Takla, 1996, EurEchinoReg, 1999)
Rhineland-Palatin.	**6.5** (4.1-9.0)	2 (Jonas & Hahn, 1984; Jonas & Dräger, 1998)	**33.8** (-)	1 (Jonas & Dräger, 1998)
Saarland	**25.6** (19.9-31.3)	2 (Ahlmann, 1996, Meine & Müller, 1996)	No data	
Saxony-Anhalt	**0.8** (-) 21/2573	1 (EurEchinoReg, 1999)	**10.2** (1.4-19.0)	2 (EurEchinoReg, 1999, Denzin et al., 2014)
Saxony	**0.0** (-)	1 (Enge, 1996)	No data	
Schleswig-Holstein	**0.2** (0.0-0.4)	3 (Lucius et al., 1988; Nebel, 1996)	**0.0** (-)	1 (Manke & Stoye, 1998)
Thuringia	**16.2** (-)	1 (Staubach et al., 2011)	**30.9** (-)	1 (Staubach et al., 2011)
Hungary	No data		**9.4** (5.0-12.7)	3 (Tolnai et al., 2013; Sreter et al., 2003; Sreter et al., 2004)
Italy				
Trento-Alto Adige	No data		**0.8** (0.6-1.0)	2 (Di Cerbo et al., 2008; Manfredi et al., 2002)
			10.0 (-) (c-PCR)	1 (Manfredi et al., 2006
other regions	**0.0** (0.0-0.0)	6 (Soldati et al., 1976; Rossi et al., 1983 ; Poglayen et al., 1985 ; Leoni et al., 1986; Iori et al., 1990 ; Guberti & Poglayen, 1991)	**0.0** (0.0-0.0)	3 (Di Cerbo et al., 2008; Calderini et al., 2009; Magi et al., 2009)
			0.0 (-) (c-PCR)	1 (Manfredi et al., 2006)

Country	1974 to 1994		1995 to 2015	
	Prevalence (%) -Median (range)-	**Number studies (references)**	**Prevalence (%) -Median** (range)-	**Number studies (references)**
Latvia	No data		**35.6** (-)	1 (Bagrade et al., 2008)
Liechtenstein	**34.9** (-)	1 (Ewald & Eckert, 1993)	No data	
Lithuania	No data		**58.7** (-)	1 (Bruzinskaite-Schmidhalter et al., 2012)
Luxembourg GD	No data		**21.0** (0.0-37.9)	8 (Ahlmann, 1996; EFSA, 2006; EFSA, 2007; EFSA, 2013a; EFSA, 2015a)
Netherlands	**0.0** (-)	1 (Borgsteede, 1984)	**9.4** (0.7-59.5)	3 (Takumi et al., 2008; Van der Giessen et al., 2004; Van der Giessen et al., 1998; Franssen et al., 2014; Maas et al., 2014)
Poland	**2.6** (-)	1 (Malczewski et al., 1999) (data until 1998)	**15.1** (1.0-20.1)	8 (Borecka et al., 2008; Karamon et al., 2008; Karamon et al., 2011; Borecka et al., 2007; Malczewski et al., 2008; Borecka et al., 2009; Pacon et al., 2006; Karamon et al., 2014)
Romania	**0.0** (-)	1 (Siko Barabasi et al., 1995)	**4.8** (-)	1 (Barabasi et al., 2010)
Russia	**16.6** (-)	1 (Peklo, 2014)	No data	
Slovakia	No data		**19.0** (16.7-30.3)	3 (Miterpakova & Dubinsky, 2011; EFSA 2013a, 2015a)
Slovenia	No data		**2.6** (-)	1 (Vergles Rataj et al., 2010)
Sweden	No data		**0.1** (0.0-0.1)	3 (Wahlström et al., 2011; Wahlström et al., 2012;, SVA 2015)
Switzerland				
North / Northeast	**48.7** (34.2-63.2)	2 (Ewald & Eckert, 1993; Althers, 1996)	**48.5** (44.3-52.7)	2 (Hofer et al., 2000; Hegglin et al., 2003)
Central (Alps)	**7.8** (3.9-11.7)	2 (Ewald & Eckert, 1993, Althers, 1996)	No data	
Berne / Northwest	**37.8** (-)	1 (EurEchinoReg, 1999)	No data	
West	**37.2** (25.5-49.0)	2 (Gottstein et al., 1996, EurEchinoReg, 1999)	**45.9** (45.7-46.1)	2 (Reperant et al., 2007; Fischer et al., 2005)
South / Southeast	**4.8** (2.2-7.1)	3 (Ewald & Eckert, 1993, Althers, 1996, EurEchinoReg, 1999)	**4.7** (3.1-6.4)	1 (Tanner et al., 2006; Guerra et al., 2014)
Ukraine	No data		**2.3** (1.6-3.0)	2 (Kharchenko et al., 2008; Kornyushin et al., 2011)

Austria (n = 3928): Older data show a clear division with high prevalence in the West (Voralberg, Tyrol) and low prevalence in the remaining country. Temporal changes are unclear, because surveys in different parts of Austria were done in different time periods. From 2004/2005, 8/105 fox without regional allocation were reported (EFSA, 2006)

Belarus (n = 94): Only southern part of Belarus studied. Cumulative data 1981-2001. Small sample size.

Belgium (n = 3755): Sample size from the previous period is far smaller than from the latter period (430 vs. 3325); no temporal decrease of prevalence can be construed from these data. There is a clear gradient with high prevalences in the south and low prevalence in the north of Belgium.

Czech Republic (n = 5413): No clear geographical pattern recognizable, a focus with high prevalence in Klatovy district (28 of 44 foxes). Possibly increasing: most recent survey (2011, n=1484) gave 33.7% prevalence.

Denmark (n = 2209): In two country-wide surveys, a total of seven positive foxes clustered in two distinct areas (on Zealand near Copenhagen and on the mainland near the German border).

Estonia (n = 17): Extremely small sample size.

France (n = 6032): Only eastern and parts of northern and central France have ever been surveyed, with a recent overall prevalence of 17.0% (n = 3307). In most the historical endemic areas (eastern France from the Ardennes to Doubs/Jura and Massif Central), prevalence increases compared to pre-1995 surveys are reported. No data are available for western and southern France.

Germany (n = 97,872: Bavaria 5660; Baden-Württ. 18200; Berlin 100; Brandenburg 7895; Hamburg 2; Hesse 2308; Lower Saxony 8488; Mecklenburg-Vorpommern 3576; North Rhine-Westphalia 2058; Rhineland-Palatinate 9824; Saarland 385; Saxony 2155; Saxony-Anhalt 9731; Schleswig-Holstein 1270; Thuringia 26220)**.** Prevalence differences are apparent both countrywide (lower prevalences in the Northeast, higher prevalences in central and southern Germany), but also within federal states (Bavaria, Baden-Württemberg, Thuringia and Lower Saxony). Generally, mountainous, sparsely populated areas show higher prevalence levels than low lying, densely populated regions. Temporal increase pronounced in most parts, although quality of evidence is variable (best documented in Lower Saxony, Baden-Württemberg and Thuringia)

Hungary (n = 1862): Country-wide survey shown gradient from northwestern to southeastern Hungary.

Italy (n = 2717): Positive records only from provinces Trento and Bolzano. Pre-1990 negative surveys (n = 778) from Piemont, Lazio, Emilia-Romagna and Sardinia, more recent negative surveys (n = 858) from Aosta, Lombardy, Veneto, Liguria, Tuscany and Lazio.

Latvia (n = 45): Extremely small sample size. According to a recent review (Marcinkute et al., accepted), a sample of 430 foxes gave a much lower prevalence ('almost halved')**.**

Liechtenstein (n = 129): Only old data available.

Lithuania (n = 269): The high prevalence appears to be evenly spread across the country, even in suburban areas (Kaunas).

Luxembourg (Grand Duchy) (n = 860): In some reviews the country was confused with the Belgian province of the same name. Fox prevalence similar to adjacent parts of Belgium and Germany. Recently AE was found to be common in muskrats.

Netherlands (n = 967): Three of the five studies focus on the only known endemic areas (Groningen and Limburg). The two wider survey (n = 589) gave 0.7% and 1.1% prevalence, which is likely to be more representative for the country.

Poland (n = 7414): Highest regional prevalences in the South (Tatra region) and the Northeast (Warmia and Mazuria), lowest in the Northwest. A trend towards increasing prevalence levels is reported for most parts of the country.

Romania (n = 1096): Surveys restricted to the central and northwestern parts of the country.

Russia: According to Bessonov (2002) and Martynenko et al. (1988), alveolar echinococcosis is endemic in at least 33 regions of the Russian Federation, mainly in the Asian parts. Maps provided in these publication place transmission foci in the Far East and in southern Siberia, while all regions bordering neighbouring countries in Europe (Finland, Baltic states, Belarus and Ukraine) are considered low endemic with morbidity of human AE of <1 / 100 000. According to Peklo (2014), records of *E. multilocularis* from animals in the northwestern part of European Russia are restricted to the tundra area of Nenetsia. The prevalence given in the table refers to red fox infection in Nenetsia Autonomous Okrug.

Slovakia (n = 5580): Prevalence range within the country between 11.5% (Bratislava) and 49.6% (Tatra region, north-central).

Slovenia (n = 428): Country-wide presence, prevalence appears to increase towards the Southeast.

Sweden (n = 8723): No positive samples from a national monitoring 2000-2009, six positive samples from four different areas of south-central Sweden from national monitoring after 2010.

Switzerland (n = 8646): Pronounced regional differences with high prevalence in the North and West, and low prevalence in the Alps and south of the Alps. Most data before 1995, no information on temporal change due to lack of recent data (except in the southern cantons, where prevalence seems to persist at a low level)

Ukraine (n = 418): Prevalence not representative for the country: recent positive records only from foxes in in western Ukraine, there possibly high prevalence (4 of 14 foxes). Old records from foxes also in Crimea region (Abuladze, 1964).

Table A5: Effectiveness of available EM deworming drugs

Reference	dosing regimens (mg/kg body weight)	Day of intervention	sample time (dpi)	SubID sample size	number of samples EM positive	Reduction in worm burden
Andersen et al., 1981	placebo isotonic saline solution once	NA	28 dpi	9	9	100%
	5 mg praziquantel / kg body weight once	21 dpi	28 dpi	9	0	
Schroeder et al., 2009	1 mg emodepside and 5 mg praziquantel/kg body weight once	11 dpi	25, 26 dpi	8	1	99.9996%
	1 mg emodepside and 5 mg praziquantel/kg body weight once	21 dpi	25, 26 dpi	8	0	
	Placebo tablets once	NA	25, 26 dpi	8	8	
Eckert, 2001	5,2-5,8 (5,4) mg/ kg body weight once	20 dpi	24 dpi	4	2	99.60%
	NA	NA	24 dpi	4	4	
	4,9 - 5,3 (5,1) mg/ kg body weight once	20 dpi	24 dpi	4	2	
	NA			4	4	
Andersen, 1985	praziquantel 1mg/kg of body weight + febantel 10 mg/kg of body weight 3 times (21, 22, 23 dpi)	21,22, 23 dpi	28 dpi	6	0	
	NA	NA	28 dpi	6	6	

Reference	dosing regimens (mg/kg body weight)	Day of intervention	sample time (dpi)	SubID sample size	number of samples EM positive	Reduction in worm burden
Rommel et al. 1986	NA	NA	30 dpi	6	6	100%
	5,0 mg/kg body weight once	27 dpi	30 dpi	5	0	
	NA	NA	30 dpi	5	5	
	5,0 mg/kg body weight once	14 dpi	30 dpi	4	0	
	10,0 mg/kg body weight once	14 dpi	30 dpi	4	0	
	NA	NA	30 dpi	4	4	
Sakashita et al., 1995	NA	17 dpi	21 dpi	2	2	
	5 mg/kg body weight	17 dpi	21 dpi	1	0	
Thomas et al., 1978	10 mg/kg body weight	24-27d	2-3 days after treatment	1	0	100%
	5 mg/kg body weight	24-27d		3	0	
	control	NA		4	4	
	control	NA		1	1	
	control	NA		2	2	
	control	NA		2	2	
	control	NA		1	1	
	control	NA		1	1	
	control	NA		2	2	
	10 mg/kg body weight	14d		4	0	
	5 mg/kg body weight	14d		2	0	
	control	NA		2	2	
	control	NA		2	2	
Sakamoto et al., 1977	none	25 dpi	27 dpi	5	5	99.996%
	5 mg/kg body weight once	25 dpi	27 dpi	5	1	
	10 mg/kg body weight once	25 dpi	27 dpi	5	0	
Kazacos et al 1994	5-7 mg /kg Praziquantel	1, 7, 14 dpi	7, 14, 21, 24, 26 , 30 dpi	6	0	100%
Kazacos et al 1993	5-7 mg /kg Praziquantel	21 dpi	28 dpi	18	0	100%

The publications taken into account consist of the selected ones by the SLR excluding those without data on worms. Two additional relevant abstracts were included (not retrieved by the SLR as without full text). This final collection of papers was also used by EMA to define the recommended treatment dose.

Appendix B – Probability of *E. multilocularis* introduction and establishment: a modelling exercise

Probability of introduction

In this appendix, the probability of introduction of the infection into a naïve population is explored through a quantitative model and a few theoretical scenario. The aim is primarily to describe the respective routes and barriers affecting the probability of introduction by the two most relevant routes, i.e. by wild canids (mainly foxes) and by domestic dogs. The difference between those two categories of DH is considerable and can be summarised in a few main points as reported in Table B1.

Table B1: Comparison between wild canids and domestic dogs as DH for EM and the respective characteristics related to the probability of introduction

Characteristic	Wild canids	Domestic dogs
Treatment	NO	Possible, and required when entering a MS listed under Commission Delegated Regulation (EU) No 1152/2011
Border compliance checks	NO	Possible in some MS where no findings of the parasite have been recorded (airports and harbours)
Environment / Habitat	Mainly rural - Suitable for EM	Mainly Urban –less suitable for EM compared to a mainly rural environment
Data on movements	Dispersal distance and population density vary according to geographical and ecological parameters and regions. Some data in scientific literature	Move by owners (Official import data from the UK and Ireland)
Prevalence data for country of origin	Available data in scientific literature	Available data in scientific literature

Because of the differences listed in Table B1, different approaches to estimate the probabilities of introduction by foxes and by domestic dogs have to be used. The two estimates may subsequently be combined in order to estimate an overall probability of introduction into a free area.

In the following sections, the first parameter that is calculated is the probability of introduction P_{intro}, defined as the probability that at least one infected DH, that is or will be excreting eggs, is introduced into a free MS during a one year period. Note that the IH's are not included in this modelling exercise as a route of introduction (the short range movements and the absence of monitoring and control measures make IH less relevant for introduction compared to canids). The important role of the IH's is however included in the probability of transmission (P_{trans}).

Probability of introduction: foxes

The quantitative model described in this appendix aims at estimating the probability that at least one infected fox will migrate from an endemic area into a free area. In the calculations, a **case (s)** is defined as any fox harbouring at least one live parasite, irrespective of its stage of development; the **population of interest (N_{WILD})** is defined as the total number of foxes crossing the borders from an endemic area to a free area in a given time period, the latter being 1 year considering the framework given by the legislation in force.

From the Binomial probability mass formula, it is possible to derive the probability that, out of the total number of foxes moving from an endemic area to a free area (N_{WILD}) in a given period, at least one is infected ($x > 0$), given a certain true prevalence (ρ_{WILD}) of infected foxes in the country of origin. This probability is the probability of introduction ($P_{introWILD}$). Equation C1 gives the formula of the probability of introduction.

$$P(x > 0) = P(x \geq 1) = 1 - P(x = 0) = 1 - [(1 - \rho_{WILD_i})^{N_{WILD_i}}] = P_{introWILD_i} \quad \textbf{B1}$$

Where N_{WILD_i} is the number of foxes moving from the i^{th} endemic area to a free area, x is the number of infected animals, ρ_{WILD_i} is the prevalence of infected foxes in the i^{th} area of origin and $P_{introWILD_i}$ is the probability of introduction in a free area from that i^{th}area of origin.

Data input

It can be seen that the two parameters needed to model the $P_{introWILD_i}$ are: i) the number of foxes moving from an endemic area to a free area (N_{WILD_i}) and; ii) the prevalence (ρ_{WILD_i}) of infected foxes in the area of origin.

Those two parameters are characterised by a large variability. The prevalence, as explained in Section 3.2, is not homogeneous across Europe and there can been large differences even between areas that are close to each other. The number of foxes crossing the borders depends mainly on three parameters: the border length, the fox population density and the distance covered during the migration phase. Several other factors actually influence the movements of foxes: natural factors (e.g. availability of food, reproductive period, competitions among adults and males, competition with other predators, presence of natural barriers) and human factors (hunting pressure, artificial barriers, etc.). However, this level of detail was considered not relevant for the scope of the modelling exercise.

For this reason, different scenarios were investigated: Figure B1 shows the behaviour of the probability of introduction as a function of the number of animals crossing the border (N_{WILD}) and of the hypothetical prevalence (different colours) in an adjacent endemic area where the animals come from.

The range of prevalence values in Figure B1 reflect realistic prevalence rates of EM infections in foxes observed across the EU member states where findings of the parasite have been recorded, the lowest one (0.1%) representing infected Swedish regions and the highest one (40%) representing high-risk areas e.g. in Germany and inthe Baltic MS (see Table A4 in Appendix A).

