SCIENTIFIC REPORT submitted to EFSA

Development of harmonised schemes for the monitoring and reporting of *Echinococcus* in animals and foodstuffs in the European Union [1]

Prepared by: Franck Boué [(d)], Jaap Boes[(i)], Pascal Boireau[(d)], Marleen Claes[(k)], Alasdair J.C. Cook[(b)], Pierre Dorny[(k)], Heidi Enemark[(j)], Joke van der Giessen[(f)], Keith R. Hunt [(b)], Mary Howell[(m)], Muza Kirjušina[(c)], Karsten Nöckler[(g)], Edoardo Pozio[(e)], Patrizia Rossi[(e)], Graham C. Smith[(a)], Lucy Snow [(b)], Mike A. Taylor[(a)], Georgios Theodoropoulos[(h)], Isabelle Vallée[(d)], Maria M. Vieira-Pinto[(l)], Irene-A. Zimmer[(a)]

ABSTRACT

Harmonised schemes are proposed for the monitoring and the reporting of *Echinococcus* in animals and foodstuffs in the European Union. Taking into account the public health perspective, the proposal focuses on *E. granulosus* sensu lato and *E. multilocularis*, which have a significant impact on human health and both are circulating to various degrees in Europe. For *E. granulosus* the monitoring of intermediate hosts (sheep, goats, pigs and cattle) at slaughterhouse level through meat inspection is recommended as well as mandatory notification of any positive cases. The genotyping to subspecies level should be performed to improve the strain identification. The development of more sensitive diagnostic methods (e.g. serology), preferably to be carried out on live animals, is also recommended. The monitoring of *E. multilocularis* should be performed in the definitive host (fox or raccoon dog) in order to identify geographical risk areas. The use of post-mortem intestine analysis as well as the notification of any positive cases are recommended. Additional information from wildlife would be needed to determine the geographical distribution of the parasite.

(a) The Food and Environment Research Agency (Fera), UK. Created on 1 April 2009 by the merger of the Central Science Laboratory (CSL) with two Defra departments
(b) Veterinary Laboratories Agency (VLA), UK
(c) Nacionalais Diagnostikas Centrs Pārtikas un Veterinārā Dienesta (NDC FVS), Latvia
(d) Agence Française de Sécurité Sanitaire des Aliments (AFSSA), France
(e) Istituto Superiore di Sanità (ISS), Italy
(f) Rijksinstituut voor Volksgezondheid en Milieu (RIVM), The Netherlands
(g) Bundesinstitut für Risikobewertung (BfR), Germany
(h) Agricultural University of Athens (AUA), Greece
(i) Danish Agricultural & Food Council (DAFC), which has been created on 3 June 2009 by the merger of the Danish Meat Association (DMA) with four other organisations, Denmark
(j) Technical University of Denmark, National Veterinary Institute (DTU), Denmark
(k) Prince Leopold Institute of Tropical Medicine Antwerp (ITG), Belgium
(l) Universidade de Trás-os-Montes e Alto Douro (UTAD), Portugal
(m) The UK Food Standards Agency, UK

KEY WORDS

Alveolar/cystic hydatid disease, descriptive analysis, Echinococcosis, *Echinococcus granulosus*, *Echinococcus multilocularis*, harmonised monitoring

[1] Question No EFSA-Q-2009-01071. Accepted for publication on 10 December 2009.

SUMMARY

In Europe, *Echinococcus* is circulating in livestock and wildlife and, being pathogenic to humans, is considered important to monitor. South-western and Eastern Europe as well as the United Kingdom are considered endemic regions for the *E. granulosus* complex. Infections in the intermediate host are usually identified during meat inspection but the sensitivity of meat inspection is rather low (Aalten et al, 2008). This means that low infection levels will not be detected and when the meat inspection procedure is carried out fast and/or not adequately supported by a sufficient number of official veterinarians (meat inspectors), some positive cases will be missed. In addition, there is a problem with improper registration and notification of partial viscera condemned for the presence of hydatid cysts during meat inspection. It is assumed that only the cases that lead to total condemnation are being notified to central authorities. It is important to improve the notification of hydatid cysts findings by improved slaughterhouse registration and meat inspection practices (low sensitivity).

For *E. granulosus,* we recommend the monitoring of intermediate hosts at slaughterhouse level through meat inspection and mandatory notification of any positive cases. Depending on the epidemiological situation in the country, a proportion of positive cases should be submitted for confirmation and genotyping of the parasite. After that the animals' producers must be notified in order to improve and adopt corrective and preventive measures to avoid animal and human infections (anthelmintic treatment of owned dogs,veterinary controls to prevent home slaughtering of sheep and goats and improved supervision of slaughtering facilities e.g. regarding destruction of infected offal). For the future, we propose further investigation and development of more sensitive methods to monitor *E. granulosus* infected animals (e.g. serology), preferably tests that can be carried out on live animals. Furthermore, the development of tests that would enable monitoring of live animals would help to address the risk of introduction of animals from endemic regions.

In the case of *E. multilocularis* information should be obtained by monitoring the definitive host (fox or raccoon dog). This will lead to a better identification of geographical risk areas and provide information for preventive action. Where population estimates exist, the sample size should be calculated in each region with reference to the local fox and raccoon dog population. Diagnosis should be carried out using the methods described by the World Organisation for Animal Health (OIE) (e.g. mucosal scraping) and positive cases should be notified to the authorities (OIE, 2008). Specific data from wildlife are needed to determine the regions in which the parasite is present and to assess public health risk in these regions. This could be achieved by dissemination of official information to medical practitioners, hunters or information to the public.

TABLE OF CONTENTS

BACKGROUND

In the EFSA Community Summary Report (CSR) (2009) on zoonoses, the information received from the Member States (MSs) is analysed and summarised specifically to identify trends in the occurrence of the zoonotic agents and the sources of human infections. As there are currently no harmonised rules or recommendations for reporting and monitoring of *Echinococcus* spp., *Trichinella* spp., *Cysticercus* spp. and *Sarcocystis* spp. in the European Union (EU), the data obtained is often difficult to analyse and interpret.

The European Food Authority's (EFSA) Scientific Panels on Biological Hazards (BIOHAZ) and on Animal Health and Welfare (AHAW) issued an opinion on the Review of the Community Summary Report on Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004. In this opinion the panels concluded among other things: parasites (Toxoplasma gondii, *Echinococcus* spp., Trichinella spp. and Taenia spp./Cysticercus spp.) have been reported less frequently in humans and have caused fewer outbreaks, than bacteria and viruses in the EU in 2004. However, in many instances the impact of these zoonotic agents (severe illness, disability, death and costs related to diagnostic procedures, hospitalisation and treatment) on vulnerable groups of the population, and often in immunocompromised persons, has probably been considerable.

The panels also stated that there is a need for a common strategy on data collection, monitoring and reporting as well as an improvement of harmonisation of definitions, in order to improve the usefulness of the data presented in the CSR.

TERMS OF REFERENCE

The objective of the call is to obtain proposals for projects, which will develop harmonised monitoring and reporting schemes for *Echinococcus* spp., respectively, in animals and, when appropriate, in foodstuffs under the Directive 2003/99/EC. The schemes shall be applicable in all EU MSs.

These schemes shall, in particular, specify:

- the animal species and/or foodstuffs, which should be monitored and the study populations (subgroups of the population) to be targeted. The animal species may cover farm animals, pet animals, zoo animals and wildlife;
- the stage when the sampling should take place (e.g. at farm, at slaughterhouse);
- sample size (the number of samples to be collected) and the procedure how to select the samples;
- the type of specimen to be taken and sampling techniques;
- the diagnostic and analytical methods to be used;
- the information to be collected at national level; and
- the information to be reported to the Commission and EFSA.

The rationale for the specifications chosen in the monitoring and reporting schemes must be given. When developing the schemes, it is advisable to take into account public health needs, the feasibility and cost-effectiveness of the schemes as well as different situations in the MSs.

The schemes shall also include suggestions for the analyses of the data at national and Community levels, and, in particular, indicate where the following of trends over the reporting years would be useful.

ACKNOWLEDGEMENTS

This contract/grant was awarded by EFSA to: The Food and Environment Research Agency (Fera), UK, created on 1 April by the merger of the Central Science Laboratory (CSL) with two Defra departments, as project co-ordinator, on behalf of the following co-beneficiaries:

- Veterinary Laboratories Agency (VLA), UK;
- Nacionalais Diagnostikas Centrs Pārtikas un Veterinārā Dienesta (NDC FVS), Latvia;
- Agence Française de Sécurité Sanitaire des Aliments (AFSSA), France;
- Istituto Superiore di Sanità (ISS), Italy;
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and in co-operation with:

- Agricultural University of Athens (AUA), Greece;
- Prince Leopold Institute of Tropical Medicine Antwerp (ITG), Belgium;
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- Universidade de Trás-os-Montes e Alto Douro (UTAD), Portugal;
- National Veterinary Institute (DTU), Denmark.

This project was supported by the UK Food Standards Agency. We would also like to thank Professor Peter Deplazes for his valuable contributions as well as all EU Member States for their assistance with collecting data and information.

Contractor/Beneficiary: The Food and Environment Research Agency (Fera), UK.

Contract/grant title: Development of harmonised schemes for the monitoring and reporting of *Echinococcus* in animals and foodstuffs in the European Union.

Contract/grant number: CFP/EFSA/Zoonoses/2007/01.

INTRODUCTION AND OBJECTIVES

INTRODUCTION

Directive 2003/99/EC (EC, 2003) forms the basis for data on zoonoses being collected throughout the MSs and reported to the EU Commission on an annual basis. These data are collected and examined by the European Food Safety Authority, who, in collaboration with the European Centre for Disease Control (ECDC) and assisted by the Zoonoses Collaboration Centre (ZCC), produce the annual CSR. The report is aimed at the detection of sources and trends within the EU MSs and to aid the long-term goal of protecting human health. *Echinococcus* is included in list A of Annex I, Directive 2003/99/EC, which determines which agents have to be monitored on a mandatory basis. *Echinococcus* prevalence is mentioned in reviews carried out for the CSRs on Trends and Sources of Zoonoses, 2005 to 2007 (EFSA, 2007; EFSA, 2008; EFSA, 2009).

Human echinococcosis (also known as "cystic hydatid disease" and "alveolar hydatid disease") is a zoonotic infection caused by the larval stages of the small tapeworms of the genus *Echinococcus.* In Europe, cystic echinococcosis (CE) (hydatid disease) is caused by *Echinococcus granulosus* (a complex of 3 or 4 sibling species). *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis (AE). Two other species of *Echinococcus* (*E. vogeli* and *E. oligarthrus*), although zoonotic, do not occur in Europe. A fifth species, *Echinococcus shiquicus*, has been isolated from a Tibetan fox in China (Xiao et al., 2006), but its zoonotic role is still unknown. Furthermore, another species, *Echinococcus felidis* has been detected only in the wildlife of Africa (Hüttner, 2008).

Pathology, epidemiology and geographical occurrence vary widely between the different *Echinococcus* taxa. As a general rule, echinococcosis caused by species mainly transmitted by wild animals is rare, due to limited contact between humans and wildlife. However, in Europe *E. multilocularis* is considered an emerging parasite probably due to the effective rabies vaccination programme, which has resulted in an increase in the fox population and possible spread of *E. multilocularis*. *E. multilocularis* is also an emerging problem where domestic dogs have been involved in the lifecycle (Schweiger et al., 2007; Takumi et al., 2008). The extension of the endemic area of *E. multilocularis* observed in Europe raises questions about the public health implications of this infection. Most forms of CE are transmitted in domestic lifecycles involving dogs and livestock and constitute an emerging public health problem, especially in regions with extensive livestock husbandry and slaughter carried out at farms without proper meat inspection (Romig, 2003).

E. granulosus is the main species of importance in relation to food producing animals. Intermediate hosts, in which hydatid cysts can be found predominantly in the lungs and liver, include cattle, sheep, pig, deer as well as many wild ruminants such as boar. Infection is through the ingestion of eggs excreted in the faeces of the canine final host (Figure 1).

A common feature of all strains (except the lion strain, *E. felidis*) is the utilisation of dogs and other canids as definitive hosts, but the strains exhibit several differences in the intermediate host spectrum, geographic distribution, adult and metacestode morphology, maturation time in definitive hosts, organ localisation of metacestodes, and protoscolex production (Eckert and Thompson, 1997). It has to be emphasised that at least seven of nine *E. granulosus* genotypes are infective to humans. Globally, most human cases of CE are caused by the sheep strain (G1) of *E. granulosus* (Eckert and Deplazes, 2004).

ECHINOCOCCOSIS GRANULOSUS

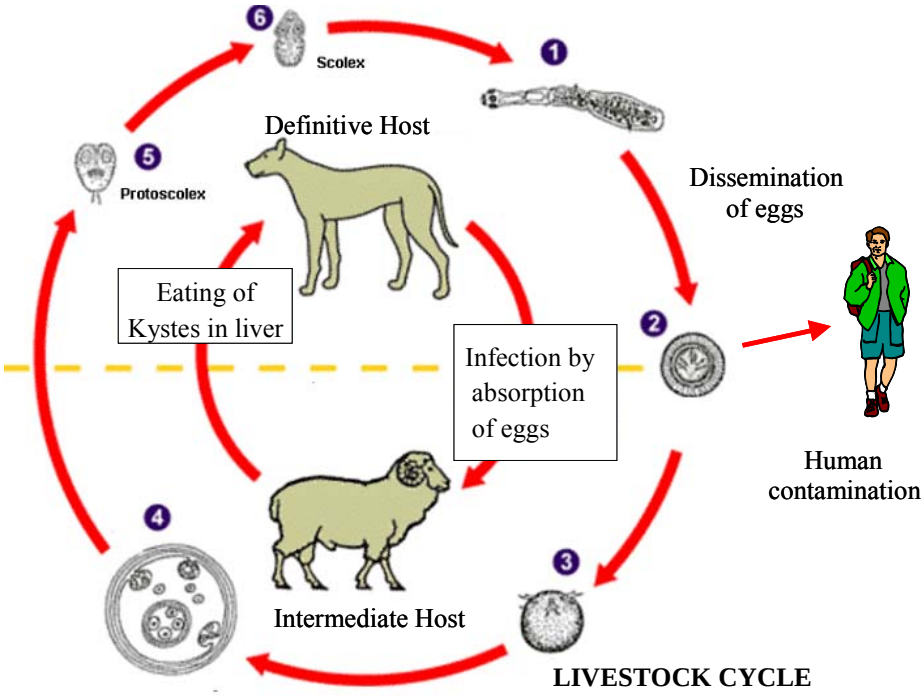

Figure 1: Life cycle of *Echinococcus granulosus*

Four (sub)species of *E. granulosus* exist in Europe. Recently, the taxonomy of *E. granulosus* was redefined (Thompson, 2008; Nakao et al., 2007). *E. granulosus* G1 has predominantly a dog-sheep cycle and is infective to man. Human infection occurs by ingestion of eggs present on the coats of dogs, or from vegetables and other foodstuffs contaminated by dog faeces and rarely, of other canids e.g. wolves and jackals (definitive host). This species is endemic in Southern and Eastern Europe. In Eastern Europe, G7 (*E. canadensis*) also exists with a cycle, involving pigs as intermediate hosts and carnivores as definitive hosts. While the infection of carnivores with immature or mature intestinal stages of *E. granulosus* does not cause morbidity, the invasion of various organs (mainly liver and lungs) of intermediate or aberrant hosts by metacestodes can cause severe and even fatal disease (echinococcosis). Another species, which has also now been reclassified as a species is *E. ortleppi* (G5), which occurs in west- and central Europe with a dog-cattle cycle. Only very few cases of this species have been reported. Another genotype, recently classified, and a species is *E. equinus* (previous G4), which has a dog-horse cycle and is present in the United Kingdom and does not seem to be zoonotic.