Results

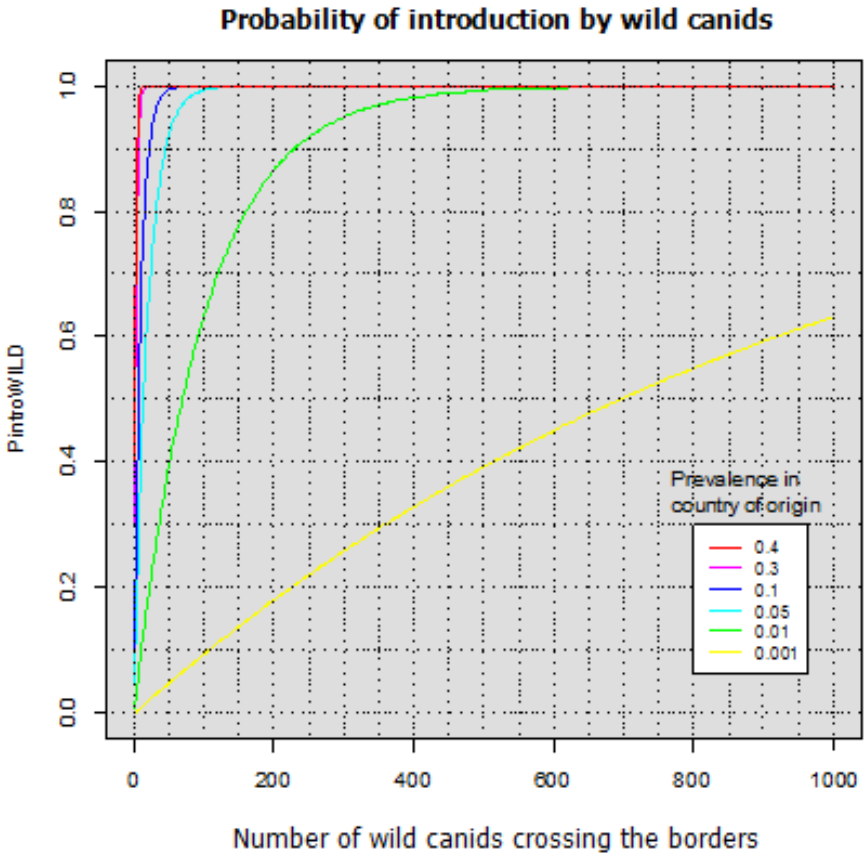

Figure B1: probability of introduction ($\boldsymbol{P_{introWILD}}$) as a function of the number of wild canids (foxes in this modelling exercise) moving from an endemic area to a free area($\boldsymbol{N_{WILD}}$) and of the hypothetical true prevalence of infected foxes in the adjacent endemic area where the animals come from (different colours)

It can be seen that, the higher the prevalence in the (endemic) area of origin and the more the foxes that cross the border, the higher the chance that at least one infected animal is introduced in the free country.

This is of course the simplest scenario, where the area of origin can be considered as a single epidemiological area, characterised by a unique prevalence value. Considering that many authors (Section 3.2 and Appendix A, Table 6) agree on the fact that the geographical distribution of EM is not homogeneous over an area, it is plausible to subdivide the area of origin in sub-areas, each of them characterised by a different true prevalence (of infected foxes) and a different number of foxes moving from an endemic area to a free area.

Figure B2 shows the graphical representation of a model which estimates the probability of introduction from an endemic area of origin subdivided into sub-areas ($Sub - area_i$), each characterised by a different true prevalence (ρ_{WILD_i}) and a different number of migrating animals (N_{WILD_i}).

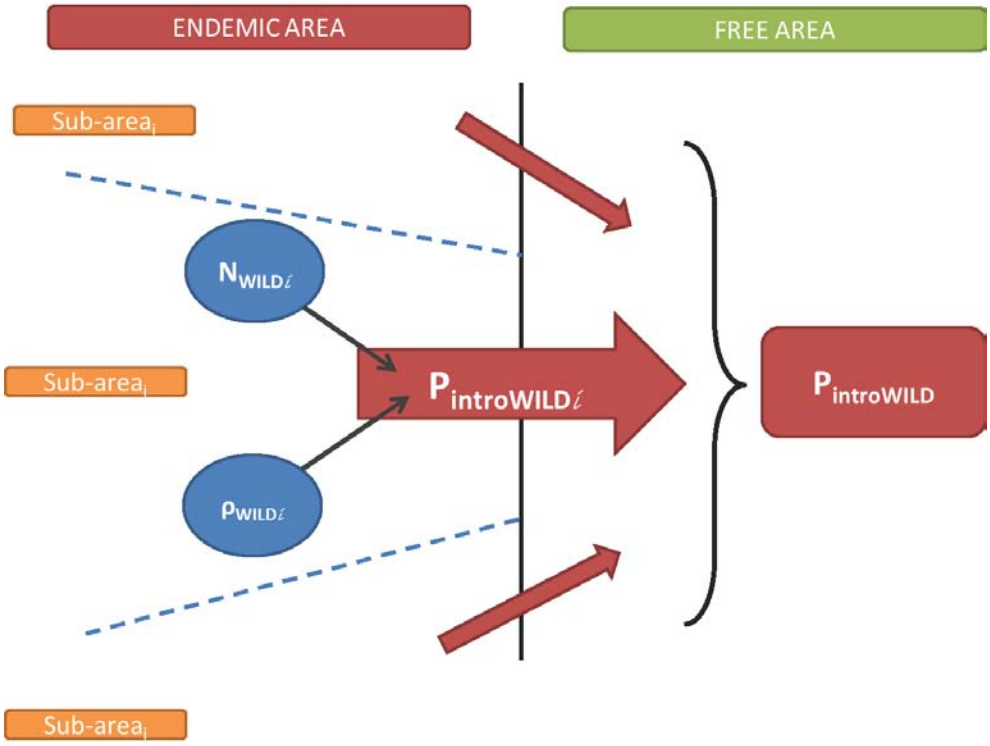

Figure B2: Graphical representation of the model used to estimate the probability of introduction from an endemic area of origin subdivided into sub-areas. NWILDi is the number of foxes moving from the ith sub-area to a free area and ρWILDi is the true prevalence in the ith sub-area.

Following the same reasoning as described above, it is possible to estimate the probability that, out of the total number of animals moving into a free area ($N = \sum_{i=1}^{n} N_i$), from at least one of the sub-areas ($Sub - area_i$), at least one animal is infected.

This probability is given by Equation C2

$$P_{introWILD} = 1 - \prod_{i=1}^{n} P_{no-introWILD_i} = 1 - \prod_{i=1}^{n} \left(1 - \mathrm{P}_{introWILD_i}\right) \qquad \textbf{B2}$$

where $P_{no-introWILD_i}$ is the probability that no infected animal will move into the free area, n is the total number of sub-areas identified in the area of origin and $\mathrm{P}_{introWILD_i}$ is the probability of introduction calculated at sub-area level using equation C1. Of course $P_{no-introWILD_i}$ and $\mathrm{P}_{introWILD_i}$ are complementary probabilities.

It has to be noted that this probability does not express the probability of establishment in the free area. The conditions for turning an introduction into an establishment are discussed in this Appendix.

Probability of introduction: domestic dogs

Estimating the possibility of an introduction by means of an infected domestic dog is completely different. The main differences between the two routes of introduction are presented in Table C1. In practice, many events may occur before an infected dog is introduced into a free area and is able to disseminate eggs in the environment.

These events are captured in the model developed for domestic dogs by the following probabilities:

- Probability that a domestic dog (potentially infected) goes through all the barriers in place ($P_{through}$). The barriers considered in the modelling are listed and described in Table C2.

- Probability that a domestic dog, coming from an endemic area *j* (with a prevalence of infected dogs equal to $Prev_{DOG_i}$), and which went through all the barriers in place is infected ($P_{introDOG_{ji}} = P_{through} \cdot Prev_{DOG_i}$)
- Probability that, out of the total number of domestic dogs showing up at the borders of the free country, at least 1 of them goes through the barriers and is infected ($P_{introDOG}$)

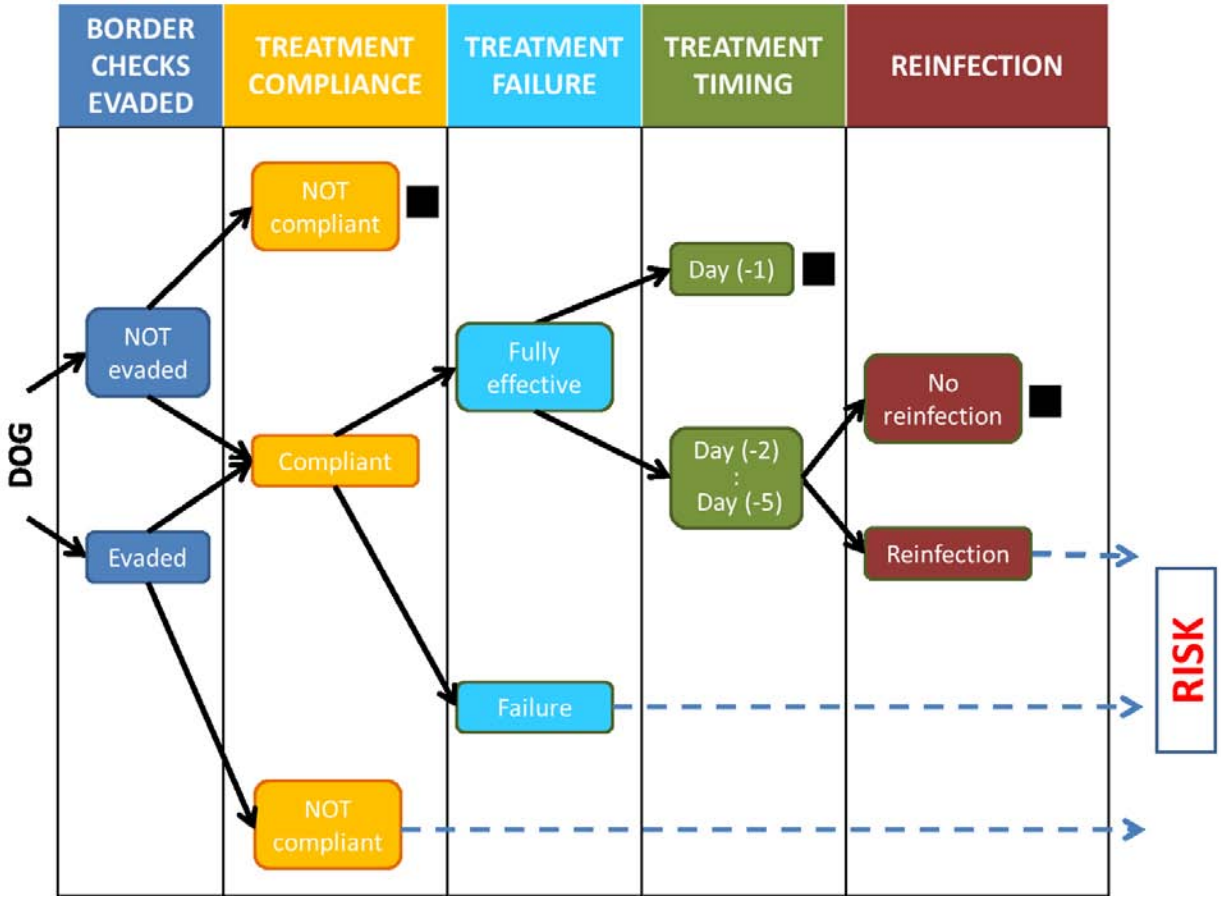

Figure B3: Scenario tree developed to estimate the probability that a domestic dog (potentially infected) goes through all the barriers in place ($P_{through}$) in a free area where border compliance checks are in place. The dashed arrows indicate the successful paths for a potential introduction. The black squares indicate the paths that are not successful.

Border compliance checks in place

Figure B3 shows the most complete model, developed assuming a free area with border compliance checks (BC) in place, in order to estimate the probability that a domestic dog (potentially infected) goes through all the barriers in place ($P_{throughBC}$).

Table B2: Barriers, preventing or reducing the probability of introduction by a potentially infected domestic dog going to an area with border compliance checks n place

Event	Description	Probability	Notation	Definition
BORDER COMPLIANCE CHECKS	Checks made by appointed staff at the entry points into the UK for travelling pets. The transport companies (airlines, ferry operators and Eurotunnel staff) are responsible for ensuring the paperwork for travelling pets is correct. In turn, the transport companies are licensed and audited by APHA to make sure they are doing a good job. (https://www.gov.uk/government/publications/pet-travel-checks-on-pets-by-transport-carriers)	Probability of evading	P_{ev}	Probability of evading the border compliance checks. (Relevant for areas where border compliance checks are in place)
TREATMENT COMPLIANCE	A dog is in compliance with Regulation EC 1152/2011 if the deworming treatment has been administered not more than 120 hours before the entrance	Probability of compliance (probability that a domestic dog has been treated not more than 120 hours before entering the free country)	P_{ke} P_{kne}	Two different values need to be considered: one for the animals that evade the border compliance checks (P_{ke}) and one for the ones that do not evade (P_{kne}). In fact, it is likely that the probability of **not** being in compliance is higher in the escaped one (usually hidden)
TREATMENT FAILURE	The deworming treatment can be not fully effective. Usually, every treatment kills the majority of the worms but it might happen that the clearance is not complete. For the purpose of this modelling and according to the case definition, these dogs still represent a risk.	Probability of failure	P_{fail}	Probability that a deworming treatment does not operate a full clearance of the treated dog.
TREATMENT TIMING	The deworming treatment is considered fully effective if it is administered not more than 24 hours before entering a free area.	Probability that a (in compliance) treatment is old.	P_{day1}	Probability that a dog has been dewormed not more than 24 hours before its entrance.
PROBABILITY OF REINFECTION	If a dog has been treated between 24 and 120 hours before it enters a free area ('old treatment'), the possibility of reinfection in the (potentially) endemic area in which it lives makes the treatment less effective.	Probability of reinfection	P_{reinf}	Probability that a treated dog gets re-infected. (Relevant for dogs treated more than 24 hours before their entrance)

It is now possible to follow the branches of the scenario tree to identify the paths that lead to a potential risk (see Table B3).

It can be seen that the probability that a domestic dog (potentially infected) goes through all the barriers in place in a free area where border compliance checks are in place ($P_{throughBC}$) is therefore:

$$P_{throughBC} = \left[(1 - P_{ev}) \cdot P_{kne} \cdot P_{fail}\right] + \left[(1 - P_{ev}) \cdot P_{kne} \cdot (1 - P_{fail}) \cdot (1 - P_{day1}) \cdot P_{reinf}\right] + \left[P_{ev} \cdot P_{ke} \cdot (1 - P_{fail}) \cdot (1 - P_{day1}) \cdot P_{reinf}\right] + \left[P_{ev} \cdot P_{ke} \cdot P_{fail}\right] + \left[P_{ev} \cdot (1 - P_{ke}) \cdot\right] \quad \textbf{B3}$$

Equation B3 estimate the probability that a single dog introduces the infection, *should this dog be infected*. Indeed, up to this point, the probability of a dog being infected as not been taken into account yet.

Table B3: Probability matrix for a domestic dog (potentially infected) going through all the barriers in place in a free area where border compliance checks are in place ($P_{throughBC}$).

BORDER CHECKS EVADED	TREATMENT COMPLIANCE	TREATMENT FAILURE	TREATMENT TIMING	RE-INFECTION	RISK
NO ($1-P_{ev}$)	NO ($1-P_{kne}$)	-	-	-	NO
NO ($1-P_{ev}$)	YES (P_{kne})	YES (P_{fail})	-	-	YES
NO ($1-P_{ev}$)	YES (P_{kne})	NO ($1-P_{fail}$)	YES (P_{day1})	-	NO
NO ($1-P_{ev}$)	YES (P_{kne})	NO ($1-P_{fail}$)	NO ($1-P_{day1}$)	NO ($1-P_{reinf}$)	NO
NO ($1-P_{ev}$)	YES (P_{kne})	NO ($1-P_{fail}$)	NO ($1-P_{day1}$)	YES (P_{reinf})	YES
YES (P_{ev})	YES (P_{ke})	NO ($1-P_{fail}$)	NO ($1-P_{day1}$)	NO ($1-P_{reinf}$)	NO
YES (P_{ev})	YES (P_{ke})	NO ($1-P_{fail}$)	NO ($1-P_{day1}$)	YES (P_{reinf})	YES
YES (P_{ev})	YES (P_{ke})	NO ($1-P_{fail}$)	YES (P_{day1})	-	NO
YES (P_{ev})	YES (P_{ke})	YES (P_{fail})	-	-	YES
YES (P_{ev})	NO ($1-P_{ke}$)	-	-	-	YES

Border compliance checks NOT in place

If the free country has no border compliance checks in place, the scenario tree and the related probabilities are different. Figure B4 shows a scenario tree for these countries and Table B4 summarises the probability values linked to the barriers (yes please include the values used.

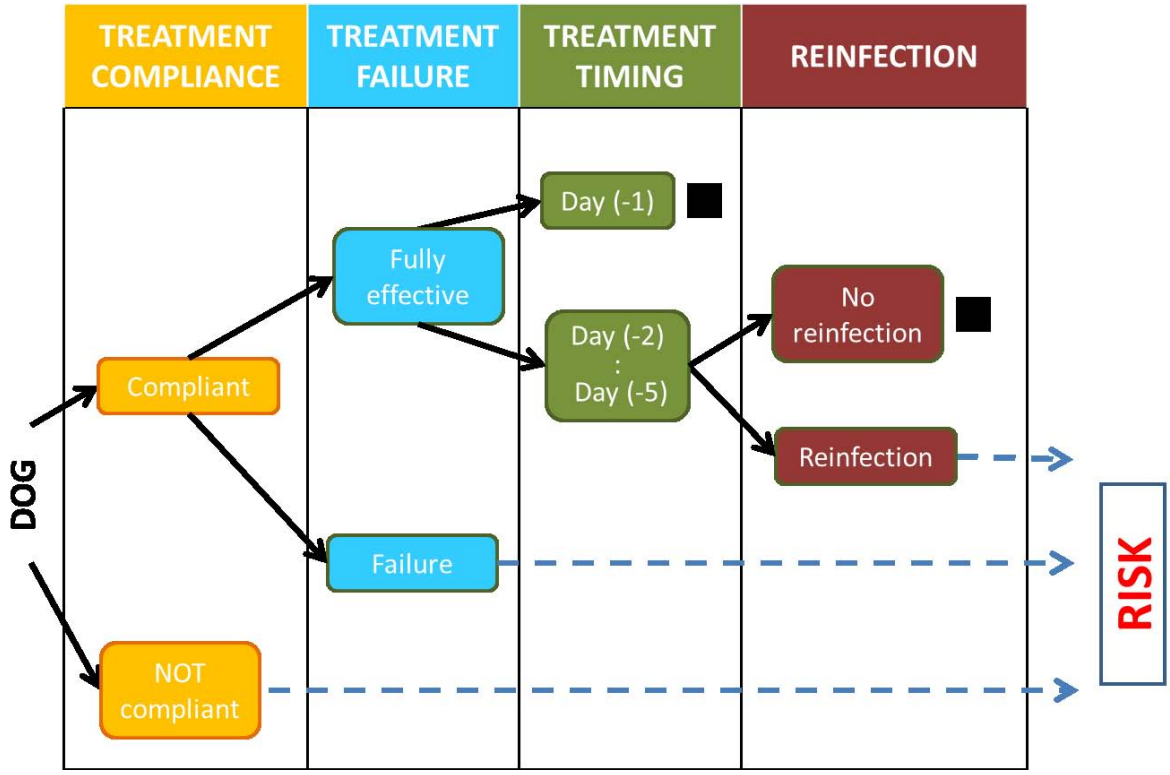

Figure B4: Scenario tree on the probability that a domestic dog (potentially infected) goes through all the barriers in place ($P_{\text{throughNBC}}$) in a free area where no border compliance checks are in place. The dashed arrows indicate successful paths. The black squares indicate no risk.