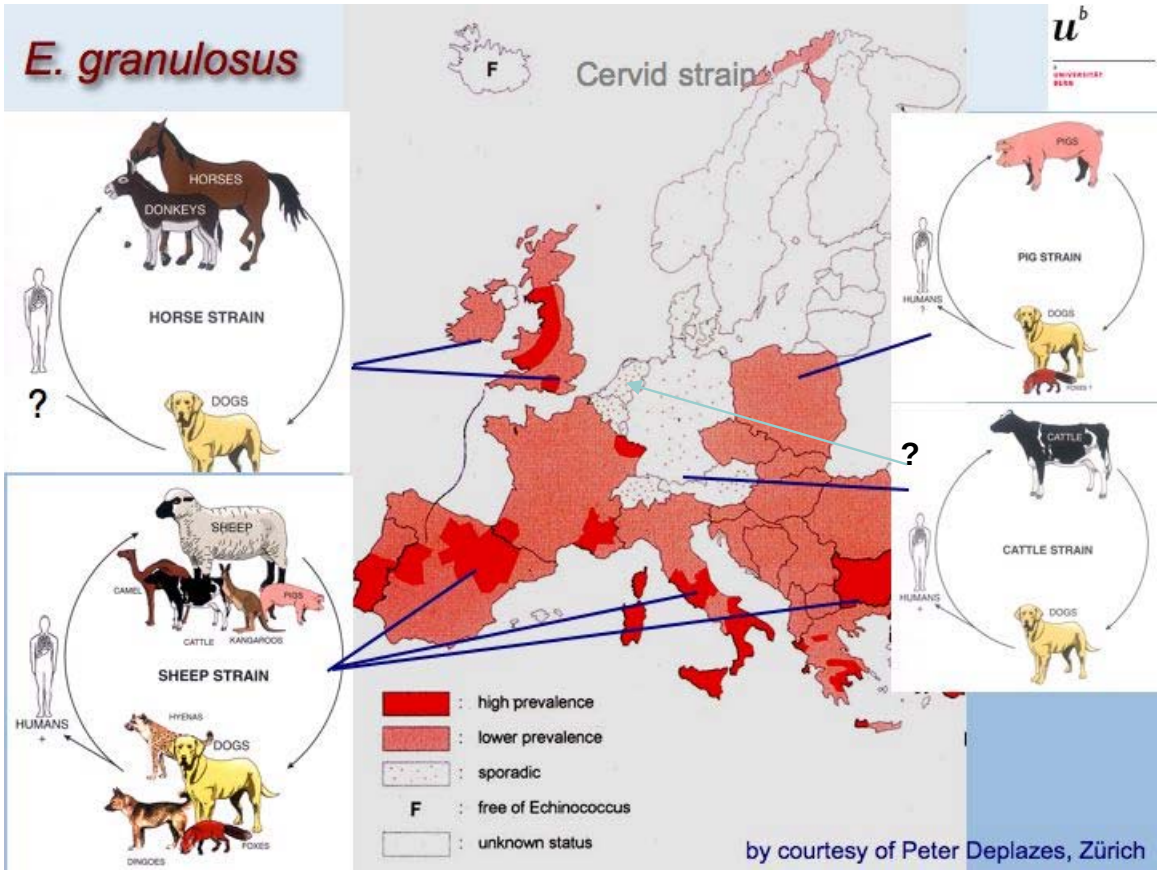

Figure 2: Map showing the distribution of *E. granulosus* strains in Europe

NB: this is not a current map and does not necessarily reflect individual findings but gives a good overview of the complex situation in Europe and demonstrates very well why strain identification is considered important.

E. multilocularis occurs throughout the northern hemisphere, although its small scale distribution and frequency is not fully understood. *Echinococcus multilocularis* has a sylvatic cycle and the typical transmission cycle in Europe is wildlife-based with red foxes as final hosts and rodents as intermediate hosts (Figure 3). Domestic animals (dogs and to a lesser extent, cats) can also be infected by the parasites, but are of secondary importance for the lifecycle's persistence (Kapel et al, 2006).

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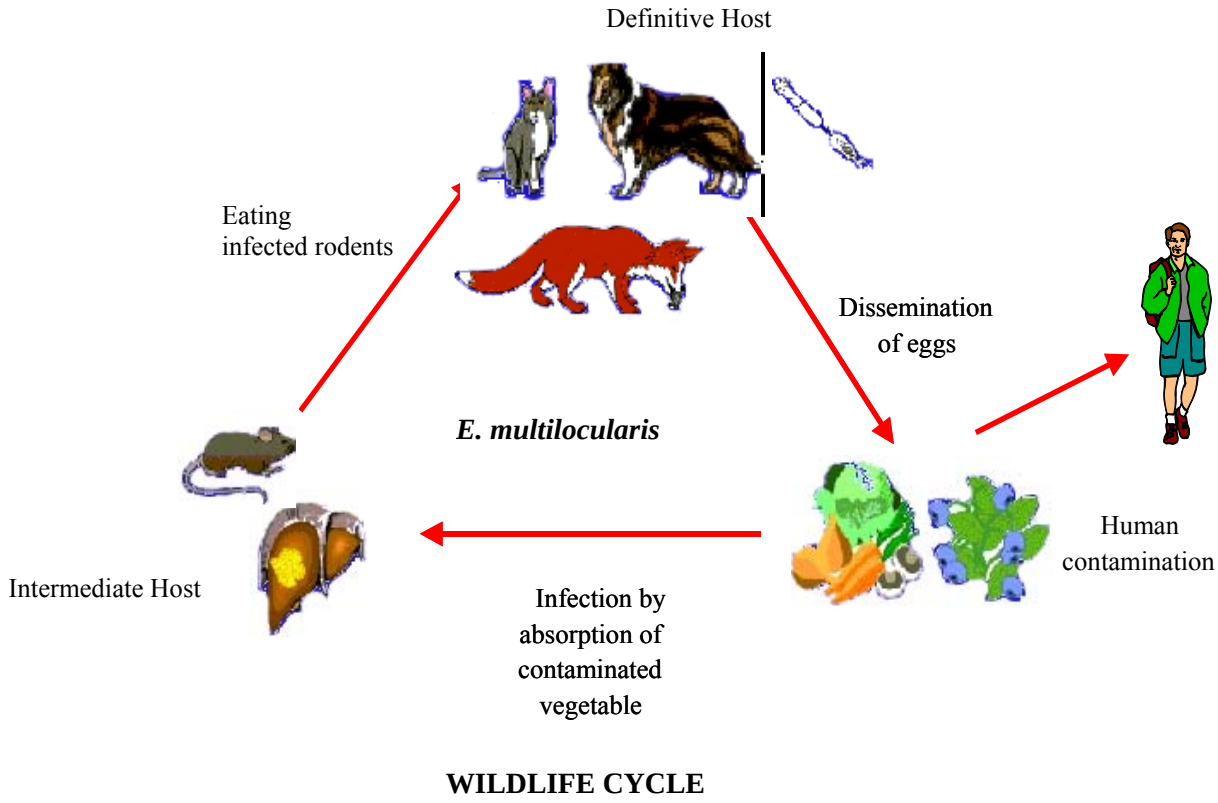

Figure 3: Life cycle of *Echinococcus multilocularis*

Human infection occurs in the same way as for *E. granulosus,* by ingestion of eggs through vegetables, berries etc. contaminated with faecal material. The adult worms of *E. multilocularis* live in the lumen of the small intestine of their carnivore hosts without any damage to the mucosa, consequently no external symptoms or clinical signs are visible.

OBJECTIVES

The objective of these specifications is to develop a harmonised scheme for the monitoring and reporting of *Echinococcus* spp. in defined animal populations in the EU. The results from the application of such a harmonised scheme should create data that would enable comparison of disease levels and status between MSs and identification of trends on a national and community level.

The overall objective was broken down into several milestones. The first milestone was to review the current disease situation and national monitoring in MSs. The rationale behind this was to identify public health needs in MSs, and to create a basis for formulating sampling plans. Other milestones assessed the agent and it's species to identify which ones are relevant to public health, their impact on human health and their epidemiology. A list of animals and foodstuffs was created for the relevant agents and their suitability within monitoring schemes was assessed. Analytical methods are one of the limiting factors in monitoring. Existing analytical methods were summarised and assessed regarding their feasibility in sampling schemes that are designed to be carried out throughout the EU.

Objective 1. Identify current disease situation in Member States and current national level of monitoring and reporting information

1.1 Rationale

In the call for proposals it is specified that harmonised schemes should consider different situations in MSs and the schemes should be designed to be applicable in all European MSs. Consideration should also be paid to testing schemes currently carried out in MSs. The table was designed to gather data needed to assess in public health needs, the current testing situation and for defining epidemiological parameters.

1.2 Approach

A spreadsheet for data and information collection was designed and circulated to MSs using personal contacts, established contacts to National Competent Authorities or networks within the project team. The spreadsheet asked for information on confirmed human cases and the current disease situation in relevant animal populations, as well as for supporting information on sampling and testing carried out in MSs. Where answers were not received a literature search was carried out in order to fill the gaps. Summary tables were compiled to give a brief overview of the current disease and monitoring situation in the different MSs, which can be found in Annex 1.

1.3 Results

Eighteen MSs responded to enquiries regarding current surveillance and 17 of these provided some information on *Echinococcus*. Monitoring for *E. granulosus* is carried out in all responding MSs through continuous surveillance of all slaughtered pigs, cattle, sheep and goats by inspection for macroscopic lesions. Some countries also sample wild boar and other wild ungulates. Many countries have legislation in place to cover this monitoring. A number of MSs also monitor solipeds. For *E. multilocularis*, a number of different wildlife species are monitored, but these are primarily foxes and racoon dogs. With regard to the current animal disease situation in the EU, limited data was provided and the EFSA CSR was felt to provide the best data.

The MSs that had been contacted provided only very little data on the current human situation therefore were supplemented with data from the CSR 2007 (EFSA, 2009). Twenty one countries reported human data on *Echinococcus*, with a total of 834 confirmed cases. Of these 87.3% of cases were of *E. granulosus*, and approximately 8.8% were *E. multilocularis* (EFSA, 2008). In Italy, the overall yearly incidence of CE in humans is about 1.3 cases per 10 inhabitants, but in a certain endemic region the incidence is up to 4.8 cases per 10^5 inhabitants. In livestock of Italy, the highest prevalence occurs in sheep and goats of southern Italy where the infection rate can be up to 50-60%. In Bulgaria, there is an average incidence of 6.3 cases per 100,000 population, with an 0.8% mortality rate, yet the incidence can reach 27.5 cases per 100,000 population in endemic areas. Data from the European Echinococcosis Registry (Surveillance for human AE in Europe, 1982-2000) shows 559 AE patients in Europe combined with the occurrence of human cases outside endemic regions (Kern *et al.*, 2003). The first human case assumed to be infected in the Netherlands was identified in the southern endemic part of the Netherlands in 2009. The patient was living in the southernmost emerging area, where the parasite was first detected in foxes in 1998 (van der Giessen et al., 1999).

Objective 2. Identify animal species and/or foodstuffs which could be affected and specify which should be monitored

2.1 Identify parasite species to be monitored

Tapeworm *Echinococcus* genus Cestoda (*Taeniidae*) is composed of several species (Jenkins et al., 2005) that exploit predatory-prey systems between carnivores (mainly canids) as principal hosts and an intermediate host that could range from rodents to livestock depending on the species. Moreover not all *Echinococcus* genotypes have been confirmed as zoonotic, but most are. Due to the different epidemiological cycles of *E. granulosus* and *E. multilocularis*, they are addressed separately from here on.

During the past four decades, considerable phenotypic and genetic variability has been observed within the species *E. granulosus* and several strains have been identified (Pearson *et al.*, 2002; Thompson and McManus, 2001, Thompson and McManus, 2002, Van Herwerchen *et al*., 2000). According to Romig (2003), new data demonstrate that '*E. granulosus*', the causative agent of CE, is an assembly of several, rather diverse strains and genotypes that show fundamental differences, not only in their epidemiology but also in their pathogenicity to humans. This fact may explain the unequal distribution of high-endemicity areas for human CE on regional scales, which previously, has been attributed to differences in human behaviour.

A common feature of all different *E. granulosus* genotypes (except the lion strain) is the utilisation of dogs and other canids as definitive hosts, but the strains exhibit several differences in the intermediate host spectrum, geographic distribution, adult and metacestode morphology, maturation time in definitive hosts, organ localisation of metacestodes, and protoscolex production (Eckert and Thompson, 1997). Globally, most human cases of CE are caused by the sheep strain (G1) of *E. granulosus* (Eckert and Deplazes, 2004).

E. multilocularis occurs throughout the northern hemisphere, although is small scale in distribution and frequency is not completely known. In Europe, due to the zoonotic potential of this parasite and the characteristics of the disease, AE is considered one of the most severe human parasitoses in non-tropical regions and it has received considerable attention in recent years.

The typical transmission cycle in Europe is wildlife based. It involves red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) as final hosts and wild rodents as intermediate hosts. Even if domestic dogs and cats could also be infected by the worms (Thompson and Eckert, 1983) under field conditions, the absolute number of infected domestic animals in Europe is small and they appear to be of secondary importance in the maintenance of the lifecycle (Kapel et al, 2006). However, infected domestic animals such as dogs are probably of major importance in terms of human infection risk because of their close contact with humans.

2.1.1 Rationale

2.1.2 Approach

Literature (scientific publications, textbooks, official websites, OIE/WHO/ECDC) on *Echinococcus* spp. was reviewed and the information/existing knowledge on zoonotic species summarised in a table. The identified species were run through a number of criteria, listed below, and their zoonotic potential assessed. A summary of the results can be found in the spreadsheet '*Echinococcus* Zoonotic species Risk Assessment' in Appendix C.

The species were run through the following criteria:

Criteria 1: Zoonotic (Y/N)?

Species, which have not been reported in literature as zoonotic were not taken further through the qualitative risk assessment, as they were considered irrelevant to this project.

Criteria 2: Pathogenicity (+ - +++)

On account of the severity of the disease, all clinical cases resulting in death, without medical intervention, and all zoonotic species were classified 'highly pathogenic'.

Criteria 3: Geographical distribution

Geographical distribution signifies the presence in the EU or the likelihood of introduction into EU MSs and likelihood of establishment if an agent were to be introduced.

Zoonotic species, currently not considered autochthonous to the MSs, were assessed as to how likely it is for them to be introduced into EU MSs and, consequentially, the likelihood of establishment. This all depends on the epidemiology of the agent and the role of human as intermediate or final/dead end host or 'vector'.

Criteria 4: Economic impact of human disease

For a qualitative assessment of the economic impact of human clinical disease, the treatment costs and/or number of sick days, and long-term effects were considered. Again, this was carried out on a qualitative scale, to give a rough guideline and justification of monitoring schemes.

2.1.3 Results

The two species relevant for monitoring in Europe are *E. granulosus* sensu lato and *E. multilocularis*. Both species have a significant impact on human health and clinical disease in humans can be fatal if left untreated. Both species are circulating to various degrees in Europe, *E. granulosus* in livestock, and *E. multilocularis* in wildlife, mainly in foxes and raccoon dogs. It needs to be stressed that, though members of the same species, due to significant differences in the epidemiology and human pathology, *E. granulosus* and *E. multilocularis* should be treated as two separate agents.

As important as the distinction between *E. multilocularis* and *E. granulosus*, is the strain (subspecies) identification of *E. granulosus*, for epidemiological and public health purposes.

In the same way *E. multilocularis* distribution areas should be defined, and the border established using the adapted sampling strategy mentioned below. Moreover, the reporting of domestic animal cases should be encouraged by MSs.

Whereas the infection of carnivores with immature or mature intestinal stages of *E. granulosus* does not cause morbidity, the invasion of various organs (mainly liver and lungs) of intermediate or aberrant hosts by metacestodes can cause severe and even fatal disease (echinococcosis) (Eckert and Deplazes, 2004). In the human host, after oral uptake of *E. granulosus* eggs, a larval stage, the metacestode, develops in the liver and possibly other internal organs (cysts may develop predominantly in the liver but can also occur in other organs following oral ingestion of eggs). This

form of echinococcosis is known as primary CE. Secondary CE, predominantly in the abdominal cavity, results from spontaneous or trauma-induced cyst rupture and the release of protoscoleces and/or small cysts, which can grow into larger cysts. Approximately 40% to 80% of patients with primary CE have single-organ involvement and harbour a solitary cyst (Ammann and Eckert, 1996; Pawlowski et al., 2001). For *E. multilocularis* the economic impact in animals is low and could be considered as unimportant for wildlife; however the human public health impact could be very important.

2.2 Identify relevant animal species and/or foodstuffs to be monitored

2.2.1 Rationale

Parasite species are often reported in a wide variety of hosts, not all of which necessarily play a role in the transmission of the disease, and have an impact on the human food chain or are suitable for surveillance in a public health context. The aim was to identify which species would be suitable for surveillance in all MSs and consideration was given to existing surveillance carried out in MSs.