Table B4: Barriers, preventing or reducing the probability of introduction by a potentially infected domestic dog going to an area where border compliance checks are NOT in place

Event	Description	Probability	Notation	Definition
TREATMENT COMPLIANCE	A dog is in compliance with Regulation EC 1152/2011 if the deworming treatment has been administered not more than 120 hours before the entrance	Probability of compliance (probability that a domestic dog has been treated not more than 120 hours before entering the free country)	P_{kNBC}	Two different values need to be considered: one for the animals that evade the border compliance checks (P_{ke}) and one for the ones that do not evade (P_{kne}). In fact, it is likely that the probability of **not** being in compliance is higher in the escaped one (usually hidden)
TREATMENT FAILURE	The deworming treatment can be not fully effective. Usually, every treatment kills the majority of the worms but it might happen that the clearance is not complete. For the purpose of this modelling and according to the case definition, these dogs still represent a risk.	Probability of failure	P_{fail}	Probability that a deworming treatment does not operate a full clearance of the treated dog.
TREATMENT TIMING	The deworming treatment is considered fully effective if it is administered not more than 24 hours before entering a free area.	Probability that a (in compliance) treatment is old.	P_{day1}	Probability that a dog has been dewormed not more than 24 hours before its entrance.

Event	Description	Probability	Notation	Definition
PROBABILITY OF REINFECTION	If a dog has been treated between 24 and 120 hours before it enters a free area ('old treatment'), the possibility of reinfection in the (potentially) endemic area in which it lives makes the treatment less effective.	Probability of reinfection	P_{reinf}	Probability that a treated dog gets re-infected. (Relevant for dogs treated more than 24 hours before their entrance)

Similarly to the previous situation, it is now possible to follow the branches of the scenario tree to identify the paths that lead to a potential risk (see Table B5).

TableB5: Probability matrix for a domestic dog (potentially infected) going through all the barriers in a free area where no border compliance checks are in place ($P_{throughNBC}$).

TREATMENT COMPLIANCE	TREATMENT FAILURE	TREATMENT TIMING	RE-INFECTION	RISK
NO ($1-P_{kNBC}$)	-	-	-	YES
YES (P_{kNBC})	YES (P_{fail})	-	-	YES
YES (P_{kNBC})	NO ($1-P_{fail}$)	YES (P_{day1})	-	NO
YES (P_{kNBC})	NO ($1-P_{fail}$)	NO ($1-P_{day1}$)	NO ($1-P_{reinf}$)	NO
YES (P_{kNBC})	NO ($1-P_{fail}$)	NO ($1-P_{day1}$)	YES (P_{reinf})	YES

The overall probability that a domestic dog (potentially infected) goes through all the barriers ($P_{throughNBC}$) in a free area where no border compliance checks are in place is therefore:

$$P_{throughNBC} = [1 - P_{kNBC}] + [P_{kNBC} \cdot P_{fail}] + [P_{kNBC} \cdot (1 - P_{fail}) \cdot (1 - P_{day1}) \cdot P_{reinf}] \quad \textbf{B4}$$

Equation B4 estimates the probability that a single dog introduces the infection, *should this dog be infected*. Indeed, up to this point, the probability of a dog being infected as not been taken into account yet.

Individual probability of introduction

As a second step, it is now necessary to include the probability that a domestic dog, which showed up at the border and went through all the barriers in place, is actually infected or not. This probability is available once the prevalence of infected dogs in the area of origin is estimated (ρ_{DOG}). However, it has to be considered that if a dog has spent only a little time in an endemic country (e.g. 2 weeks, as an average holiday period), the probability of being infected will be lower than the prevalence in that country. In this specific example of a dog living in a free country, spending 14 days in an endemic country and then coming back home, the probability of getting infected will be 14/90 (see also Section 3.5). Therefore, the probability of introduction by means of a domestic dog is given by Equation B5

$$P_{introDOG_j} = P_{through(BC\ or\ NBC)} \cdot F_{inf} \cdot \rho_{DOG} \quad \textbf{B5}$$

Where F_{inf} is the reduction factor of the probability of being infected (ρ_{dog}) as a function of the time spent in an endemic area (this will be 1 for dogs living in endemic areas and then entering a free country and 14/90 for dogs living in a free country, spending 14 days in an endemic country and then coming back home) and $P_{introDOG_j}$ is the probability that a single dog (the j^{th} dog) introduces the

disease into a free area with or without border compliance checks in place, i.e. it is infected and went through all the barriers.

In case more than one area of origin needs to be included in the calculation, Equation B5 can be modified as in Equation B6

$$P_{introDOG_{ji}} = P_{through(BC\ or\ NBC)} \cdot P_{inf} \cdot \rho_{DOG_i} \quad \textbf{B6}$$

where $P_{introDOG_{ji}}$ is the probability that a dog from area i will introduce the infection into a free area.

Overall probability of introduction

It is now possible, following the same principle used for the wildlife, to estimate the probability that at least one infected dog, out of the total number of dogs showing up at the border, will introduce the infection into a free area ($P_{introDOG_i}$; see Equation B7)

$$P_{introDOG_i} = 1 - \left[\left(1 - P_{introDOG_{ji}}\right)^{N_{DOG_i}}\right] \quad \textbf{B7}$$

where N_{DOG_i} is the total number of dogs showing up at the border originating from area i.

When more than one area of origin has to be taken into account, the $P_{introDOG}$ needs to be calculated for each area by means of with Equation B7. The overall probability will then be estimated by Equation B8 and will represent the probability that, out of the total number of dogs showing up at the border from a given number of endemic areas, at least one of these dogs from at least one of the endemic areas will introduce the infection.

$$P_{introDOG} = 1 - \prod_{i=1}^{n}\left(1 - \mathrm{P}_{introDOG_i}\right) \quad \textbf{B8}$$

where n is the total number of areas of origin.

Probability of transmission

In the previous section, the probability of introduction has been extensively defined for wildlife and foxes.

In order to estimate the probability of establishment, however, another step needs to be considered. In fact, once an infected-infectious definitive host (DH) has entered a free area, it will transmit the infection, with a given probability, to a suitable intermediate host (IH), which in turns will infect, with a given probability, a naïve suitable DH in the free area. Only if these events take place, the infection will remain in the new (previously free) area.

Many factors are involved in the definition of these probabilities which can be actually only estimated and explored, at this time, by means of '*what if* scenarios'. This is because of the lack of specific quantitative data. As a consequence, the function itself that links the two transmission probabilities to the overall probability of transmission is not known.

Some examples of the data that would be needed are: the probability that a DH disseminates eggs in a habitat which is suitable for the IH and therefore for the parasite; the probability that a DH, depending on its behaviour and feeding conditions, eats an infected IH. It has to be considered that these probabilities may differ consistently between domestic dogs and wild red foxes, making the latter more effective in initiating and maintaining an EM life cycle although no or little evidences are available.

Probability of establishment

In the case of EM, the incursion and establishment are two separate events which involve three populations of animals: imported dogs (incursion); resident rodents (arvicolid rodents most commonly; establishment) and a wildlife carnivore, the red fox or raccoon dog either as a resident

(establishment) or trans-boundary from neighbouring area where findings of the parasite have been recorded (incursion).

Given the probability of transmission ($P_{transWILD}$ and $P_{transDOG}$) and the number of infected animals entering the free area ($N_{introWILD}$ and $N_{introDOG}$), it is then possible to estimate the probability of establishment, which is:

$$P_{estWILD} = 1 - (1 - P_{transWILD})^{N_{introWILD}}$$

$$P_{estDOG} = 1 - (1 - P_{transDOG})^{N_{introDOG}} \quad \textbf{B9}$$

When both foxes and domestic dogs need to be accounted for in the calculation, the overall probability of introduction will be estimated by Equation B10.

$$P_{est} = 1 - \{[(1 - P_{transWILD})^{N_{introWILD}}] \cdot [(1 - P_{transDOG})^{N_{introDOG}}]\} \quad \textbf{B10}$$

It is now possible to consider different theoretical scenarios for the probability of introduction at member state level accounting for both the introduction by means of foxes moving from one or more endemic areas and by means of domestic dogs.

Data needs

The data needed in order to solve the equations presented in the previous sections are summarised in the following tables (see Table B6 and Table B7).

Table B5: Definitions of the probabilities needed to estimate the probability of establishment. All parameters refer to 1 year period.

$P_{through(BC\ or\ NBC)}$	Probability that a domestic dog showing up at the border, goes through all the barriers
$P_{introWILD}$	Probability that, out of the total number of foxes entering a free country from at least 1 of the adjacent endemic countries and/or endemic sub-areas, at least 1 animal is infected.
$P_{introDOG(BC\ or\ NBC)}$	Probability that, out of the total number of domestic dogs that show up at the border from at least 1 of the endemic countries and/or endemic sub-areas, at least 1 dog is infected.
$P_{trans(WILD\ or\ DOG)}$	Probability of transmission of the parasite from DH to a naïve IH and from the latter to a naïve DH.
P_{est}	Probability that the parasite, given its introduction, finds the optimal conditions to start a biological cycle in the naïve area where it has been introduced.

Table B6: Data and estimates needed (left column) to calculate the probabilities of interest (top row). The crosses indicate that the quantity is always needed, 'BC' are the estimates needed in case of the free area having border compliance checks in place and 'NBC' in case of no border compliance checks being in place

		$P_{introWILD}$	$P_{through}$ (Border checks in place)	$P_{through}$ (NO border checks)	$P_{introDOG}$	P_{est}
ρ_{WILD_i}	Prevalence of infected foxes in the ith country of origin	X				X
ρ_{DOG_i}	Prevalence of infected domestic dogs in the ith country of origin				X	X
N_{WILD_i}	Number of foxes crossing the borders in 1 year from the ith country of origin	X				X
N_{DOG_i}	Number of domestic dogs showing up at the borders in 1 year				X	X
n	Total number of adjacent countries and/or sub-areas with endemicity	X			X	X
P_{ev}	Probability that a dog showing up at the borders evades the border compliance checks in place		X		BC	BC
P_{kEV}	Probability that a dog is in compliance, given that it evaded		X		BC	BC
P_{kNEV}	Probability that a domestic dog is in compliance, given that it did not evade		X		BC	BC
P_{kNBC}	Probability that a domestic dog showing up at the borders where no check is in place is in compliance			X	NBC	NBC
P_{oldT}	Probability that a in compliance domestic dog underwent a treatment between 2 and 5 days		X	X	X	X
P_{neff}	Probability that the treatment was NOT effective, given that it is was administered between 2 and 5 days		X	X	X	X

Estimation of the probability of introduction and establishment by means of theoretical scenarios

As discussed in the previous sections, the data needed to estimate the probability of introduction, transmission and of establishment are rather specific. It is unlikely that all the relevant countries have valid evidences to support the estimates for the parameters listed above.

Nevertheless, even if the aim of estimating the exact probability of introduction for a given free Member State is not realistically achievable, it is still possible to better understand the relationship between input (the model parameters) and output (probability of introduction and/or establishment). This is commonly known as 'sensitivity analysis' (Saltelli et al., 2008)

Two main aspects were investigated in this modelling exercise: i) the impact of the border compliance checks (in place / not in place) on the overall probability of introduction; ii) the impact of the red fox on the overall probability of introduction.

Parameterisation of the model

Parameters related to dogs

Number of dogs showing up at the borders in 1 year (N_{DOG_i}):

Official data were provided by UK (DEFRA – APHA, Helen Roberts, personal communication).

Table B7: Data on number of dogs showing up at the borders in UK from 2010 to 2014

	2010	2011	2012	2013	2014
Number of dogs	82,512	85,786	139,644	152,075	155,445
Rate of variation		0.003	0.627	0.089	0.022

Number of dogs moving to the free area (N_{dogs}):

This is an independent variable. The range explored in the modelling exercise was limited to 0 – 30,000 as sufficient to reach a 100% probability of introduction.

Probability of evading the border compliance checks (P_{ev}):

The number of dogs evading the border compliance checks has been estimated by the UK representatives to be around 10% (worst case scenario) of the dogs entering the country (Helen Roberts, personal communication). The last observed data point is 155,445 (Table B8, year 2014). Therefore, the number of dogs that evaded the border compliance checks in 2014 are estimated to be 15,545.

The total number of dogs that leave their countries to go to UK is therefore given by the sum of the number of dogs showing up at the border (155,445) and the number of dogs that evade the border compliance checks (15,545). The total is estimated to be around 170,990.

It is therefore possible to estimate the probability of evading, which is given by the ratio between the number of dogs evading the border compliance checks (15,545) and the total number of dogs that leave their countries to go to UK (170,990). The best guess for the probability of evading is 0.09.

The estimated values were used as parameters of a Beta probabilistic distribution, with uniform prior, in order to include a degree of uncertainty around the best guess (Figure B6).

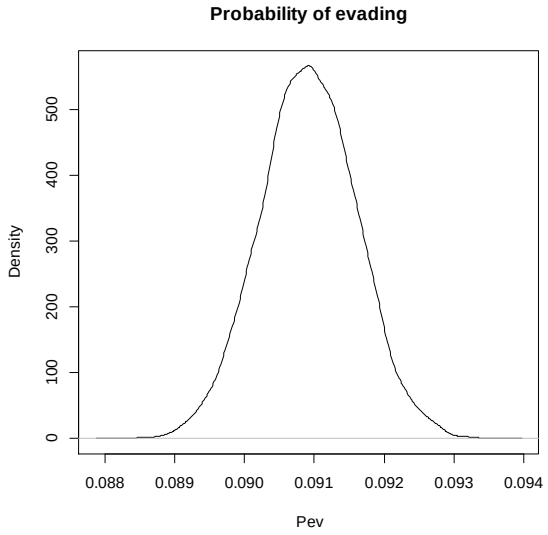

Figure B5: Probabilistic density distribution of the probability of evading.

Probability that a dog NOT evading the border compliance checks is in compliance with the deworming treatment (P_{kne}):

Official data from UK (DEFRA – APHA, Helen Roberts, personal communication) report a number of deworming treatment failure of 2,145 in 2014 (Table B9). These represent the number of domestic dogs which are checked and found to not be in compliance with the deworming treatment. They will be withheld and treated before being allowed to travel. As border checkpoints are not continuously manned (for example at night), there will be a number of pets which are not checked and a proportion of these are likely to be non- in compliance as well.

Table B8: Data on number of dogs showing up at the borders in UK from 2010 to 2014

	2010	2011	2012	2013	2014
EM deworming treatment failures	1,571	1,455	1,762	1,887	2,145
EM deworming compliances	80,941	84,331	137,882	150,188	153,300

It is therefore possible to estimate the probability that a dog that does not evade the border compliance checks is in compliance, which is given by the ratio between the number of EM deworming compliances (153,300) and the total number of dogs that do not evade the border compliance checks (155,445). The best guess for the probability of being in compliance in not evading dogs is 0.99.

The estimated values were used as parameters of a Beta probabilistic distribution, with uniform prior, in order to include a degree of uncertainty around the best guess (Figure B7).

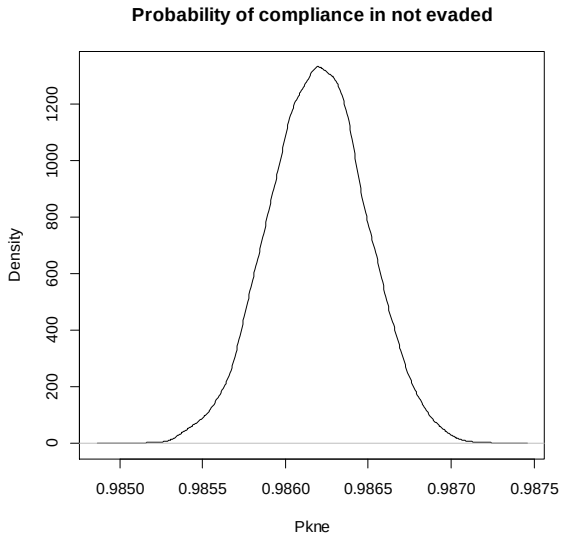

Figure B6: Probabilistic density distribution of the probability of compliance with the deworming treatments in dogs not evading the border compliance checks.

Probability that a dog evading the border compliance checks is in compliance with the deworming treatment (P_{ke}):

Non official data from UK (Helen Roberts, personal communication) allowed to estimate the probability of a dog evading the border compliance-checks to be around 80%. Although this value is much above the known level of compliance (at ~97%), it was considered the most appropriate conservative value. The same percentage was used as the worst level in the quantitative risk model for rabies introduction (Defra, 2011). No uncertainty was considered around this estimate.

Probability that a deworming treatment does not operate a full clearance (P_{fail}):

The scientific literature reports some cases of failure of the deworming treatment (Table A4). More specifically, in some cases not all worms were killed by the treatment. For the purpose of this modelling exercise, those dogs which still host some worm do represent a risk. The value used for this probability comes from the scientific literature and it is the ratio between the number of not-complete clearance out of the total number of dogs treated (0.08). No uncertainty was considered around this estimate.