2.2.2 Approach

A table was compiled with animal species in which the zoonotic agent has been reported. The animal species were then assessed as to their role in the epidemiological chain and the human food chain.

2.2.3 Results

Due to the difference between the species, *E. granulosus* and *E. multilocularis* are considered separately and an overview can be found in Appendices D & E 'Relevant animals and foodstuffs to be monitored'.

For *E. granulosus* the highest prevalence rates among humans and animals occur where livestock production is extensive (e.g. large-scale sheep farming), where large numbers of dogs are kept (e.g. for guarding livestock), and where dogs have access to carcasses of dead livestock or offal after uncontrolled slaughter (Schantz et al., 1995). For that reason, the animals that should be monitored at slaughterhouse are:

- sheep;
- pigs;
- goats; and
- cattle.

Foodstuffs other than carcasses are not relevant for monitoring purposes.

To increase the identification of the strain, genotyping to subspecies level (G1 etc.) should be encouraged.

Increased range *E. multilocularis* is a result of expansion or intensified investigations. However, there is evidence of an increase of the parasite density in many areas. For *E. multilocularis*, the wildlife cycle of the parasite necessitated the organisation of a sampling strategy to monitor the fox and raccoon dog infection (definitive hosts) particularly at the border of its distribution.

Concerning the role of cats as a final host of *E. multilocularis* and their role as a source of infection for humans, the opinion of Professor Peter Deplazes, a recognised expert in this field, was sought. He commented that cats are not very good definitive hosts and the egg production of the worms is heavily reduced. Furthermore, cats are known to be rather clean animals compared to dogs and, as we know from *Toxoplasma*, cats excreting parasite stages are not contaminated with such infective stages. Prevalence of infected cats are around 0.3% in Switzerland or Germany. Professor Deplazes strongly supported the hypothesis that cats are not important as a source of infection, unlike dogs. Due to high fox populations in the cities, cycles of this parasite exist, so does a very high urban contamination with *E. multilocularis*. Professor Deplazes was aware that the importance of cats was stressed by some authors and there was a large interest of the industry to give consideration to cats as a risk because this would justify recommendations for a higher frequency of anthelmintic treatment. Furthermore, in one risk analysis in Austria, cats were described as a risk factor, however, several epidemiologists have commented very critically on this paper and this paper should not be cited without a critical comment (Peter Deplazes, personal communication).

Objective 3. Identify most suitable diagnostic and analytical methods to be used

3.1 Rationale

For most agents more than one detection method exists, applicable to different sample materials and producing results that often vary from method to method. These methods were compiled to identify the limitations of what can be achieved diagnostically, to compare the cost benefits of various methods and to assess practical aspects. Not every test can be used for every sample type. However, if two different methods produce the same result, e.g. the measuring of national prevalence to a certain level, the results of both methods could be directly compared. A cost estimate was also included as this is an important criterion when recommending analytical methods.

3.2 Approach

Existing analytical methods, as cited in publications or official methods (OIE manual, 2008/Regulation (EC) No 854/2004 (EC, 2004)) were compiled and test specifics (sensitivity, specificity), as far as available, listed. Also considered were the expenditure and complexity of test methods. Costs were estimated roughly, where possible, bearing in mind variations from country to country and depend on the daily throughput in a diagnostic facility.

3.3 Results

Feedback from questionnaires to MSs revealed that surveillance is performed during official meat inspection as part of Regulation (EC) No 854/2004 (EC, 2004) of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption.

The diagnosis of intestinal *Echinococcus* infection in living dogs or foxes is difficult because the small proglottids spontaneously discharged with faeces are usually overlooked and eggs detected by routine coproscopic techniques cannot be differentiated by light microscopy from the eggs of the *Taenia* species. ELISAs for detecting parasite antigens in faecal samples (coproantigens) of dogs have been used in specialised laboratories in recent years (Deplazes et al., 1994; Craig et al., 1995) but the commercial test is not actually available. A Polymerase Chain Reaction (PCR) for specific detection of DNA from *Echinococcus* eggs has been developed. The coproantigen ELISA can be used as a screening test for individual dogs/foxes or for canid populations, however the sensitivity should be validated for regions where the prevalence of the parasite and the worm burdens are low. PCR can be used as a sensitive and specific test for *E. granulosus* or *E. multilocularis* infection. *Post mortem* examination of definitive hosts for *Echinococcus* species requires special techniques, which are described in detail elsewhere (Eckert et al., 2001a & b).

Due to this diagnostic difficulty in the definitive host in the case of *E. granulosus*, the monitoring of the disease must be performed on the intermediate host at slaughterhouse level. Infections with *E. granulosus* cysts in intermediate hosts (sheep, goat, cattle, horses, etc.) are typically asymptomatic, and there are no reliable methods for the routine diagnosis of the infection in living animals. Serological assays might be useful to monitor the occurrence and prevalence in a population and to follow trends in time. However, because of the lack of serological assays, the only possible diagnostic method is cyst detection during meat inspection or at post mortem examination (Eckert and Deplazes, 2004). However, the sensitivity of meat inspection is low especially when low infection levels in slaughter animals occur or the speed of slaughtering hampers the time for proper individual meat inspection when large numbers of animals have to be slaughtered. It is likely that in a number of instances there is improper registration and notification of partial (viscera) condemned for the presence

of hydatid cysts during meat inspection and therefore only the cases that lead to total condemnation are being reported.

At this point, meat inspection remains the diagnostic method of choice, which should be carried out in the intermediate hosts. However, it is important to improve the level of hydatid cyst identification and to improve reporting by mandatory notification.

Due to the difficulty of diagnosis in the intermediate host in the case of *E. multilocularis*, the definitive host (foxes and raccoon dogs) should be targeted, and the only referent methods is the post mortem examination of or faecal examination using PCR. The post mortem intestine examination requires special techniques and is time consuming. Analytical methods are summarised in Appendices F and G.

Objective 4. Define sample size, collection procedure, specimen types and sampling techniques

4.1 Sample sizes

4.1.1 Livestock

All animals destined for the human food chain are inspected for *Echinococcus* under the current EC legislation. *E. granulosus* is a risk for those countries free of *E. granulosus* and that are importing cattle (other livestock). In 2007, Berends et al. (2009) carried out a risk assessment based on the number of imported *E. granulosus* infected cattle in The Netherlands, which varied from 0 (Cyprus) to 4,934 from Romania (90% of all positive cattle). This risk assessment calculated the number of potentially exposed dogs based on the low sensitivity of detection of infected animals in slaughterhouses (assumed 10%) and the fact that lungs and livers approved for human consumption could be processed into dog food. If dogs are infected with *E. granulosus* by these products, they can then infect humans. This demonstrates the need and the importance of good diagnostic tools (development of specific and sensitive serological assay) for non-endemic regions to prevent the risk of importing *E. granulosus* and furthermore for strong veterinary controls and the adoption of good hygiene practices to prevent dogs coming in contact with infected viscera.

4.1.2 Foxes and raccoon dogs

In regions where *E. multilocularis* is present in foxes the prevalence of infection in foxes usually varies between approximately 10%-20%, with higher prevalences in some of up to 50-60% (EFSA, 2009; Losson et al, 2003).

The sample size for the monitoring of *E. multilocularis* in populations where it is endemic is therefore determined to allow the estimation of prevalence at 50% with 5% accuracy and 95% confidence.

In the absence of more definitive information it is assumed that the red fox or racoon dog population density is 1 per km^2 and so the population size is equal to the area of the region in km^2. For simplicity, raccoon dogs, if present, are assumed to be present at a similar density to red foxes and may replace foxes within the sample.

Table 1: Number of fox (or raccoon dog) carcasses to be collected per region for given estimated population size

Fox or raccoon dog population	*E. Multilocularis* free regions Detect disease at 1% (95% confidence)	Endemic/emerging regions Estimate prevalence at 50% (5% accuracy, 95% confidence)
>=5,000	300	360
4,000	288	351
3,000	284	341
2,000	277	323
1,000	258	278
500	225	218

If accurate regional fox population estimates are available the sample size may be calculated using these.

Where fox populations are low, it is recommended that regions are large enough to ensure that the required number of foxes can be collected over the period without a detrimental effect on the local fox population. This may require combining two regions for sampling.