Probability that a deworming treatment has been administered within 24 hours before entering the free area (P_{day1}):

The available options for a deworming treatment being in compliance are 5 (i.e. 1, 2, 3, 4 or 5 days before entrance). The same probability of occurrence has been assumed for each option (0.2 probability each). Therefore, the probability of a treatment being administered within 24 hours before the entrance is 0.2. No uncertainty was considered around this estimate.

Probability that a dog treated between 24 and 120 hours before entering a free country (i.e. between 2 and 5 days before) gets re-infected after a fully effective deworming treatment (P_{reinf}):

The data derive from the deterministic model described in Section 3.5. The probability of re-infection is summarised in Table B10. Considering that an adult worm does not live more than 90 days in its host, a dog can be at maximum getting infected 90 days before its entrance to represent a risk. This means that a dog living or travelling in an endemic country will have 1/90 probability each day of getting re-infected.

Table B9: Probability of re-infection for each treatment day.

Treatment day (before entrance)	Probability of re-infection
-2 (within 48 hrs)	1/90
-3 (within 72 hrs)	2/90
-4 (within 96 hrs)	3/90
-5 (within 120 hrs)	4/90

Therefore, if a treatment is administered 2 days before the entrance into a free country and considering that the dog cannot be re-infected within 24 hours from the treatment, it will have only 1 day to get re-infected (1/90). The overall probability of a dog getting re-infected, given that the treatment has been administered between 24 and 120 hours, is therefore the average of the four probabilities listed in Table B9 (0.03). No uncertainty was considered around this estimate.

Probability that a dog entering a free country where no border compliance checks are in place is in compliance with the deworming treatment (P_{kNBC}):

This probability was considered analogous to the probability that a dog evading the border compliance checks is in compliance with the deworming treatment (P_{ke}). However, it was considered plausible that a dog's owners could be less incentivised in treating their pets before entering a free country if they know that no border compliance checks are in place. On the other hand, it could not be excluded that dog's owners actually treat their animals independently from a potential control measure. In a study conducted in Sweden (Helene Wahlström, personal communication based on Hirvonen, 2010[7]) where no border compliance checks are in place, it was estimated that about 40% of owners were in compliance with the regulation. In a report from the Norwegian Committee on Food safety (VKM, 2012) it was estimated that about 80% of dogs entering Norway by road was in compliance. The present modelling exercise explores both scenarios using 40% and 80% probability of compliance. No uncertainty was considered around these estimates.

Prevalence of infected dogs in the country from which the entering dog comes (ρ_{DOG_i}):

The data were retrieved from the most recent paper published on the matter (Dyachenko, 2008). A number of 43 dogs were found positive out of 21,588 examined and tested from Germany and other European countries, giving a raw prevalence of 0.002. The study used faecal samples sent to a veterinary laboratory for routine coprological examination. This implies a likely bias towards well-kept dogs which are dewormed frequently and do not roam unsupervised. The prevalence found is therefore likely to be an underestimate compared to the general dog population. However, this fraction of the dog population is also likely to travel as pets with their owners, so we consider it justified to use this figure. The reported values were used as parameters of a Beta probabilistic distribution, with uniform prior, in order to include a degree of uncertainty around the best guess (Figure B7).

Another scenario with a higher prevalence value (0.01) was considered. No uncertainty was considered around these estimates.

[7] Dogs on the move – a study of the travel habits of Swedish dogs and their owners' awareness of infectious diseases, Examensarbete inom veterinärprogrammet. Fakulteten för Veterinärmedicin och husdjursvetenskap Institutionen för Kliniska Vetenskaper, Sveriges Lantbruksuniversitet, Uppsala, 2010. http://stud.epsilon.slu.se/2118/1/Hirvonen_K_110110.pdf

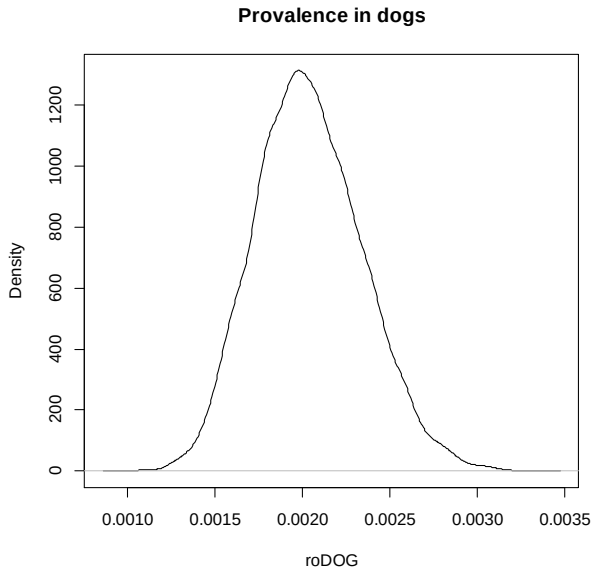

Figure B7: Probabilistic density distribution of the prevalence in domestic dogs

Parameters related to foxes

Number of foxes moving to the free area (N_{foxes}):

This is an independent variable. The range explored in the modelling exercise was limited to 0 – 50 as sufficient to reach a 100% probability of introduction.

Prevalence of infected foxes in the country from which the entering fox comes (ρ_{WILD_i}):

The data were retrieved from the most recent paper published on the matter (Karamon J, 2014). A number of 256 foxes were found positive out of 1,546 examined and tested in Poland, giving a raw prevalence of 0.166.

However, this study was conducted in a highly endemic area and cannot represent the European situation. As an example, the prevalence of infected foxes in the Southern borders of Sweden was estimated to be 0.01, i.e. 10 times higher than the national prevalence (Helene Wahlström, personal communication based on Wahlström et al., 2012 and SVA, 2015[8]) and a similar prevalence (0.008) has been estimated from a survey implemented in a restricted area, known to be infected, in Middle Sweden in 2011 (Wahlström et al., 2015).

In order to cover a range of plausible prevalence values, two scenarios were explored: a first scenario where the number of infected foxes is extremely low, as in Sweden and a second one where the proportion of infected foxes is considerably higher. The two values assigned to these two scenarios are 0.001 and 0.16. For the latter, the reported values in Karamon's paper were used as parameters of a Beta probabilistic distribution, with uniform prior, in order to include a degree of uncertainty around the best guess (Figure B8), while no uncertainty was considered around the estimated prevalence of the low-prevalence scenario.

[8] http://www.sva.se/globalassets/redesign2011/pdf/djurhalsa/zoonoser/em-rav-slutredovisning-per-lan.pdf

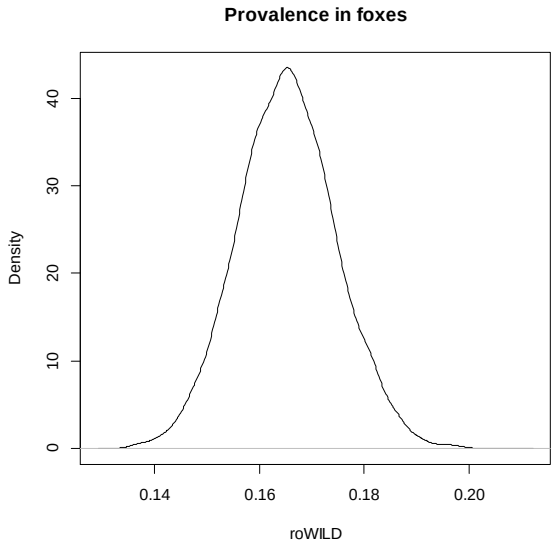

Figure B8: Probabilistic density distribution of the prevalence in foxes

Table B10 summarises the values used in the modelling exercise.

Table B10: Summary of the values used in the modelling exercise.

Notation	Short description	Values	Source
ρ_{WILD_i}	Prevalence of infected foxes in the i^{th} country of origin	0.0001	Romig T and Wahlström H., personal communication
		0.16 (stocastic)	Karamon J, 2014
ρ_{DOG_i}	Prevalence of infected domestic dogs in the ith country of origin	0.002 (stocastic)	Dyachenko, 2008
		0.01	(Worst case scenario) Romig T, personal communication
P_{ev}	Probability that a dog showing up at the borders evades the border compliance checks in place	0.09 (stocastic)	Roberts H, personal communication
P_{ke}	Probability that a dog is in compliance, given that it evaded	0.8	Roberts H, personal communication
P_{kne}	Probability that a domestic dog is in compliance, given that it did not evade	0.99 (stocastic)	Roberts H, personal communication
P_{kNBC}	Probability that a domestic dog showing up at the borders where no check is in place is in compliance	0.4 0.8	Wahlström H, personal communication
P_{fail}	Probability that a deworming treatment does not operate a full clearance of the parasite in the treated dog	0.08 (stocastic)	Table A4
P_{day1}	Probability that the treatment has been administered within 24 hours before the dog enters a free country	0.2	Roberts H, personal communication
P_{reinf}	Probability that a dog gets re-infected following a fully effective deworming treatment administered between 24 and 120 hours	0.03	Bødker R, personal communication (Section 3.5)
P_{inf}	Probability that a dog which is not in compliance (and it evades or no border compliance checks are in place) or on which the treatment did not operate a full clearance gets reinfected	0.16 (2 weeks in an endemic area)	Bødker R, personal communication (Section 3.5)
		1 (living in an endemic area)	

The Probability that a fox has to initiate the EM lifecycle ($P_{transWILD}$) and the Probability that a dog has to initiate the EM lifecycle ($P_{transDOG}$) were not parameterised in this modelling exercise as no data were available (see Section 3.3.3)

Results

The results of the modelling exercise are shown in Figure B10. In order to improve the readability of the outcomes, letters were assigned to the columns and numbers to the rows of the trellis plot, as shown in Figure B13.

Plots	A	B	C	D
1	A1	B1	C1	D1
2	A2	B2	C2	D2
3	A3	B3	C3	D3
4	A4	B4	C4	D4

Figure B9: Identification of the plots in the trellis graph shown in Figure B14

Columns A and B refer to dogs living in a free country, travelling in an endemic country for two weeks and then coming back home.

Columns C and D refer to dogs living in an endemic country and then entering a free country.

Columns A and C consider a low-prevalence fox scenario.

Columns B and D consider a high-prevalence fox scenario (worst case).

Rows 1 and 2 refer to a probability of compliance in countries where no border compliance checks are in place equal to 40%.

Rows 3 and 4 refer to a probability of compliance in countries where no border compliance checks are in place equal to 80%.

Rows 1 and 3 consider a low-prevalence dog scenario.

Rows 2 and 4 consider a high-prevalence dog scenario (worst case).

The red and the blue lines refer to dogs (border compliance checks in place / no border compliance checks in place, respectively), while the green lines refer to foxes.

The black x-axis in each small plot indicates the number of dogs. Relevant for blue and red lines.

The green x-axis at the bottom of the figure refers to foxes. Relevant for the green line.

It can be seen that in all scenarios, independently from the prevalence of infected dogs in the country of origin and from the probability of compliance when no border compliance checks are in place, the presence of border compliance checks always reduce the probability of introduction (the red line increases always less rapidly than the blue line).

It can also be observed that in case the prevalence of foxes reaches a value of 16% (columns B and D), the relative impact of the dogs on the probability of introduction is considerably small.

In order to make the comparison easier and less qualitative, Table B11 reports the number of dogs and foxes needed to reach a probability of at least 75% (value arbitrary chosen).

Table B11: Number of dogs and foxes for a probability of introduction of at least 75% in the different scenarios

				$P_{reinf} = 0.16$		$P_{reinf} = 1$	
				$\rho_{fox} = 0.001$	$\rho_{fox} = 0.16$	$\rho_{fox} = 0.001$	$\rho_{fox} = 0.16$
				A	**B**	**C**	**D**
$P_{kNBC} = P_{kEV} = 0.4$	$\rho_{dog} = 0.002$	**1**	*Dogs BC*	17400	17400	4800	4800
			Dogs NBC	6000	6000	1200	1200
			Foxes	1386	8	1386	8
	$\rho_{dog} = 0.01$	**2**	*Dogs BC*	3600	3200	1200	1200
			Dogs NBC	1200	1200	<600	<600
			Foxes	1386	8	1386	8
$P_{kNBC} = P_{kEV} = 0.8$	$\rho_{dog} = 0.002$	**3**	*Dogs BC*	20400	20400	6600	6600
			Dogs NBC	9600	9600	1800	1800
			Foxes	1386	8	1386	8
	$\rho_{dog} = 0.01$	**4**	*Dogs BC*	4200	4200	1200	1200
			Dogs NBC	2400	2400	<600	<600
			Foxes	1386	8	1386	8

BC: Border compliance-checks in place
NBC: No Border compliance-checks in place

It can be seen that the presence of the border compliance checks implies that the number of dogs that need to show up at the borders of the free country in order to reach at least 75% probability of introduction is 1.75 to 4 times higher, compared to a situation where no border compliance checks are in place.

The figures confirm what is shown in the trellis plot about the impact of the foxes. In case a free country is adjacent to an endemic area (prevalence of foxes equal to 16%, scnearios B and D) the number of dogs that need to show up at the borders to reach at least the same probability of introduction of 75% is 75 to 1200 times higher that the number of foxes.

In case a free country is adjacent to a country where findings of the parasite have been recorded with a very low prevalence in foxes (0.01%), the situation is not so dissimilar. In case the considered dogs are all coming back to their free country, where no border compliance checks are in place, after a 2 weeks period in an endemic area (scenario A), where the prevalence in dogs is 0.2% or 1%, still the number of dogs that need to show up at the borders (coming back home) to reach at least the same probability of introduction of 75% is 1.7 to 6.9 times higher than the number of foxes. Only in one case the number of dogs and foxes is almost comparable, i.e. when the probability of compliance is only 40% (scenario A1). In all other cases, where the border compliance checks are in place, the foxes still represent the main route of introduction (scenarios from A2 to A4).

The situation slightly changes if the free country is adjacent to a country where findings of the parasite have been recorded with a very low prevalence in foxes (0.01%), and if the considered dogs all live in an endemic country and enter the free country, where no border compliance checks are in place, for a given period (scenario C). In this case the situation is the opposite, although the proportions are not even comparable. In order to reach at least the same probability of introduction of 75%, the number of foxes that need to cross the borders is at max 2.3 times higher than the number of dogs that need to shouw up at the border. It has to be noted that, in the same conditions, if we consider a country where border compliance checks are in place, foxes play again a prominent role in the introduction: only if the dogs all live and come from an endemic area with a high prevalence (1%) can have a relatively higher impact on the probability of introduction, i.e. the number of dogs needs to be 1.16 times higher than the number of foxes.

Finally, it has to be highlighted that, considering the number of dogs entering a free country every year (Appendix B, Table B8) the probability that at least one of them will introduce a worm or its eggs is 100%.

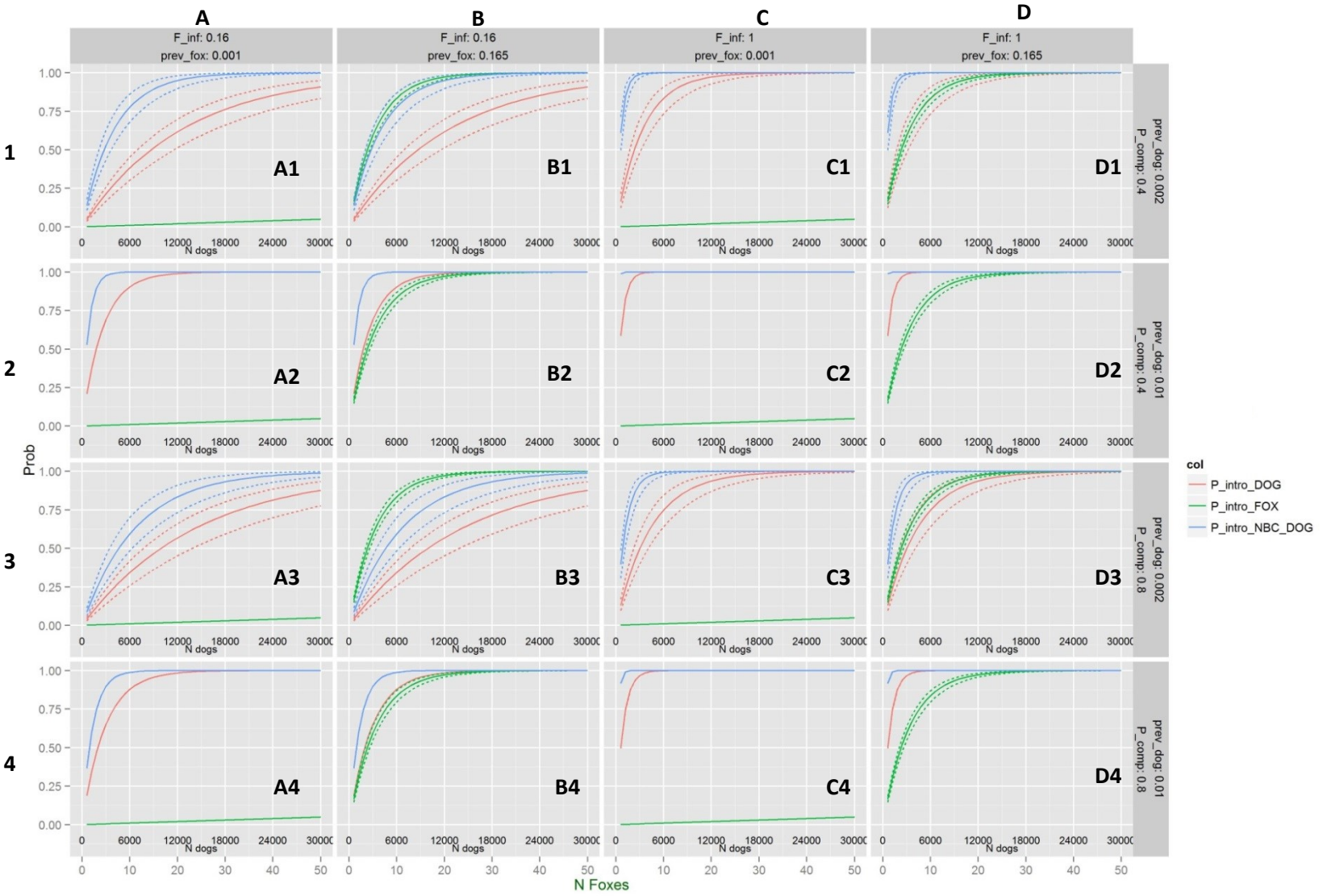

Figure B10: Trellis plot with 16 different scenarios. X-axis=number of dogs/foxes. Y-axis=probability of introduction. Red line: dogs & border checks in place. Blue line: dogs & no border chacks in place. Green line: foxes. The black x-axis in each small plot indicates the number of dogs (blue and red lines). The green x-axis at the bottom of the figure refers to foxes (green line). Dashed lines=95% confidence bounds

Appendix C – *E. multilocularis* surveillance and monitoring programmes in EU Member States

Surveillance and Monitoring in EU Member States where no findings of the parasite have been recorded

Malta is a small island of just 246 km^2area. There is a population of 60,000 dogs, of which an estimated 2,000 are stray dogs. The dog is considered the epidemiological unit for surveillance purposes. There are no wild definitive hosts and the risk of introduction is limited totravelling pets, but the risk of establishment is negligible due to the absence of wild canids.

Ireland forms part of an island (shared with Northern Ireland, a devolved administration of the UK). The risk of introduction is from the movement of travelling pets as any fox movement across the land border is from another area where no findings of the parasite have been recorded. The surveillance is designed to detect establishment of disease/infection. The Regulation is currently worded to allow Ireland to retain disease/infection freedom even if Northern Ireland would detect a positive fox. This in effect would mean the risk to Ireland will have changed, as it should now consider the movement of infected wild definitive hosts into the area where no findings of the parasite have been recorded, not only the movement of travelling pets. Ireland carries out 100% checks of (identified) travelling pets arriving from countries where findings of the parasite have been recorded; pets arriving from the UK are not checked.

UK consists of two epidemiologically distinct areas, GB and NI. Although pets may travel between the two areas without the requirement for tapeworm treatment, there is no movement of foxes. The Regulation is worded at present that the detection of an infected fox in GB would mean that NI would also lose infection free status, or that an infected fox in NI would mean GB would lose free status. This should be addressed so that infection absence should be associated with the distinct area, as is allowed in EU legislation. The entry points for pet movement into mainland GB are numerous, however at the entry points there are 100% checks in place for compliance, carried out by the ferry operators and Eurostar. The movement of pets into Northern Ireland will generally be from GB by ferry (limited checks) or across the border (no checks) from republic of Ireland).

Finland is the largest of the four Member States where no findings of the parasite have been recorded, has two populations of wild definitive hosts and has land borders withan EU MS with low prevalence (Sweden), and EU MS with medium to high prevalence (Estonia) and a third country with unknown prevalence (presumed also medium to high; Russian Federation).

In 2011, all four Member States provided evidence for ten years of infection absence from EM. Sweden was not able to apply given their finding of EM in a wild fox in 2011. Different health prevention regimes were in place in these countries prior to the harmonisation under the Pet Travel Scheme. In Finland, a treatment window of 30 days prior to entry was permitted; in Sweden 10 days prior to entry (similar to Norway) and in Malta, UK and Ireland a window of just 48 to 24 hours was permitted. Under the new harmonised controls (date), a treatment window of 5 days to 24 hours prior to entry into the Member State, based on was put in place for all countries listed in Annex I Part A.

Table C1: Change in tapeworm treatment regimes and changes in risk of introduction of the parasite after harmonisation of control, for the countries considered EM free prior to 2011 harmonisation.

	Finland	Ireland	Malta	UK	Sweden[(a)]	Norway[(b)]
Pre 2011	30 days	24–48 hr	24–48 hr	24–48 hr	10 days	Before arrival and 7 days after
Post 2011	1–5 days	1–5 days	1–5 days	1–5 days	N/A	Same
Change in risk	Decrease	Increase	Increase	Increase	Increase	Status quo

(a): Although Sweden lost official freedom in 2011 after detection of a positive fox, the authorities were part of the initial negotiation with the EU on harmonisation and retention of tapeworm requirements.
(b): Norway, although not an EU member state, also provided information on their history of disease freedom as part of the negotiations.

Surveillance programmes for the four Member States followed recommendations made in the Regulation (EU) No 1152/2011, but there were differences, appropriate to the situation in the Member

State. Finland reports for two definitive host populations, the red fox (*Vulpes vulpes*) and the Raccoon Dog (*Nyctereutes procyonoides*) as both have been shown to be suitable hosts for EM infection. Raccoon dogs are more populous than red foxes particularly in areas bordering Estonia and the Russian Federation which represent a risk for disease/infection introduction through movement of wildlife. Ireland, like the UK, reports on surveillance in red foxes, as there are no other wild definitive hosts for EM present; Malta reports on surveillance in domestic dogs as the red fox or other wild definitive hosts are absent from the island, but the analysis takes account of different risk factors for different sub groups of dog. The UK is separated into devolved administrations and Northern Ireland forms a separate epidemiological unit therefore reporting is done for the two areas, both for red foxes, but using different tests.

Malta implements a risk-based surveillance system, testing only dogs as no wild definitive hosts are assumed to be present on Malta. Two risk indicators are used: exposed to intermediate hosts or not (which depended on the dog being a hunting or rural dog) and: imported dogs (imported and stray dogs (as stray dogs could originate from illegally imported dogs). The relative risk for the groups was given as 1.2 for both risk categories. However, evidence forthe assigned relative risk ratios is required (EFSA, 2014).

Norway has also recently provided details of their surveillance programme to show evidence of disease infection absence. Since 2002, 3,405 fox carcases have tested negative (none positive) of an estimated population of between 70,000 – 120,000 red foxes. Of these, 1600 were tested prior to 2009. Surveillance is carried out on the mainland, covers all counties but does not include the islands of Svalbard. Pet treatment is required (and non-in compliance pets are placed in quarantine until in compliance) but border compliance checks are not complete and there is a long land border with Sweden, where positive fox carcases have been detected just 80 km from the border (Wahlström et al. in press).

Surveillance and Monitoring in non-'free' EU Member States

Sweden: following the first report of EM in a fox carcase in 2011, intensive surveillance was carried out to determining the geographical extent of the infection. Fox carcases (2985 animals) were collected from all over Sweden and tested. Positive results were found in three areas, with a very low national prevalence (0.1%) suggesting very low endemicity (Wahlström et al. 2011). A second survey, using probabilistic sampling was conducted to obtain a more accurate prevalence estimate, a baseline to be used for comparison in the future to evaluate if prevalence changes over time. Furthermore, for cost-efficiency reasons, fox faecal samples instead of fox carcasses was collected and analysed with a new magnetic probe PCR method (Isaksson et al 2014). Three infected areas were identified two previously known and one new. Import controls in Sweden between 1994 and 2011 required pet dogs to enter with a veterinary certificate of deworming treatment having taken place in the ten days prior to entering Sweden. There are currently import recommendations for pet owners travelling from highly endemic areas to deworm their dogs. Guidance is also given for all pet owners in Sweden to regularly deworm their pets every month if they are concerned.

Denmark first detected EM in fox carcases in 2000 in Copenhagen, when 3 of 340 animals tested positive, but no further studies in Denmark were carried out until 2011. Between 2011 and 2014 1,500 carnivores were tested: 1,169 foxes, 265 raccoon dogs and 66 other wild carnivores. The first positive result was found in November 2011 near the border with Germany, but further surveillance in this area of 28 foxes showed high local prevalence as 9 tested positive (32%) as well as two raccoon dogs. Current national prevalence is estimated at 1.2% for foxes and 0.75% for raccoon dogs (95% confidence) (Wahlström et al. in press). Import controls for dogs entering Denmark have relied on the voluntary deworming of dogs from highly endemic areas following the recommendations of the Danish Veterinary and Food Administration in 2013.

Norway (Islands of Svalbard) first reported EM in Arctic foxes (*Vulpes lagopus*) in 2000 (Henntonen et al. 2001 & Fuglei et al. 2008). As a result of surveillance of the sibling vole (*Microtus levis*) which has a very limited geographical distribution, bounded by the availability of suitable food plants. Surveillance of fox faeces in areas of high vole distribution showed these areas are the highest prevalence of EM. It is thought that while the arctic foxes roam freely across pack ice and may have already acquired the parasite in Russia, it only became established in Svalbard following the anthropogenic introduction of the sibiling vole at the end of the twentieth Century.

Appendix D – Survey design for demonstrating absence of infection

Introduction

The basic principle when dealing with absence of infection involves estimation of the so called sensitivity of the surveillance system (SSe), i.e. the probability that qualifies a statement on the presence/absence of a given infection (Cameron and Baldock, 1998). Since 1982, when Cannon and Roe published their manual on disease surveys techniques, many authors contributed to the development of methodologies to increase the performance of a survey. In a recent publication Cameron (2012) outlined this evolution, starting from early tools to calculate the sample size for representative surveys (Cannon and Roe, 1982) up to recent sophisticated methodologies. These latter imply the use of risk based sampling techniques. More insight is required to implement such sampling techniques compared to a simple random sampling, and some of this insight may not be properly documented and has to be assumed for the population in question. However, the advantages in terms of effectiveness/efficiency of such methods are substantial and should be considered when dealing with Disease Detection or Demonstration of Freedom from Disease.

The full methodology is described in detail in the EFSA Technical Report published in 2012[9] related to the implementation of a absence of infection framework specifically on *Echinococcus multilocularis*.

Design prevalence in the 'freedom from disease' framework

It has to be noted that, analytically speaking, it is not possible to demonstrate 'freedom' from a given disease/ infection. If equation D1 is solved setting DP equal to 0, the sample size required (n) would be equal to $-\infty$.

$$n = \frac{log(1 - RSe)}{log(1 - DP \cdot TSe)} \quad \text{D1}$$

To estimate the probability that the actual prevalence is below a given threshold (DP), this value must be greater than zero. There are no right or wrong values, but some considerations are useful to define a proper threshold.

The closer to 'zero', the more similar to 'absence'. As an example, it can be decided to define the design prevalence as the reciprocal of the target population (1/N), i.e. the worst case scenario of only 1 infected individual in the population. The drawback here is that the lower the DP-value, the greater the sample size. So, for large populations, as for foxes, the required sample size would be impracticable (see also Figure D1).

A more applicable threshold can be set using a value which can be considered and accepted as synonymous with absence. E.g. if a disease is characterised by a very high R_0, the threshold can justifiably be set above 1%, because shortly after the infection has been introduced in a free area, the prevalence would increase to over 1%. In such case, saying that the prevalence is below 1% or saying that the infection is absent is approximately the same thing. Unfortunately, in most situations, E.m. is a slow-moving infection, i.e. it has a low R_0, and therefore, ideally, a relatively low design prevalence value should be set. However, the EU-regulation defines the DP to be 1% when evaluating possible absence of EM.

[9] EFSA (European Food Safety Authority), 2012. 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. Supporting Publications 2012:EN-366. 44 pp. Available online: www.efsa.europa.eu/publications

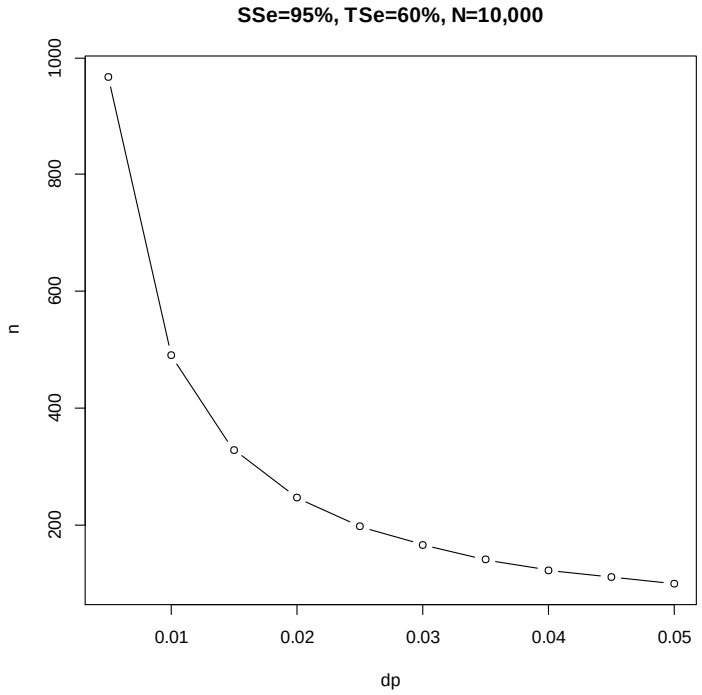

Figure D1: Sample size as a function of the design prevalence; dp: design prevalence. SSe: required surveillance system sensitivity (confidence); TSe: sensitivity of the test; N: target population size.

System Sensitivity (SSe) and Probability of Freedom (P_{free}) in the 'freedom from disease' framework

The concept of the system sensitivity (SSe) as outlined above, foreseen in Regulation (EU) No 1152/2011, allows an increased efficiency and flexibility when compared to the input-based type of survey. However, it does not address how to take into account, among others aspects, the outcome of previous surveillance activities. Briefly, the confidence that MS 'A' is free from a given infection (i.e. DP < 1%) would be higher if previous surveillance activities had been carried out without finding infected animals when compared to MS 'B', which is implementing a survey for the first time. To address this issue, another parameter was introduced in the framework of demonstrating absence of infection: the Probability of Freedom (P_{free}).

In probabilistic language, the System Sensitivity (equation D2) is the probability of getting at least one positive test given that the infection is present at or above, say, 1% prevalence in the target population:

$$SSe = P(S+|D+) \quad \text{D2}$$

In contrast, the Probability of Freedom is the probability of the infection being absent (i.e. DP < 1%) given that all samples tested negative:

$$P_{free} = P(D-|S-) \quad \text{D3}$$

which is also known as the Negative Predictive Value of the Surveillance System.

P_{free} can then be calculated incorporating historical evidence of infection absence from previous surveillance activities using Bayes' theorem as follows:

$$P_{free} = \frac{PriorP_{free}}{PriorP_{free} + [(1 - PriorP_{free}) \cdot (1 - SSe)]} \quad \text{D4}$$

where $PriorP_{free}$ is the prior probability of infection absence and SSe is the System Sensitivity.

A stepwise analysis of historical data provides a progressively updated estimate of the probability of infection absence. However, in order to take into account the decreased value of historical data, each prior estimate of the probability of infection absence must be adjusted to take into account the risk of introduction of infection since the surveillance was undertaken. This latter adjusted probability of infection absence will be the $PriorP_{free}$ for the next year (see Figure D2). When no information is available from previous surveillance activities, the first $PriorP_{free}$ can be set at 0.5 (50%).

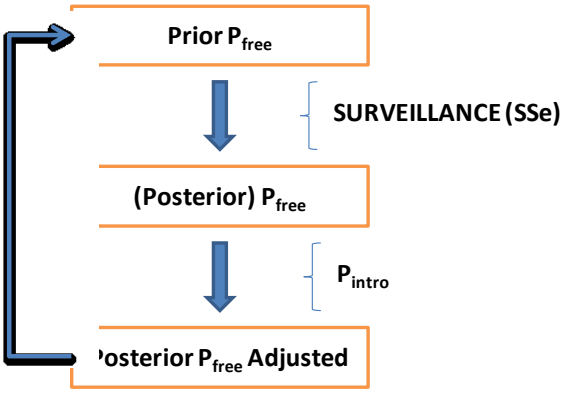

Figure D2: Continuous update of the probability of infection absence taking into account the probability of introduction and the additional evidences gathered from both the recent and the past surveillance activity

In order to harmonise the process of demonstrating absence of infection and to ensure equivalence between different Surveillance Systems, Regulation (EU) No 1152/2011 refers to the the 'confidence', which according to the available scientific literature is equal to the SSe. Therefore the concerned MS will have to implement each year a survey with an overall SSe of at least 95%. In this situation, and assuming a probability of introduction equal to 48% (i.e. relatively high) the P_{free}, if calculated, would reach the value of 100% in 3 years (Figure D3).

Including the P_{free}, (i.e.the Negative Predictive Value of the surveillance system) in the regulation, rather than the SSe, would potentially lead to a reduction of the required annual sample size.

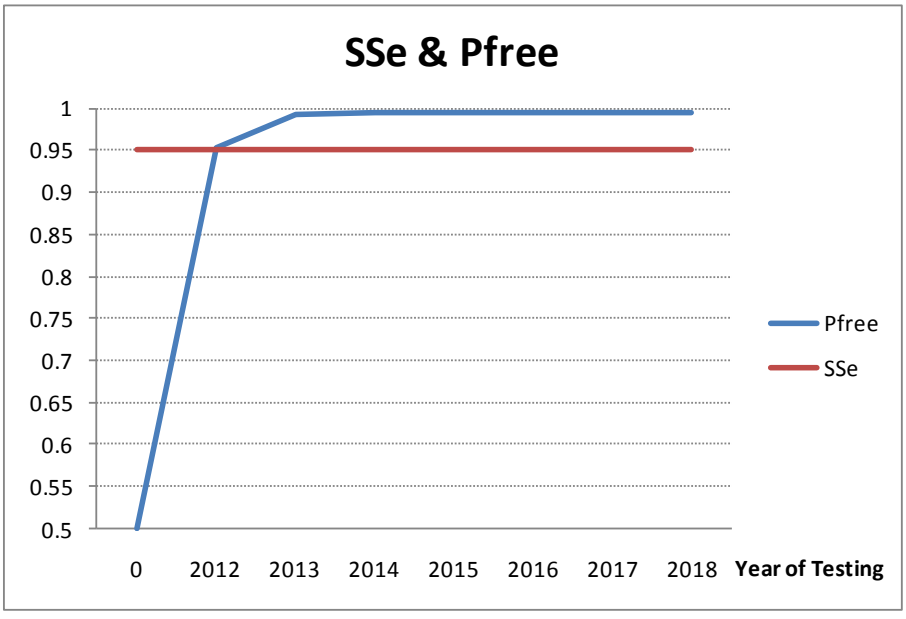

Figure D3: Trend over time of the System Sensitivity and of the probability of infection absence assuming a probability of introduction equal to 48%

Appendix E – Human alveolar echinococcosis

Questionnaire circulated to …

REQUEST FOR INFORMATION REGARDING ECHINOCOCCUS MULTILOCULARIS in addition to what is already published or submitted for EUSR

Prevalence data in the period 2012-2014:

- Number of samples tested per year? *Intra vitam*
- Number of positive samples detected per year?
- Locations where samples were taken (NUTS2 or NUTS3 if possible).
- Which diagnostic procedure was used and provide a reference, if possible.
- Which matrix was sampled (individual intestinal content, faeces taken from an animal or from the environment) and from which species: individual
- What was the reason for sampling (e.g. prevalence estimation, evaluation of control measures taken, …)?
- If sampling was not homogeneous throughout your country, please specify regional differences.