Monitoring

In order to detect changes in prevalence between reporting years in endemic and emerging regions Table 2 outlines the minimum sample size required for given changes.

Table 2: Sample sizes to detect changes in prevalence (95% confidence, 80% power)

Change in prevalence to detect	From 1% to 5%	From 5% to 10%	From 10% to 20%	From 20% to 30%	From 30% to 40%	From 40% to 50%
Required minimum sample size per year (or reporting period e.g. 2 years)	283	443	198	292	355	386

Table 2 demonstrates that although changes in prevalence may not be detected between successive sampling years or reporting periods (required sample sizes greater than 250), monitoring over 10+ years will enable the detection of changes in prevalence of 5%-10% even when the prevalence is high or the detected change is small.

Objective 5. Propose harmonised monitoring and reporting schemes

5.1 Monitoring methodology

5.1.1 Parasite species to be monitored

For *E. multilocularis*, post-mortem intestine analysis in the wild definitive host, red foxes or racoon dogs, is recommended. This requires harmonisation of the sampling strategy at European level to monitor the fox/raccoon dog infection levels and permit comparison of results between MSs.

It is recommended that the monitoring for *E. granulosus* in the intermediate host is carried out at slaughterhouse level through meat inspection analysis with mandatory notification of every positive case. All positive cases detected must be sent to the National Reference Laboratory (NRL) Parasites or the Community Reference Laboratory (CRL) Parasites to be identified and genotyped. In case of an endemic region, the genotyping should be limited to *E. granulosus* genotypes to describe the epidemiological situation in different intermediate host animals in that region. Also the animals' producers must be notified in order to improve and adopt corrective and preventive measures to avoid animal and human infection (anthelmintic treatment of owned dogs, strong veterinary control to avoid home slaughtering of sheep, goats and other livestock (e.g. cattle and pigs), and supervision of slaughtering facilities, i.e. destruction of infected offal). Since the main transmission route of *E. granulosus* from the intermediate to the definitive host is the home slaughtering of sheep and goats and other livestock without any veterinary control and official meat inspection and infected offal often used to feed dogs, the education of farmers and shepherds is a key action in the control of this infection in animals and humans.

Cysts, both fertile and unfertile, should be collected at the slaughterhouse in the context of meat inspection, stored in 90% ethyl alcohol and sent to the NRL. The cyst fertility, host species and locality of origin should be registered.

5.2 Sampling for *Echinococcus multilocularis*

5.2.1 The animal populations to be sampled and time of year

E. multilocularis is primarily found in wild foxes, which therefore provide the best species for monitoring. Racoon dogs may also harbour infection and may be sampled instead of foxes where they exist. The required sample size may comprise of foxes, racoon dogs or both. Since population densities of foxes and racoon dogs vary within the year, it is important to harmonise sampling times. Sampling in the winter period from October until March is recommended.

Sampling strategy

E. multilocularis is spatially heterogeneous and thought to be absent in a number of MSs. It is therefore proposed that monitoring be carried out according the status of the region with respect to *E. multilocularis*. In this case region may be a country or an area of the country with a defined boundary (e.g. NUTS region), dependent on the epidemiological situation in the MS. Three status categories can be applied:

Endemic regions:

Disease is known to be present and monitoring is carried out in foxes to confirm disease presence and monitor any changes in prevalence.

A regional survey should be carried out every five years or small numbers cumulatively over the period to meet sample size requirements. The sample size will be dependent on the size of the region and the size of the local fox population and will enable an estimate of the prevalence of disease if the prevalence is 50%, with 5% accuracy and 95% confidence. See section 5.6.

Since the prevalence does not reflect the real risks to human health, it is also important to know quantitative data about the level of infections. In this way, the biomass of eggs and thus the exposure to humans can be determined better. For example, prevalence can remain the same, but the number of parasites per animal can be increased (Takumi et al., 2005). Therefore, as well as the prevalence (positive/negative) of infected foxes, there is also a need to count the number of parasites in each fox. In an endemic area, the monitoring of changes in the prevalence in foxes is important to determine environmental contamination that could help in public information and human risk prevention.

Emerging regions

Areas where *E. multilocularis* is emerging and spreading. Sampling of foxes is carried out every five years to detect disease and monitor the spread of disease. Any region directly adjacent to an endemic region is either endemic or emerging.

As in regions where *E. multilocularis* is endemic the sample size will enable an estimate of the prevalence of disease if the prevalence is 20% with 5% accuracy and 95% confidence. See section 5.2

Disease free regions

Infection is assumed to be absent in the host (i.e. fox and/or raccoon dog) by sampling to confirm true prevalence is less than 1%. See section 5.6. Samples must be taken every five years or cumulatively over the period to meet the sample size requirements.

Which category a region falls into will depend on the status of *E. multilocularis* in foxes. If previous history of positive foxes exists covering more than 10 years and autochthonous human cases occur, the region is considered to be endemic. If infection is present in part of the region and considered to be spreading the region is 'emerging'. If no infection is shown to be present at the given level despite adequate sampling, the region is considered to be free from infection.

In all regions sampling should be carried out as far as possible homogeneously across the region and effort should be made to record accurately the geographical coordinates of all carcases (negative and positive). Where foxes and racoon dogs are also collected for the monitoring of *Trichinella* the samples can be tested for both. Sampling should ideally take place in the winter as the highest levels of contamination are recorded during winter.

5.3 Sampling for *Echinococcus granulosus*

5.3.1 Populations to be sampled

All regions should continue to inspect sheep, goats, cattle and pig carcasses for hydatid cysts at slaughter, under Regulation (EC) No 854/2004 (EC, 2004).

Additionally it is recommended that all samples should be genotyped with the exception of highly endemic areas where an agreed proportion of the samples should be tested. The aim of the genotyping is to identify the strains present in the region and to correlate the strain with cyst fertility and the host species, not to estimate the prevalence. For an infinite population, 300 samples are required to detect a prevalence of 1% with 95% confidence. Assuming that within the population of positive *E. granulosus* cases, each genotype is present at 1% or more, the same sample size can be applied. Therefore, each MSs should aim to genotype all positive cases, or where this exceeds 300 positives, submit a random sample of 300 positive cases for genotyping. This should be stratified by slaughterhouse to ensure adequate epidemiological and geographical coverage.

5.4 Reporting

Recommendation: The reporting of *E. granulosus and E. multilocularis* in humans is to become mandatory across the EU. It is recommended that notification of *Echinococcus granulosus* in livestock be compulsory for the central competent authority. Monitoring of echinococcosis/hydatidosis is already covered in the Directive 2003/99/EC (on the monitoring of zoonoses and zoonotic agents).

The information to be collected by MSs is described below and consists of two categories:

1. the description of the monitoring programme, which is to be reported to the EU; and
2. the individual data for each wildlife sample to be used for national reporting. MSs are encouraged to use Food Chain Information (FCI) where possible for livestock data, as collection of information on origin of carcases is mandatory under the new food hygiene legislation. Currently only aggregated data (section 6.1) can be reported to the EU but MSs are encouraged to put in place tools for capturing and storing more detailed results.

5.5 Description of surveillance programme for EU level reporting (*E. multilocularis and E. granulosus* to be treated separately)

- MS name;
- region name (NUTS region);
- parasite species/strain;
- monitoring scheme employed;
- date of start and end of surveillance;
- animal species (e.g. fox, racoon dog, sheep, goats, cattle and pig);
- overall results:
  - number of animals tested;
  - number of positive animals; and
  - prevalence of *Echinococcus*.

5.6 Individual sample information for wildlife (for national reporting)

- surveillance status (Endemic, emerging, free)
- animal species
- status* (positive/negative – see case definition)
- analysis method used
- species/strain of parasite detected
- worm burden per animal if post mortem examination is carried out in the case of *E. multilocularis*
- strain of parasite detected e.g. G1 and cyst fertility
- geographical origin of carcass (coordinates of carcass if wildlife)

Reporting of data is currently via electronic submission on the EFSA website. It is felt that this is an adequate method and no recommendations for change are made. To fully utilise the data available and information/knowledge gained regarding *Echinococcus*, it is recommended that some attempt be made in future to capture and/or analyse the national reporting data (animal level) at EU level.

5.7 Population data

Reporting of adequate population data is not only important for *E. multilocularis* and *E. granulosus* but for all zoonoses reporting to EFSA and consideration should be given to the central collection of this data. For the current work, the following information is of interest:

- species (e.g. sheep, goat);
- production type if applicable (e.g. fattening pig); and
- total slaughtered population in each MS.