Host populations

- Is there a homogeneous distribution of definitive hosts (foxes, wild canids and domestic dogs) in your country? If not, could you explain the regional differences?
- Has *E. multilocularis* been detected in an intermediate host in your country? If yes, please provide details (species, date, location, diagnostic method, sampling design, …)
- Any other species found to be positive for *E. multilocularis*? (e.g. imported beavers (which the UK has had) or zoo species)

Notification of E. multilocularis:

- Is *E. multilocularis* in animals notifiable in your country? (If yes, since when? and isthere a discrimination between *E. multilocularis* and *E. granulosus* in the reporting?)
- Is *E. multilocularis* in humans notifiable in your country? (if yes, since when? and is there a discrimination between *E. multilocularis* and *E. granulosus* in the reporting?)

Alveolar echinococcosis (AE) cases in humans:

- Is the number of AE cases known in your country?
- If yes, what has been the number of annual AE cases in your country since 2000? Is the origin of these cases known (domestic, foreign or not known)? Please provide details.

Surveillance

- How did you first detect that *E. multilocularis* was present in your country? (to give info on what type of activity detected the parasite).
- How did you detect the first case in a definitive host?
- How did you detect the first case in an intermediate host?
- How did you detect the first human case?

Dog movements

- Do you have data on dog movements across your external borders?
- If yes, how many dogs are entering (transit or stay) and leaving the country every year? How are the numbers collected//estimated?

Table E1: Notification of AE human cases in EU and AC

COUNTRY	NOTIFICATION of HUMAN AE CASES	DIFFERENTIATION BETWEEN CE and AE CASES
Albania		
Austria	Yes	
Belgium	Yes	No
Belarus	Yes	
Bosnia Herzegovina		
Bulgaria	Yes	
Croatia	Yes	
Cyprus	No	
Czech Republic	Yes	
Denmark	Yes	No
Estonia	Yes	No
Finland		
France	No	No
FYR Macedonia		
Germany	Yes	
Greece		
Hungary	Yes	Yes
Iceland	Yes	
Ireland	Yes	
Italy	No	
Latvia		
Lithuania		
Liechtenstein		
Luxembourg		
Malta	Yes	No
Kosovo		
Moldova		
Montenegro		
Netherlands	No	No
Norway	Yes	No
Poland	Yes	
Portugal	Yes	No
Romania		
Serbia		
Slovakia	Yes	Yes
Slovenia	Yes	
Spain	Yes	No
Sweden		
Switzerland	No	
Turkey		
Ukraine		
United Kingdom	Yes	

Table E2: Estimation of annual number of AE human cases in the EU and AC

COUNTRY	ESTIMATED ANNUAL NUMBERS of CASES	REFERENCES
Albania		
Austria	7	Kern et al., 2003; Auer and Aspock, 2001; Torgerson et al., 2010
Belgium	1	Torgerson et al., 2010
Belarus	6	Torgerson et al., 2010
Bosnia Herzegovina		
Bulgaria	1	Torgerson et al., 2010
Croatia		
Cyprus		
Czech Republic	1	Torgerson et al., 2010
Denmark		
Estonia	9	Torgerson et al., 2010
Finland		
France	21	Abdullaev et al., 2006; Torgerson et al., 2010
FYR Macedonia	1	Torgerson et al., 2010
Germany	61	Torgerson et al., 2010
Greece	1	Torgerson et al., 2010
Hungary	1	Torgerson et al., 2010
Iceland		
Ireland		
Italy		
Latvia	9	Torgerson et al., 2010
Lithuania	9	Torgerson et al., 2010
Liechtenstein		
Luxembourg		
Malta		
Kosovo		
Moldova	1	Torgerson et al., 2010
Montenegro		
Netherlands		
Norway		
Poland	3	Torgerson et al., 2010
Portugal		
Romania		
Russia	1.180	Torgerson et al., 2010
Serbia		
Slovakia	4	Torgerson et al., 2010
Slovenia	2	Torgerson et al., 2010
Spain		
Sweden		
Switzerland	20	Torgerson et al., 2010
Turkey	>100	Torgerson et al., 2010
Ukraine	10	Bessanov et al., 2003; Torgerson et al., 2010
United Kingdom		

Table E3: Case series reporting AE human cases in the EU and AC

COUNTRY	CASE SERIES (PERIOD)	REFERENCES
Albania		
Austria	2.4 cases/100,000 (1991–2000) and 2,8/100,000 (2001–2010); 13 cases (2013)	Schneider et al., 2013
Belgium	1 case reported (2002); 3 cases reported in 2004	Landen et al., 2013 ; Detry et al., 2005
	1 case per year	Questionnaire from MS
Belarus		
Bosnia Herzegovina		
Bulgaria		
Croatia		
Cyprus		
Czech Republic	20 cases (1998–2014)	Kolářová, et al., 2015
Denmark	2 cases	Samuelsson and Kapel, 2004
	15 cases (2010–2013)	Questionnaire from MS
Estonia		
Finland		
France	509 diagnosed cases (1982–2011)	Said-Ali et al., 2013
FYR Macedonia	1 case	Druschky et al., 1995
Germany	114 cases (2003–2013)	Jorgersen et al., 2008
Greece	1 case (1980–2000)	Vuitton et al., 2003
Hungary	1 case (2004); 1 autochthonous case (2015)	Horvat et al., 2008; personal communication: Balázs Dezsényi, 15/09/15, St. Ladislau Hospital, Budapest (Hungary) and Thomas F.E. Barth, 14/09/15, Institute of Pathology, University of Ulm, (Germany)
Iceland		
Ireland		
Italy		
Latvia	29 cases from Pauls Stradins University Hospital (1996–2010)/ 14 cases from Infectivology Centre of Latvia (1999–2010)	Tulin et al., 2012
Lithuania	80 cases at the State Hospital for Tuberculosis and Infectious Diseases in cooperation with the Santariškių Clinic (Vilnius University) (1997–2006)	Bružinskaitė et al., 2007
Liechtenstein		
Luxembourg		
Malta		
Kosovo		
Moldova		
Montenegro		
Netherlands	Approx. 1 case per year (2008–2014) (most likely autochthonous cases)	Questionnaire from MS
Norway		
Poland	121 cases (1990–2011)	Nahorski et al., 2013
Portugal		
Romania	2 cases	Siko' et al., 2011
Russia		
Serbia		
Slovakia	26 cases (2000–2013)	Antolova et al., 2014
Slovenia	0.45 per 100,000 inhabitants (2001–2005)	Logar et al., 2007
Spain		

COUNTRY	CASE SERIES (PERIOD)	REFERENCES
Sweden	2 cases (imported)	Wahlström et al., 2015
Switzerland	incidence: 0.12– 0.15 (1956–1992)/ 0.10 (1993–2000) / 0.26 (2001–2005)	Schweiger et al., 2007
Turkey	162 cases	Miman et al., 2012
Ukraine		
United Kingdom	1 case (unknown origin)	Cook et al., 1991

Table E4: Overview of risk factors for AE

Risk factor	Study area		Cross-sectional studies			Case-control studies		
			Number of studies/number of participants	Odds Ratio (M-H, Fixed, 95% CI)	Reference	Number of studies/number of participants	Odds Ratio (M-H, Fixed, 95% CI)	Reference
Dog ownership	**Europe**	**China**				4/1011	2.30 [1.56, 3.40]	*Casulli et al, 2015*
			5/13883	2.88 [2.30, 3.62]	*Casulli et al, 2015*	5/1068	2.50 [1.73, 3.62]	
Playing with dogs	**Europe**	**China**	3/5916	3.48 [2.20, 5.52]		2/216	1.42 [0.75, 2.66]	
Hand washing before eating	**Europe**					–	–	–
	Global		3/5348	6.94 [4.99, 9.66]		–	–	–
Gender: female	**Europe**					–	–	–
	Global		10/42812	1.50 [1.35, 1.67]		–	–	–
Age>20	**Europe**					–	–	–
	Global		8/24988	2.96 [2.39, 3.68]		–	–	–
Ethnic group: Tibetan	**Europe**					–	–	–
	China		4/25952	2.03 [1.56, 2.63		–	–	–
Low Income	**Europe**					–	–	–
	China		2/4124	3.92 [2.42, 6.36]		–	–	–
Source of drinking water other than well or tap	**Europe**					–	–	–
	Global		5/23714	1.81 [1.52, 2.17]		–	–	–
Occupation: farming	**Europe**					–	–	–
	Global		5/17878	1.29 [0.97, 1.72]		4/1011	4.50 [2.74, 7.39]	*Casulli et al, 2015*
Occupation: herding	**Europe**					–	–	–
	Global		5/21045	2.22 [1.76, 2.81]		–	–	–
Drinking not boiled water	**Europe**					–	–	–
	China		2/7096	0.63 [0.48, 0.84]		–	–	–
Hunting /handling foxes	**Europe**					–	–	–
	Global		3/9442	1.29 [0.97, 1.71]		4/959	2.27 [1.35, 3.81]	–
Low education	**Europe**					–	–	–
	China		2/5297	4.81 [2.73, 8.48]		–	–	–

Risk factor	Study area	Cross-sectional studies			Case-control studies		
		Number of studies/number of participants	Odds Ratio (M-H, Fixed, 95% CI)	Reference	Number of studies/number of participants	Odds Ratio (M-H, Fixed, 95% CI)	Reference
Allowed dog into the house	**Europe**				2/216	1.80 [0.90, 3.62]	
	Global				2/216	1.80 [0.90, 3.62]	*Casulli et al, 2015*
Playing with dogs	**Europe**				1/159	2.07 [0.97, 4.42]	
	Global				2/216	1.42 [0.75, 2.66]	
Cat ownership	**Europe**				2/265	2.63 [1.42, 4.85]	
	Global				2/265	2.63 [1.42, 4.85]	
Living in rural area	**Europe**				2/746	3.12 [1.95, 5.01]	
	Global				3/803	3.44 [2.19, 5.41]	
Having a kitchen garden	**Europe**				2/746	5.21 [2.65, 10.22]	
	Global				2/746	5.21 [2.65, 10.22]	
Did haymaking in meadows not adjacent to water	**Europe**				2/238	3.50 [1.63, 7.55]	
	Global				2/238	3.50 [1.63, 7.55]	
Went to forests for vocational reasons	**Europe**				2/266	2.61 [1.13, 6.05]	
	Global				2/266	2.61 [1.13, 6.05]	
Ate unwashed strawberries	**Europe**				4/1006	1.39 [0.87, 2.23]	
	Global				4/1006	1.39 [0.87, 2.23]	
Chewed grass	**Europe**				2/252	3.20 [1.65, 6.20]	
	Global				2/252	3.20 [1.65, 6.20]	
Hunting	**Europe**				4/1007	1.25 [0.73, 2.15]	
	Global				5/1064	1.13 [0.69, 1.83]	
Handling foxes	**Europe**				3/902	2.84 [1.57, 5.15]	
	Global				4/959	2.27 [1.35, 3.81]	
Eating mushrooms	**Europe**				2/255	0.72 [0.38, 1.39]	
	Global				2/255	0.72 [0.38, 1.39]	
Consumption of wild vegetables and fruit	**Europe**				4/990	1.50 [0.98, 2.31]	
	Global				5/1046	1.38 [0.90, 2.10]	
Protective HLA	**Europe**				1/604	0.55 [0.34, 0.88]	
	Global				2/743	0.50 [0.32, 0.80]	

Appendix F – Modelling *E. multilocularis* treatment protocols

A mathematical model was developed at the National Veterinary Institute DTU by René Bødker. The model was used to quantitatively compare the effects of different treatment protocols. The model allows for treatment of dogs up to 90 days before moving to a free area, and also up to 90 days after entering the free area as the latter will also reduce the number of excreted eggs in the free area. The model has been used to quantify the relative treatment effect of dogs from, endemic areas imported or visiting free areas (section 3.5.4), and of dogs from free areas visiting endemic areas and then returning to their free areas (section 3.5.5). Finally the model was used estimate the level of compliance (with the deworming requirements) that was needed in order not to increase the risk when treating earlier than day -1 (default) and instead treating day -2 and up to day -6 (section 3.5.6).

The magniture of the probability of transmission is unknown, however in the model, it is assumed that the probability of establishing the parasite in a free area is linearly proportional to the number of eggs excreted in this area, given the area has an environment that is suitable for transmission. Therefore, a deterministic mathematical model (built in an Excel spread sheet) was used to calculate the average number of eggs excreted in a free country by an infected dog entering a free country. The probability that a dog is infected depends on the prevalence level and the duration of exposure in the endemic area and also on dog behavior i.e. probability of eating rodents. In the model, calculations do not quantify the actual infection risk in the endemic areas but merely measure the relative effect of different treatment protocols regardless of the absolute exposure level. The model does however calculate the effect of the duration of exposure in the endemic area and also of the duration of stay in the free country as the relationship between the risk and these two measures is not linear and in some cases complex.

In a previous EFSA risk assessment (2007), risk was defined as the probability of introduction of an infected dog. In this section of the present scientific opinion, risk is defined as being proportional to the number of eggs deposited in a free country. Therefore, the focus is no longer on the number of dogs crossing the border but on the number of worms crossing the border and specifically on how many eggs these worms will be able to produce in a free area before the dog leaves the area or before the worms dies of either natural causes or from treatment. Hence, a dog with only immature worms entering and leaving before worms mature, is not considered a risk albeit the infection is technically temporarily introduced to the free country. This is because these immature worms will not excrete any eggs in the free country. Also a dog with many worms is considered a relatively higher risk than a dog with few worms. In addition, a dog with a young worm is considered a relatively higher risk than a dog with an old worm, since young worms will live longer and produce more eggs in the free area. Also the duration of the visit in a free country is important since a long visit will result in more eggs being deposited. The timing of the treatment is important because the drug is excreted in just 24 hours after which the dog may be reinfected. Treating five days before crossing the border will therefore leave 4 days where the dog can be reinfected. Since the worms only live for 90 days the maximum exposure period is effectively just 90 days after which the maximum worm load is reached. Thus a four days re-exposure period is equivalent to 4.4% of the maximum risk (4/90) and therefore relatively important compared to e.g. treatment failure.

An important effect of defining risk as the number of deposited eggs is that preventive treatment can be applied after entering a free area since delayed treatment will still reduce the number of excreted eggs. Somewhat surprisingly, delaying treatment until after crossing the border may in some cases reduce the risk compared to the traditional protocol of applying treatment in the endemic area shortly before entering a free country (Figure F2).

The lifespan of a worm is assumed to be 90 days, meaning that a maximum worm load will be reached after 90 days exposure (although the absolute worm load is specific for the prevalence level in the area). The egg production per mature worm is assumed constant over time regardless of the age of the worm, and it also assumes the eggs produced are excreted by the worm within 24 hours. Essentially the model calculates the daily rate of infection as 1/90 of that maximum. The model assumes each infecting worm goes through a 30 days pre-patent stage followed by a 60 days egg excreting stage. The model therefore keeps track of the age of each daily infecting worm cohort. The model either assumes a full 90 days exposure followed by visits to free areas of various durations (dogs living in endemic areas visiting or being imported to free countries) or a full 90 days stay in a

free country that follows exposure periods of various durations in endemic areas (dogs living in free countries and visiting endemic areas before returning to the free area). Prevalence levels and duration of exposure is not taking into account in EU legislation. For practical reasons, present legislation simply distinguishes between dogs moving from free areas to not-free areas. But in the present model duration of exposure as well as duration of the period spend in the free country are important drivers of risk.

Model structure

The model describes an average dog and the unit of time is one day. The rows in the spread sheet are days relative to the crossing the border (at day 0) ranging from 100 days before until 100 days after. The columns in the spread sheet represent different development stages of worms and also count the eggs produced in each time step. One column counts the number of prepatent worms infecting an average dog each day (as 1/90 of the maximum worm burden for a specific exposure area). Another column counts the number of immature worms maturing to adult worms each day (the number of infecting worms 30 days previously). A third column calculates the number of patent worms dying (the number of infecting worms 90 days earlier). A fourth column calculates the daily number of adult worms as the number of patent worms the day before plus the daily number of worms maturing minus the number of worms dying. Another column then calculates the daily number of excreted eggs based on the number of adult worms that day. This assumes the egg production per worm is always the same regardless of the age of the patent worm and also assumes that all eggs produced by a worm is excreted within 24 hours both assumptions are simplifications. A final column calculates the cumulative number of eggs produced by a dog, but only while the dog stays in a free area. This total number of eggs produced in the free area is the 'risk'.

In the model it is then possible to treat the dog at any day before or after crossing the border (equal to day 0 in the model). Treatment will affect both the prepatent and patent worms equally. Treatment is assumed to kill a proportion of the worms while leaving the remaining worms unharmed and able to produce eggs. While this and other assumptions are an important simplification of the biological effect of deworming it is likely to capture the key average effect. In the model, non-compliance is simply treated as another form of treatment failure.

Model results

The model distinguish between dogs being exposed for a full 90 day period and then visiting a free country for shorter periods and dogs being exposed for various periods less than 90 days and then staying a full 90 days period in a free area. Combinations of short exposure periods combined with short periods in a free area were not explored here. Figure F1 shows dogs with a 90 days exposure that then visits a free area for ninety, sixty, twenty, ten or five days. As the maximum lifespan of a worm is 90 days, a 90 day visit is equal to permanent import. When the treatment effectiveness is very high, the risk stems almost only from re-infection of the dog between treatment and entering the free country. The best time is to treat is day -1 as this prevents re-infection of the dog and ensures all eggs are excreted before crossing the border and therefore prevent excretion of eggs in the free country.

When the dogs are exposed for 90 days and then stay 90 days in the free area, the risk after treatment is symmetrical over treatment days, in the sense that the risk is the same whether the dog is treated e.g. ten days prior to crossing the border or ten days after. The risk is symmetrical because treating dogs before entry will result in a few re-infecting worms that will live a long time in the free area, while treating the dog after entry will result in many worms that only live the short period until the treatment day. Thus, the number of eggs produced in the free area is the same whether the dog carries a few worms that live long or many worms that live short (figure F1-A).

However, if a dog from an endemic area is going to a free country for a short visit and is treated earlier than day -1 this may not always affect risk. This is because potentially re-infecting worms will not be able to mature in the free area before the dog returns to the endemic country, as long as the dog returns within 30 after beting treated. If the dog is treated prior to crossing the border, the risk is therefore always smaller the shorter the visit is (left side of Figure F1-B to E). However if the dog is treated after crossing the border, the duration of stay has little importance as only the period until treatment allows for egg excretion. Only when the treatment effectiveness is low, the duration of stay after treatment will add to the risk. Hence at visits shorter than 90 days the risk is no longer symmetrical around day-1 (figure F1-B to E).

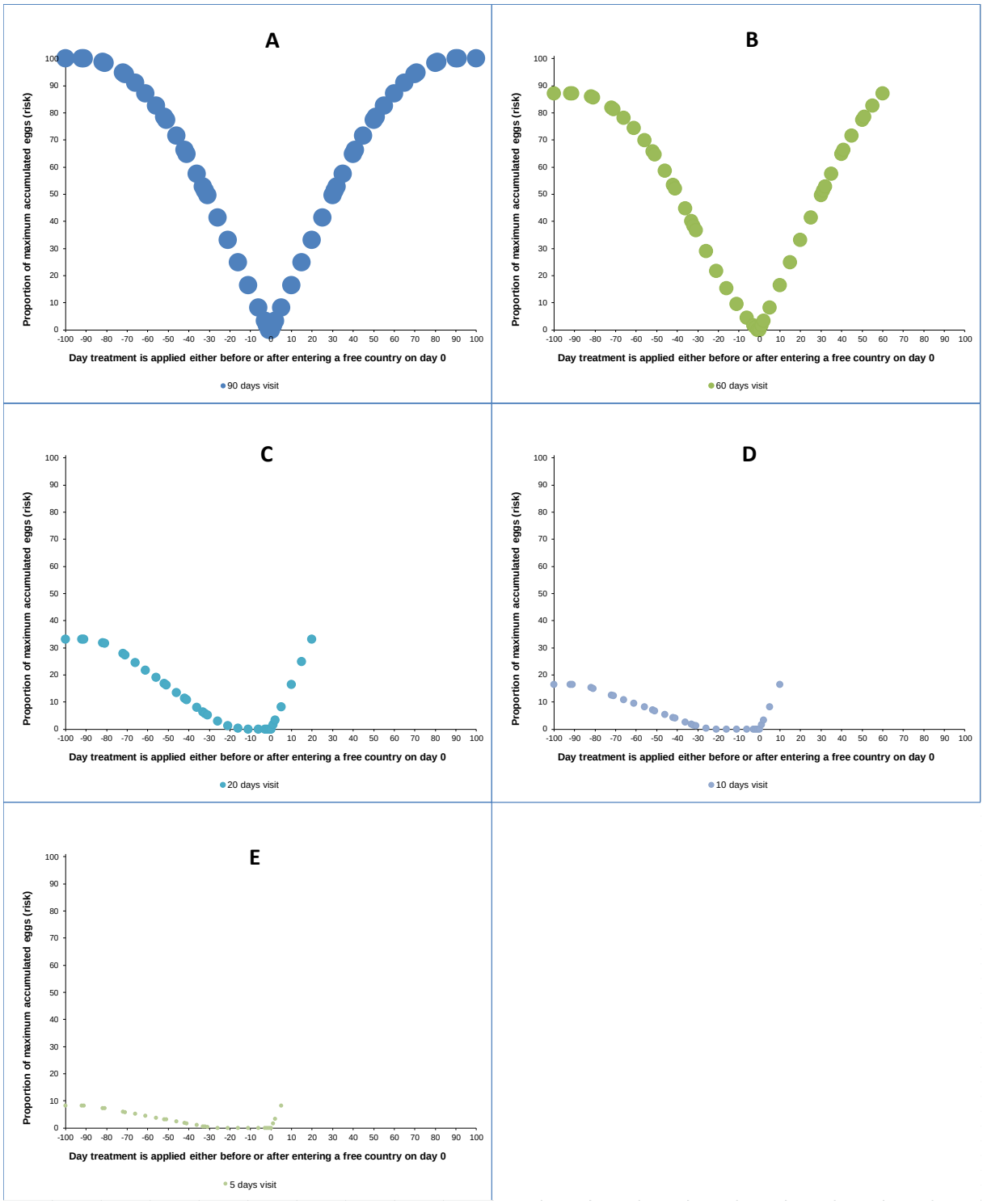

Figure F1. Proportion of number of eggs deposited in a free area by dogs living in endemic areas and visiting free countries for five different duration scenarios. Different treatment days are considered. Treatment day -1 results in the lowest risk while treatment earlier than day -90 or later than day +90 has no effect at all. If the visit in the free area is more than 90 days then the risk is symmetric around day -1, so whether treatment is done n days before entry on n days after does not affect the number of eggs excreted in the free area. If a dog from an endemic area visits a free area for 60 days the risk is no longer symmetric. It is possible to treat the dog at any day before the visit to the free area or during any of the sixty days in the free area. However treating n days before crossing the border results in fewer eggs deposited in the free area than treating n days after crossing the border. If a dog from an endemic area visits a free area for 20 days the risk is more asymmetric. Treating n days before crossing the border will result in

much fewer eggs being deposited in the free area compared to treating n days after crossing the border. If a dog from an endemic area visits a free area for 10 days the risk is very asymmetric. Treating such a dog day -5 results in minimal risk since any reinfecting worms in the dog will not be able to mature before the dog returns from the free area. If a dog from an endemic area visits a free area for just 5 days the risk is highly asymmetric. Treating such a dog later than day -25 results in minimal risk since any reinfecting worms in the dog will not be able to mature before the dog returns from the free area up to 30 days later.

The risk is very different in the reverse situation where dogs from a free county are exposed for various short periods before returning home (Figure F2-A to D). If treated prior to returning the re-infection risk is almost the same regardless of the duration of the visit. However, if treated after returning to the free area, the risk may be much lower at short visits because many of the worms or even all worms aquirred during the short stay in an endemic area will still be in the prepatent stage (right side of Figure F2 A to D).

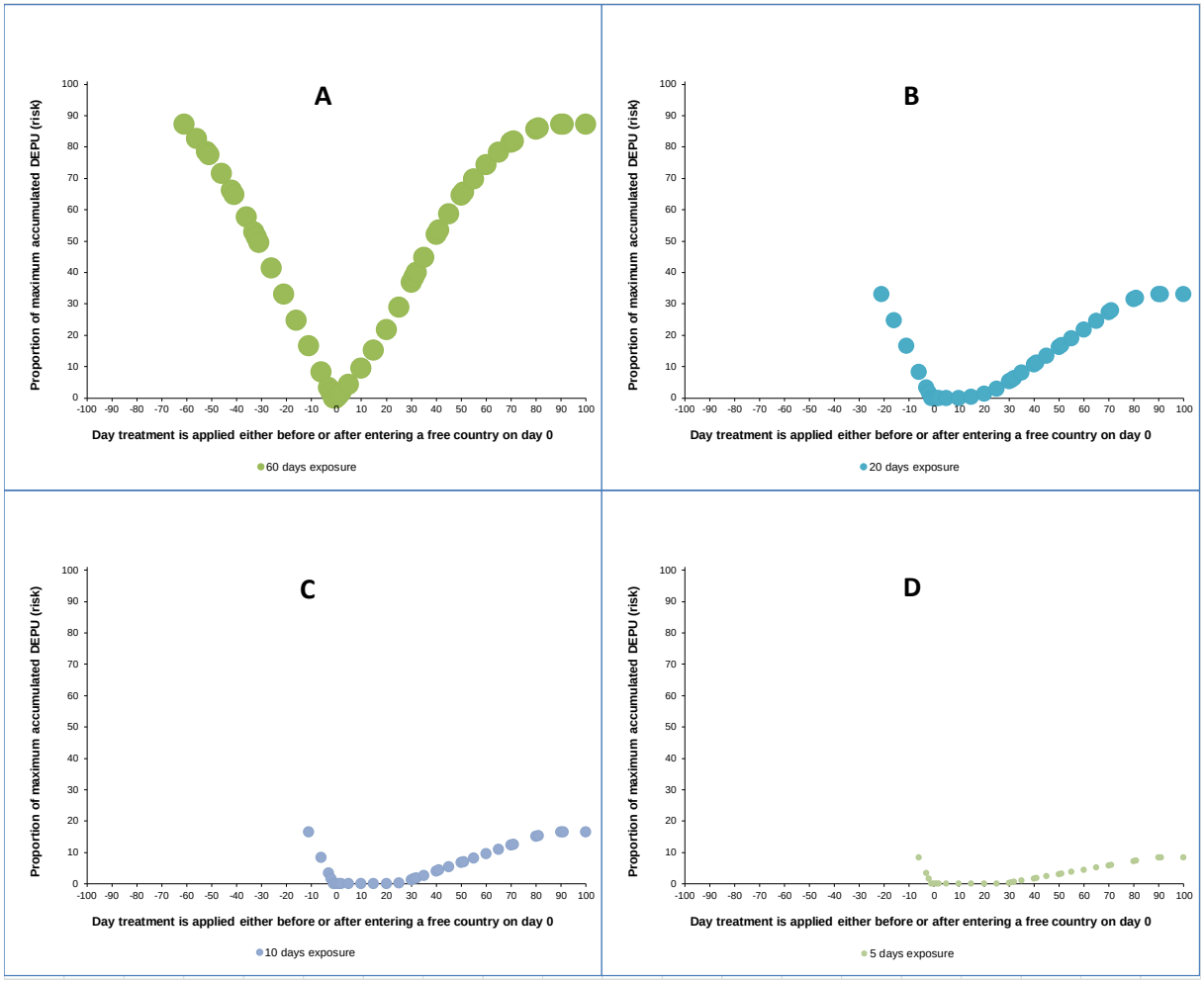

Figure F2: If a dog from a free area visits an endemic area for 60 days before returning to the free area the risk is also asymmetric. But now it is slightly better to treat n days after crossing the border than treating n days before crossing to the free area. If a dog from a free area visits an endemic area for 20 days before returning to the free area the asymmetric relationship increases. And it is now markedly better to treat n days after crossing the border than treating n days before. If a dog from a free area visits an endemic area for just 10 days before returning to the free area the asymmetric relationship increases. Treating such a dog five days after entry results in minimal risk since any acquired worms in the dog will be unable to mature before the dog is treated

in the free area. If a dog from a free area visits an endemic area for just 5 days before returning to the free area the risk is highly asymmetric. Treating such a dog up to 25 days after entry results in minimal risk since any acquired worms in the dog will be unable to mature before the dog is treated in the free area.

Increasing the treatment window may increase the number of eggs deposited in the free area (figure F1 and F2). An increased treatment window from 1 to 5 days may also increase treatment compliance because it makes it considerably easier for the owners to plan the treatment during their travel and thus more likely to comply with legislation as it may be difficult to find a veterinarian in e.g. weekends. Increased compliance during this time period may thus counteract the increased risk of re-infection. A mathematical equation has not been generated for the relationship between the size of the treatment window and the breakeven compliance level. The relationship will also be affected by the treatment efficacy of the drug as well the type of movement (permanent import or short exposure in endemic areas or short visits to free countries). Instead, the risk for six selected scenarios has been estimated (Tables 1-6 in Section 3.5.5.):

- a scenario where long term exposed (more than 90 days) dogs were permanently imported into a free country (Figure F3 A),
- three scenarios where long-term exposed dogs stayed 60, 20 and 5 days in the free country (Figur F3 B, C and D)
- and two scenarios where dogs were exposed for 20 or 5 days before returning to the free area and staying there permanently (Figur F3 E and F).

For each scenario, the risk was first calculated as a function of treatment day for three compliance levels ranging from 50% to 99% and then the results were interpolated for each compliance level (Figures F3). Only treatment prior to entering the free area was examined as present legislation does not allow for treatment after entering a free country.

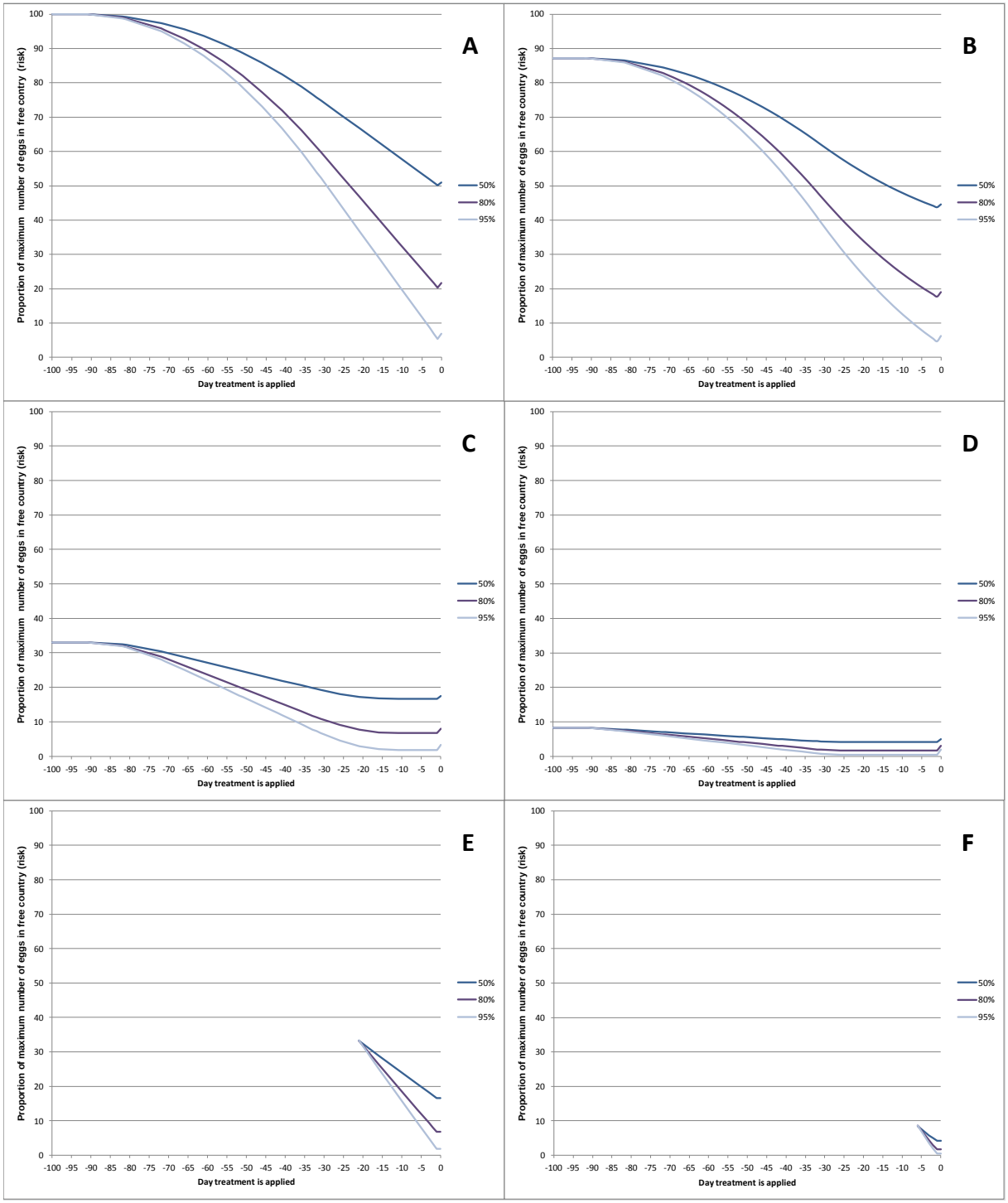

Figure F3. Treating dogs early will increase risk of reinfection after treatment but may also increase compliance. The six graphs show the interpolated relationship between day of treatment and risk (number of egg deposited in a free country). The relationship is calculated for three levels of compliance ranging from 50% to 95%. If treatment is given earlier (towards the left of the chart) then the more eggs will be deposited (increased risk) and compliance therefore needs to increase to counteract this increase in risk. A) The dog is long term exposed and then move permanently to a free area. E.g. if the initial compliance at day -1 is only 80% then the risk is about 20%. If a wider treatment window is desired for the convenience of owners and the risk at the same time has to be kept at 20%, then treatment can be allowed as early as day -10 if compliance at the same time increases to about 95% since the risk is also 20% for treatment day -10 at a 95% compliance level. B) The dog is long term exposed in an endemic area and visits a free country for 60 days. If treatment is given earlier

compliance needs to increase to prevent the risk from increasing, but not as much as for permanent import. E.g. if the initial compliance at day -1 is only 80% and risk is about 18% then treatment can be allowed as early as day -15 without increasing risk as long as compliance increases to about 95%. C) The dog is long term exposed and visits a free country for 20 days if the initial compliance at day -1 is only 80% and risk is about 7% then treatment can be allowed as early as day -31 without increasing risk as long as compliance increases to about 95%. D) The dog is long term exposed and visits a free country for just 5 days. E.g. if the initial compliance at day -1 is only 80% and risk is about 1.8% then treatment can be allowed as early as day -40 without increasing risk as long as compliance increases to about 95%. E) The the dog is not long term exposed but only exposed in an endemic area for 20 days before returning to stay in a free area for more than 90 days. If treatment is given earlier then compliance need to increase relatively much to prevent the risk from increasing. E.g. if the initial compliance at day -1 is only 80% and risk is about 6.5% then treatment cannot be allowed earlier than about day -4 without increasing risk even if compliance increases to about 95%. F) The dog is only exposed in an endemic area for 5 days before returning to stay in a free area for more than 90 days. If treatment is given earlier then compliance need to increase dramatically to prevent the risk from increasing. E.g. if the initial compliance at day -1 is only 80% and risk is about 1.8% then treatment cannot be allowed earlier than about day -2 without increasing risk even if compliance increases to about 95%.