Objective 6. Propose information to be analysed by the Commission and EFSA for detecting trends

The results for the two *Echinococcus* species should be reported and analysed separately.

6.1 *Echinococcus multilocularis*

6.2 Descriptive

Regions where no disease is detected are considered to have a prevalence of less than 1% prevalence and 95% confidence intervals for all other regions can be estimated.

Suggested analysis can include:

- prevalence of positive samples from the fox/raccoon dog population in each region or MS. Where reported at MS level, this must include data from all regions in the MS including regions considered free;
- regional estimates (NUTS 1) of the prevalence and worm burden of *E. multilocularis* in the fox/raccoon dog populations for the reporting period;
- the number of endemic/emerging/free regions in the EU according to status, for example a bar chart showing how many regions are endemic, emerging and free; and
- at Community level an estimate of the overall prevalence of *E. multilocularis* can be estimated using the total number of positive animals and the total number tested. However, if sampling is not carried out randomly within a region (e.g. targeted to where infection is known to be highest) then this may bias estimates.

6.2.1 Monitoring of trends over time

Different surveillance approaches are used in different regions depending on the status with respect to *E. multilocularis.* This means that several approaches for the analysis of results are suitable:

- monitoring the change in the number of regions according to category e.g. increasing number of endemic regions and decreasing number of free regions over time; and
- monitoring the change in prevalence by area e.g. prevalence in endemic areas, prevalence in emerging areas.

6.2.2 Spatial analysis

Geographical analysis can be carried out at the regional level where surveillance is also carried out regionally. Choropleth maps showing 1) the status of each region with respect to *E. multilocularis*, and 2) the prevalence of *E. multilocularis* within each region. Where prevalence in endemic regions is presented, cluster methods could be used to identify clusters of regions with higher and lower prevalence, regardless of national boundaries (only regional boundaries). Analysis at national level could include spatial analysis of the point location of carcasses but currently this data cannot be analysed at EU level.

6.3 *Echinococcus granulosus*

6.3.1 Descriptive analyses

Analysis of monitoring at slaughterhouse will be at MS level, rather than regionally. The results of the report from the EFSA Working Group on Statistical Analysis of Temporal and Spatial Trends in Zoonotic Agents in Animals and Food should also be taken into consideration.

Suggestions for descriptive analysis include:

- tables showing the proportion of positive samples per MS for each animals species monitored; and
- an estimation of Community prevalence of *E. granulosus* in each species. Where MSs do not sample all animals, weighting to account for the proportion of animals sampled within the MSs may be required to estimate prevalence using the reported population data.

6.3.2 Monitoring trends over time

For determining trends in the prevalence of *E. granulosus* over time within a MS, methods such as logistic regression can be used when the number of reporting periods exceeds two. Different models could be used for each animal species. Data can be analysed cumulatively or using a 10 year rolling window. Other non-parametric tests to compare consecutive reporting periods can also be used.

At Community level approaches such as multilevel (e.g. random effects, GEE) modelling can be applied to all EU data while still taking into account differences in trends between MSs.

6.3.3 Spatial analysis

Choropleth maps to show the prevalence of *E. granulosus* at MS level in each animal species. The distribution of livestock is aggregated in the EU with some countries producing a large number of the total population. It is possible to use maps such as cartograms to illustrate where the highest number of animals is present and use additional choropleth layers to illustrate prevalence.

REFERENCES

Aalten M, Züchner L, Bruinier E, Holzhauer M, Wouda W, Borgsteede F, Sprong H and van der Giessen J, 2008. Reintroduction of echinococcosis in The Netherlands via import of cattle Tijdschrift voor Diergeneeskunde Nov 1, 133, 21, pp. 898-902.

Abbasi I, Branzburg A, Campos-Ponce M, Abdel Hafez SK, Raoul F, Craig PS, Hamburger J, 2003. Copro-diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified repeated DNA sequence. The American Society of Tropical Medicine and Hygiene, 69, pp. 324–330.

Allan JC, Craig PS, Garcia Noval J, Mencos F, Liu D, Wang Y, Wen H, Zhou P, Stringer R, Rogan M, et al., 1992. Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans, Parasitology, Apr, 104, Pt 2, pp. 347-56.

Ammann, R W and Eckert J, 1996. Cestodes: *Echinococcus.* Gastroenterology Clinics of North America, 25, pp. 655-689.

Bacciarini LN, Gottstein B, Wenker C, Gröne A, 2005. Rapid development of hepatic alveolar echinococcosis in a cynomolgus monkey (Macaca fascicularis), 225. The Veterinary Record, 15, 156, 3, pp. 90-1.

Berends IM, Holzhauer M, van der Giessen JW, van Schaik G, in press 2009. Risk of *Echinococcus granulosus* becoming endemic in Dutch cattle. Tijdschrift voor diergeneeskunde, 134, 3, pp. 104-9.

Berke O, Romig T, von Keyserlingk M, 2008. Emergence of *Echinococcus multilocularis* among Red Foxes in northern Germany, 1991–2005, Veterinary Parasitology, 155, 3-4, pp. 319-22.

Borgsteede FH, Tibben JH, van der Giessen JW, 2003. The musk rat (Ondatra zibethicus) as intermediate host of cestodes in the Netherlands, Veterinary Parasitology, 117, 1-2, pp. 29-36.

Boucher JM, Hanosset R, Augot D, Bart JM, Morand M, Piarroux R, Pozet-Bouhier F, Losson B, Cliquet F, 2005. Detection of *Echinococcus multilocularis* in wild boars in France using PCR techniques against larval form**,** Veterinary Parasitology, 129, 3-4, pp. 259-66.

Boussinesq M, Bresson S, Liance M, Houin R, 1986. A new natural intermediate host of Echinococcus multilocularis in France: the muskrat (Ondatra zibethicus L.) Annales de Parasitologie Humaine et Comparée, 61, pp. 431-4.

Bowles J, Blair D, Mc Manus D, 1995. A molecular phylogeny of the genus *Echinococcus,* Parsitology, 110, pp. 317-328.

Casulli A, Manfredi MT, La Rosa G, Cerbo AR, Genchi C, Pozio E. 2008. *Echinococcus ortleppi* and *E. granulosus* G1, G2 and G3 genotypes in Italian bovines, Veterinary Parasitology, 155, 1-2, pp. 168-72,

Christodoulopoulos G, Theodoropoulos G, Petrakos G, 2008. Epidemiological survey of cestode-larva disease in Greek sheep flocks, Veterinary Parasitology, 153, 3-4, pp. 368-73.

Craig PS, Gasser RB, Parada L, Cabrera P, Parietti S, Borgues C, Acuttis A, Agulla J, Snowden K, Paolillo E, 1995. Diagnosis of canine echinococcosis: comparison of coproantigen and serum antibody tests with arecoline purgation in Uruguay. Veterinary Parasitology., 56, pp. 293-301.

D'Alessandro A, Rausch L, 2008. New Aspects of Neotropical Polycystic (*Echinococcus vogeli*) and Unicystic (*Echinococcus oligarthrus*) Echinococcosis, Clinical Microbiology Reviews, 21, 2, pp. 380-401.

Deplazes P, Jimenez-Palacios S, Gottstein B, Skaggs J, Eckert J, 1994. Detection of *Echinococcus granulosus* coproantigens in stray dogs of northern Spain. Applied Parasitology, 35, pp. 297-301.

Deplazes P, Alther P, Tanner I, Thompson RCA, Eckert J, 1999. *Echinococcus multilocularis* coproantigen detection by enzyme-linked immunosorbent assay in fox, dog, and cat populations. Journal of Parasitology, 85, 1, pp. 115-121.

Deplazes P, Eckert J, 2001. Veterinary aspects of alveolar echinococcosis--a zoonosis of public health significance. Veterinary Parasitology, 98, 1-3, pp. 65-87

Dinkel A, Nickisch-Rosenegk MV, Bilger B, Merli M, Lucius R, Romig T, 1998. Detection of *Echinococcus multilocularis* in definitive host: Coprodiagnosis by PCR as an alternative to necropsy, Journal of Clinical Microbiology, 36, 7, pp. 1871-1876.