The actual compliance levels for dogs entering the free Member States has been estimated to be between 40% and 80% in countries without border control (NO and FIN) and higher in contries with border controls (UK, Ireland). Therefore, three different suboptimal compliance levels of 95%, 80% and 50% were explored at the optimal treatment time (day -1). These compliance levels capture the problems and advantages of increasing the treatment window given that a wider window will result in better compliance.

The increase in compliance required to keep the risk at the same level as treatment day -1 was calculated given treatment instead was applied at day -2, day -3, day -4, day -5 or day -6. Because the re-infection risk increases as treatment is given earlier, it is calculated how much compliance would be required to increase to at least hold the risk stable. This break-even point is presented in Tables 1-6 (Section 3.5.5).

There are two conclusions regarding size of treatment windows, compliance and risk. One conclusion applies to dogs living in endemic areas and visiting free areas and another conclusion applies to dogs living in free countries and visiting endemic areas before returning to remain in their free countries:

- The shorter the period a dog from an endemic area visits a free country, the more likely the risk is to break even if a wider treatment window will lead to an increase in compliance above the compliance level at the optimal treatment time (which is one day before entry). If the visit is so short that the dog from an endemic area will return within 30 days after being treated, then re-infection does not increase risk and therefore any improvement in compliance will actually reduce the overall risk of eggs being deposited in the free area. Increasing the treatment window may therefore be beneficial if this makes dog owners more likely to comply better with treatment regulations. And especially where the initial compliance is low as may be the case in countries without border control.
- However, this only applies to dogs from endemic areas visiting free areas. Dogs living in free countries and visiting endemic areas for shorter periods before returning to their free countries constitutes a risk of egg excretion in their home countries that will require large increases in compliance to counteract the the reinfection risk resulting form an increase in treatment window. Increasing the treatment window from e.g. day -1 (-24 hours) to day -5 (-120 hours) is therefore likely to increase the risk of establishment of the infection unless the initial compliance at day-1 is very low and the increase in compliance with a larger window at the same time is very high.

The over all effect of increasing the treatment window for a specific country therefore depends on the compliance level before increasing the treatment window (which may depend on presence or absence

of border control), the resulting increase in compliance resulting from the increases treatment window, the proportion of dogs crossing the border that are either foreign or domestic and finally on the duration of exposure in the endemic area and the duration of stay in the free country. Thus if initial compliance is high (as it may be in countries with border controls) and travelling dogs are mostly domestic and only visit endemic areas for short periods then increasing the treatment window will increase the risk of introduction (but allowing treatment of domestic dogs after returning to the free country could keep the risk low even with a wider treatment window). But if the initial compliance is low with a narrow treatment window and compliance will increase with a bigger treatment window and the visiting dogs are mostly foreing and only visiting the free country for short periods then increasing the treatment window may be safe and may even reduce the overall risk while at the same time making travelling between EU countries easier for dog owners.

Appendix G – Diagnostic tests in animals

SEDIMENTATION AND COUNTING TECHNIQUE (SCT) – Post mortem test

Target: intestine of dead animals.

Description: at necropsy, the intestine is removed, opened and incubated in physiological saline. The intestinal mucosa is scraped between two fingers, after sedimentation the worms can be counted from the sediment with a binocular microscope. The SCT is considered as the gold standard and it is based on identification of morphologic features of EM. Despite that the sensitivity is dependent on the worm burden as shown by Karamon and colleagues, (2010). They estimated the limit of detection of the SCT by testing samples of small intestines, experimentally enriched with known numbers of EM tapeworms. Forty samples containing 2, 5, 10 and 30 tapeworms were examined and EM was detected in 30% using two spiked adult worms, increasing to 40% (five worms), 60% (ten worms) and 100% (30 worms). These results show that the sensitivity of the SCT depends on the worm burden. The worms that were used for spiking the samples, however, had been stored in 70% ethanol, potentially influencing the SCT test. However using spiked samples is expected to over-estimate the sensitivity as the quality of spiked samples is expected to be better compared to natural samples, also worms are more easily accessible in spiked samples where the worms are free in the lumen, compared to natural samples where worms can be located in the intestinal villi, finally it is easier to identify a small number of worms in a section of an intestine (spiked samples) compared to natural samples where the whole small intestine has to be examined. The detection limit of the SCT needs to be further investigated to confirm the reported results by Karamon et al., 2010.

In high endemic areas, the worm distribution is skewed with a small proportion of foxes with a high worm burden (Hofer et al., 2000; Tackmann et al., 2001). The sensitivity of SCT might be lower in low endemic areas compared to high endemic areas.

Moreover, bias of the investigators by overlooking worms cannot be excluded. Therefore, Eckert (2003) **suggested a sensitivity for SCT of about 98%**.

Limitations: SCT is a time-consuming technique. The use of SCT is restricted to the examination of necropsy material with related costs to manage the carcasses.

Approximate working intensity per person per day (according to Conraths and Deplazes, 2015): 50-100 animals depending on worm burdens and quantification (necropsy included).

Safety for laboratory personnel: precautions must be strictly followed when using this diagnostic test. Carcasses of definitive hosts from which samples are collected have to be frozen at −80°C for 7 days in order to achieve thorough deep-freezing and inactivate the eggs.

SEGMENTAL SEDIMENTATION AND COUNTING TECHNIQUE (SSCT) – Post mortem test

Target: intestine of dead animals.

Description: Similar to SCT, but intestine is divided in 5 segments and 3 are selected for analysis. In SSCT the examination of S1 (or S2) and S4 segments have a **sensitivity of 98.3%** compared to SCT (Umhang et al., 2011). This method is able to detect pre-patent period. SSCT is suitable for routine examination of fox intestines for large epidemiological studies.
Limitations: SSCT is an optimization of SCT because only selected segments (with higher probability to find EM) from the intestine are analysed, instead of analyzing the whole intestine. Reported sensitivity compared to SCT was 98.3% (Umhang et al., 2011) but sensitivity as lower worm burdens has not been reported. It remains a time-consuming technique, it is however less time consuming than SCT. The detection limit of the SSCT needs to be further investigated to confirm the reported results. Managing the carcasses remains costly.

Approximate working intensity per person per day (according to Conraths and Deplazes, 2015): around 100 animals depending on worm burdens and quantification (necropsy included).

Safety for laboratory personnel: precautions must be strictly followed when using this diagnostic test. Carcasses of definitive hosts from which samples are collected have to be frozen at −80°C for 7 days in order to achieve thorough deep-freezing and inactivate the eggs.

INTESTINAL SCRAPING TECHNIQUE (IST) – Post mortem test

Target: intestine of dead animals.

Description: fifteen deep mucosal scrapings are made from the intestines after necropsy. These scrapings can be performed with microscope slides. With (light and stereo) microscopy, the adult worms can be counted. The specificity of the IST is very high like the SCT because the diagnosis is based on the morphologic features of EM. In contrast to SCT, only small parts of the mucosa are investigated and therefore parasites present in low numbers may be overlooked. This method is not able to detect pre-patent period. In comparison with the SCT, the **sensitivity of the IST was 78%** (WHO OIE manual on Echinococcosis). This method is less time consuming compared to SCT and SSCT.

Limitations: it is a time-consuming and expensive technique (but not as labour intensive as SCT). The use of IST is restricted to the examination of necropsy material.

Approximate working intensity per person per day (according to Conraths and Deplazes, 2015): 100-150 animals depending on worm burdens (necropsy included).

Safety for laboratory personnel: precautions must be strictly followed when using this diagnostic test. Carcasses of definitive hosts from which samples are collected have to be frozen at −80°C for 7 days in order to achieve thorough deep-freezing and inactivate the eggs.

SHAKING IN A VESSEL TECHNIQUE (SVT) – Post mortem test

Target: intestine of dead animals.

Description: SVT is a modified sedimentation technique to examine intestines for smaller helminthes such as EM (Duscher et al., 2005). The opened small intestines with all its contents have to be placed into the vessel. After filling with water, the vessel is closed with a lid, covered by a steel mesh. By shaking the vessel, the water was decanted out. After filling the vessel again, the process must be repeated until the decanted water was clear. After stripping the intestines between two fingers, the vessel with intestines was refilled and shaken again. The remaining sediment can be microscopically scanned for worms. No sedimentation is necessary which saves time, and the method reduces the risk to lose worms by the decantation process. The sensitivity was better than the IST to which it was compared in the study, and the specificity of the SVT is very high, like the SCT, because the diagnosis is based on the morphologic features of EM. When SVT was applied after IST, **sensitivity resulted in 96.2%**. This method is not able to detect pre-patent period. The detection limit of the SVT needs to be further investigated to confirm the reported results.

Limitations: Very small worms can be washed through the sieve, worms can also be overlooked.

Approximate working intensity per person per day (according to Conraths and Deplazes, 2015): 100 animals depending on worm burdens (necropsy included).

Safety for laboratory personnel: precautions must be strictly followed when using this diagnostic test. Carcasses of definitive hosts from which samples are collected have to be frozen at −80°C for 7 days in order to achieve thorough deep-freezing and inactivate the eggs.

COPRO-ANTIGEN ENZYME-LINKED IMMUNOSORBENT ASSAY (cELISA) – Ante/Post mortem test

Target: faeces.

Description: fecal samples can be examined by ELISA to detect pathogen-specific antigens in the feces (copro-antigens, further referred to as cELISA). The excretion of copro-antigens is closely correlated to the presence of the intestinal worms. The detection rate rises with increasing worm burden. Deplazes and colleagues (1999) defined the overall diagnostic sensitivity of the cELISA in foxes with a known worm burden, as determined by using the SCT. **The sensitivity of the cELISA ranged from 40% to 100%** in fecal samples of animals with worm burdens ranging from 4-20 to 520-60.000 (REF)Deplazes et al., 1999) The overall sensitivity was 84%, with a higher sensitivity of the cELISA in fecal samples with a moderate to high worm burden (REF)Deplazes et al., 1999)

The specificity against *Echinococcus* antigens is often reported to be high, but in the field, cross reactivity can easily occur with antigens from *Taenia* species or other helminthes. It is important to note that the cELISA can detect the antigens already during the prepatent period. Commercial test kits are available. In fact, comparing this method with conventional PCR on DNA (isolated directly

from the faecal samples and from the eggs obtained by the flotation/sieving procedure), cELISA was the most sensitive to detect pre-patent infections (63%; Al-Sabi et al., 2007). Samples from the low patent infections were positive in 77% by microscopy and in 80% by egg-DNA PCR, being significantly more sensitive than cELISA and copro-DNA PCR (Al-Sabi et al., 2007). The detection limit of the cELISA needs to be further investigated to confirm the reported results.

Limitations: it can be performed in living and dead animals and is useful for population studies, but testing animals in areas with a low or unknown endemicity, the ELISA is less useful. In fact, this test has been shown to have high sensitivity when worm burdens are moderate to high, but the occurrence of false negatives when worm burdens are lower such as 50 or less worms (Allan and Craig, 2006). Cross reactions are affecting the specificity of the test.

Approximate working intensity per person per day (according to Conraths and Deplazes, 2015): 500-800 samples (for procedures see Sakai et al., 1998; Deplazes et al., 1999; Allan et al., 1992; Craig et al., 1995).

Safety for laboratory personnel: precautions must be strictly followed when using this diagnostic test. Carcasses of definitive hosts or feces from which samples are collected have to be frozen at −80°C for 7 days in order to achieve thorough deep-freezing and inactivate the eggs.

DNA-BASED TESTS – Ante/Post mortem test

Target: feces, eggs, worms.

Description: Polymerase Chain Reaction (PCR) tests are used to detect EM DNA in fecal samples. At least three steps are included in the diagnostic procedure: DNA extraction, specific amplification of *E. multilocularis* DNA and subsequently visualization or measurement of the PCR products. Various methods exist for the different steps.

Extraction of taenid DNA is the first step and it can be achieved in three different ways:

1) Concentration of taenid eggs by a combination of sequential sieving and flotation (Mathis et al., 1996). This method only retrieves particles of a size close to the size of taenid eggs. This means that detached parts of worms such as proglottids will not be detected. On the other hand the method can handle large sample sizes (3-20 g). After concentration, the eggs are digested by alkaline lysis and DNA is extracted. Often the extraction is done by using a Boom-silica spin column kit.

2) Extraction directly from feces (Dinkel et al., 1998; Knapp et al., 2014) by the use of a general DNA extraction method, often Boom-silica (Boom et al., 1990) in the form of a spin column extraction kit. This method generally cannot handle more than a maximum of 0.5 g, but will extract all taenid DNA and also DNA from other organisms from the sample.

3) DNA fishing method/magnetic capture: selective extraction of taeniid DNA by the means of a more or less specific hybridization probe connected to magnetic beads, Magnetic Capture (MC) (Isaksson et al., 2014; Øines et al., 2014). This method will also be able to retrieve taeniid DNA from other sources than just eggs, e.g. disintegrated worms. The probe will hybridize to the taeniid DNA target selectively, thus excluding the vast amounts of bacterial DNA present in feces. This method can handle 3 g of sample material, but could be automized and optimized to up to 10 g of sample material.

In approach 1 and 3, more sample material can be used in the extraction with less risk of inhibition of the following PCR, thus potentially increasing the sensitivity of the test. The reason is that these methods in different ways selectively enrich the target, allowing more material to be used in the assay. The floatation method achieves the enrichment by concentrating taeniid eggs, and the Magnetic Capture method by concentrating the target gene itself from both eggs and disintegrated worms. The advantage of enrichment (besides potentially 'catching' more of the target from the larger sample size) is that a large part of the PCR inhibitory substances are effectively removed. Inhibition considered to be a problem when trying to do direct nucleic acid extraction from more than a few hundred mg of feces. A comprehensive comparison between approach 1 and approach 3 is presented in Øines et al., 2014. Since this comparison, the MC-PCR has been improved somewhat by exchanging one of the primers and by automation of the washing of the magnetic beads.

For as concern the methodologies to use for the DNA extraction, they mainly consist of classical phenol-chloroform DNA extraction from faeces (e.g. Bretagne et al., 1993; Monnier et al., 2005), commercial DNA isolation kits (Al Sabi et al., 2007; Jiang et al., 2012) or the DNA fishing method/magnetic capture (Isaksson et al., 2014; Øines et al., 2014). The second step is amplification of the target sequence by PCR-based methods. Mitochondrial DNA molecules are most important PCR target because of their amount present in several copies per mitochondrion, and total up to several tens of thousands of copies per cell. Examples of mitochondrial DNA targets are: 12S, 12S,*nad1* and *cox1* genes. An example of a repeated target sequence present in the genomic DNA is ITS1 which is present in hundreds of copies per cell. PCR-amplification performed on DNA extracted directly from worms should not pose any problems whatsoever regardless of the chosen assay.

Furthermore, DNA amplification can be done in a single target conventional-PCR, a multiplex-PCR (Trachsel et al., 2007), a nested-PCR (Dinkel et al., 1998) or in a real time-PCR (Dinkel et al., 2011; Knapp et al., 2014). New techniques like the DNA fishing method/magnetic capture followed by real time-PCR show high sensitivity and high specificity, especially with worm burdens > 100 worms. It can be done partially automated, making it well-suited for nationwide EM surveillance programmes (Isaksson et al., 2014; Øines et al., 2014). The detection limit of the SSCT needs to be further investigated to confirm the reported results.

An obvious advantage of using PCR is the possibility to use feces as sample. The saving of not having to shoot, transport and perform necropsies on foxes in order to be able to do SCT/SSCT is probably quite large, although no one has published data on this. On the contrary, the disadvantages of using faeces is the incorrect identification of the host species and oversampling same individuals.

Limitations: due to the large variability in both the DNA extraction and the DNA amplification methods, it is difficult to compare studies with exactly the same PCR-methods. For this reason there are **very sparse data about the sensitivity of the diagnostic tests**. In general, when targeting a specific gene fragment of *Echinococcus multilocularis*, PCR can be highly specific. Some information are coming from Isaksson and colleagues (2014) that were evaluating the sensitivity of MC-PCR using the SCT as the golden standard. In this study **sensitivity was evaluated as 88% compared to SCT positive panel, and 95.7% considering samples with more than 100 worms** (Isaksson et al., 2014).

To increase the sensitivity, larger volumes of feces are required, but this is often hampered by the DNA extraction method. Inhibition of the PCR may result in false negative results, lowering the sensitivity of the PCR. A solution for this problem is extracting DNA of purified taeniid eggs or using an internal control (Mathis et al., 1996). PCR gives no information about the worm burden, though a Q-PCR gives information on the (relative) amount of DNA in the sample. Even though there is a strong negative correlation between worm burden and Cq-value when using real time-PCR assays, molecular assays can hardly be called quantitative. This is most likely due to that immature worms do not shed eggs and that shedding of eggs is not continuous even for mature worms.

It is important to note that PCR approaches usually haven't a so high sensitivity because cannot detect pre-patent period with the exception of the above mentioned PCRs using DNA fishing. PCR is also a laborious and expensive technique (as far as SCT) but the automation of processes and the decreasing of the costs will enormously simplify the approach to this methodology in surveillance programmes on *E. multilocularis*.

Approximate working intensity per person per day (according to Conraths and Deplazes, 2015):

- Conventional-PCR or Multiplex-PCR withsieving procedure for egg isolation from faeces: 40-80 samples depending on taeniid prevalence (for procedures see Mathis et al., 1996; Trachsel et al., 2007);
- Nested-PCR for total DNA isolation from faeces: around 70 samples (for procedures see Monnier et al., 1996; Dinkel et al., 1998; Van der Giessen et al., 1999);
- Real Time-PCR for total DNA isolation from faeces: 70 samples (for procedures see Dinkel et al., 2011; Knapp et al., 2013);
- MC-PCR with manual DNA fishing from faeces: 70 samples (for procedures see Isaksson et al., 2014);
- MC-PCR with automated DNA fishing from faeces: 240 samples (for procedures see Isaksson et al., 2014).

Safety for laboratory personnel: Precautions must be strictly followed when using this diagnostic test when using samples coming from definitive hosts (feces, eggs, worms). Samples have to be frozen at −80°C for 7 days in order to achieve thorough deep-freezing and inactivate the eggs.