Dinkel A, Njoroge EM, Zimmermann A, Wälz M, Zeyhle E, Elmahdi IE, Mackenstedt U, Romig T, 2004. A PCR system for detection of species and genotypes of the *Echinococcus granulosus* - complex, with reference to the epidemiological situation in eastern Africa. International Journal for Parasitology, 34, pp. 645-653.

Duscher G, Prosl H, Joachim A, 2005. Scraping or shaking--a comparison of methods for the quantitative determination of *Echinococcus multilocularis* in fox intestines, Parasitology Research 95, pp. 40-42.

EC (European Community), 2003. Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC, OJ L325, 12.12.2003, pp. 31-40.

EC (European Community), 2004. Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption, OJ L 139, 30.4.2004, pp. 206-320.

Eckert J, 1997. Epidemiology of *Echinococcus multilocularis* and *Echinococcus granulosus* in central Europe Parassitologia, 39, pp. 337-344.

Eckert J, Thompson RCA, 1997. Intraspecific variation of *Echinococcus granulosus* and related species with emphasis on their infectivity to humans. Acta Tropica, 64, pp. 19-34.

Eckert J, Conraths F, Tackman K, 2000. Echinococcosis: An emerging or re-emerging zoonosis? International Journal for Parasitology, 30, pp. 1283-1294.

Eckert J, Gottstein B, Heath D, Liu F-J, 2001a. Prevention of echinococcosis in humans and safety precautions, pp. 238-247. In J. Eckert, M. A. Gemmell, F.-X. Meslin, and Z. S. Pawlowski (ed.), WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. World Organisation for Animal Health, Paris, France.

Eckert J, Deplazes P, Craig PS, Gemmell MA, Gottstein B, Heath D, Jenkins DJ, Kamiya M, Lightowlers, M, 2001b. Echinococcosis in animals: clinical aspects, diagnosis and treatment, pp. 72-99. In: J. Eckert, M. A. Gemmell, F.-X. Meslin, and Z. S. Pawlowski (ed.), WHO/OIE Manual on echinococcosis in humans and animals: a public health problem of global concern. World Organisation for Animal Health, Paris, France.

Eckert J, Deplazes P, 2004. Biological, Epidemiological, and Clinical Aspects of Echinococcosis, a Zoonosis of Increasing Concern, Clinical Microbiology Reviews, January, 17, 1, pp. 107–135.

EFSA, 2006. Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004. The EFSA Journal 2005, 310 pp.

EFSA Scientific Panels on Biological Hazards (BIOHAZ) and on Animal Health and Welfare (AHAW), .2006. Opinion on the Review of the Community Summary Report on Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union, 2004. The EFSA Journal, 2006, 403, pp. 1-62, http://www.efsa.europa.eu/en/science/biohaz/biohaz_opinions/biohazahaw_ej403_zoonoses.html.

EFSA, 2007. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005. The EFSA Journal 2006, 94 pp..

EFSA, 2008. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006. The EFSA Journal 2007, 130 pp..

EFSA, 2009. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. The EFSA Journal 2009, 223 pp..

Hansen F, Jeltsch F, Tackmann K, Staubach C, Thulke HH, 2004. Processes leading to a spatial aggregation of *Echinococcus multilocularis* in its natural intermediate host *Microtus arvalis*. International Journal for Parasitology, 34, pp. 37-44.

Hofer S, Gloor S, Muller U, Mathis A, Hegglin D, Deplazes P, 2000. High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zürich, Switzerland. Parasitology 120, Pt 2, pp. 135-142.

Hüttner M, Nakao M, Wassermann T, Siefert L, Boomker JD, Dinkel A, Sako Y, Mackenstedt U, Romig T, Ito A. 2008. Genetic characterization and phylogenetic position of *Echinococcus* felidis (Cestoda: Taeniidae) from the African lion. International Journal for Parasitology, 38, 7, pp. 861-8.

Jenkins DJ, Romig T, Thompson RC, 2005. Emergence/re-emergence of *Echinococcus* spp. - a global update. International Journal for Parasitology; 35, 11-12, pp. 1205-19.

Kapel CM, Torgerson PR, Thompson RC, Deplazes P, 2006. Reproductive potential of *Echinococcus multilocularis* in experimentally infected foxes, dogs, raccoon dogs and cats. International Journal for Parasitology, 36, 1, pp. 79-86.

Lavikainen A, Lehtinen MJ, Meri T, Hirvelä-Koski V, Meri S, 2003. Molecular genetic characterization of the Fennoscandian cervid strain, a new genotypic group (G10) of *Echinococcus granulosus*, Parasitology., 127, pp. 207-215.

Losson B, Kervyn T, Detry J, Pastoret PP, Mignon B, Brochier B, 2003. Prevalence of *Echinococcus multilocularis* in the red fox (*Vulpes vulpes*) in southern Belgium. Veterinary Parasitology, 117, 1-2, pp. 23-8.

Maillard S, Benchikh-Elfegoun MC, Knapp J, Bart JM, Koskei P, Gottstein B, Piarroux R, 2007. Parasitology Research,100, pp. 495-503.

Malczewski A, Gawor J, Malczewska M, 2008. Parasitology Research. Infection of red foxes (*Vulpes vulpes*) with *Echinococcus multilocularis* during the years 2001-2004 in Poland. Parasitology Research, 103,3, pp. 501-5.

Martín-Hernando MP, González LM, Ruiz-Fons F, Garate T, Gortazar C, 2008. Parasitol Res. Massive presence of *Echinococcus granulosus* (Cestoda, Taeniidae) cysts in a wild boar (Sus scrofa) from Spain, Parasitology Research, 103, 3, 705-7..

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

Mc Manus D, Thompson R, 2003. Molecular epidemiology of cystic echinococcosis**.** Parasitology, 127, S37-S51.

Moks E, Jõgisalu I, Valdmann H, Saarma U, 2008. First report of *Echinococcus granulosus* G8 in Eurasia and a reappraisal of the phylogenetic relationships of 'genotypes' G5-G10, Parasitology, 135, pp. 647-654.

Monnier, P., Cliquet, F., Aubert, M., Bretagne, S., 1996. Improvement of a polymerase chain reaction assay for the detection of *Echinococcus multilocularis* DNA in faecal samples of foxes. Veterinary Parasitology, 67, pp. 185-195.

Morishima Y, Tsukada H, Nonaka N, Oku Y, Kamiya M, 1999. Evaluation of coproantigen diagnosis for natural *Echinococcus multilocularis* infection in red foxes. Japanese Journal of Veterinary Research, Feb, 46, 4, pp. 185-9.

Moro P and Schantz P, 2008. The Pediatric Infectious Disease Journal, pp. 13-20.

Nonaka N, Tsukada H, Abe N, Oku Y, Kamiya M, 1998. Monitoring of Echinococcus multilocularis infection in red foxes in Shiretoko, Japan, by coproantigen detection, Parasitology, August, 117, Pt 2, pp 193-200.

OIE (World Organisation for Animal Health), 2005. Manual for Terrestrial Animal.

OIE (World Organisation for Animal Health), 2008. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees). Sixth Edition. Published by the Office International des Epizooties. ISBN 978-92-9044-718-4. Available online at: www.oie.int/Eng/Normes/Mmanual/A_summry.htm.

Pawlowski ZS, Eckert J, Vuitton DA, Ammann RW, Kern P, Craig PS, Dar FK, De Rosa F, Filice C, Gottstein B, Grimm F, Macpherson CNL, Sato N, Todorov T, Uchino J, von Sinner W, Wen H. 2001. Echinococcosis in humans: clinical aspects, diagnosis and treatment, pp. 20-66. In: J. Eckert, M. A. Gemmell, F.-X. Meslin, and Z. S. Pawlowski (ed.), WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. (OIE) World Organisation for Animal Health, Paris, France.

Pearson MS, Blair D, Dai N, Zhang LH, McManus DP, 2002. Complete mitochondrial genomes confirm the distinctiveness of the horse-dog and sheep-dog strains of *Echinococcus granulosus*. Parasitology, 124, pp. 97-112.

Petavy A, Deblock S, Prost C, 1990. Journal of Parasitology, 77, pp. 133-137.

Pfister T, Schad V, Schelling U, Lucius R, Frank W, 1993. Parasitol Res., 79, pp. 617-8.

Romig T, 2002. Spread of *Echinococcus multilocularis* in Europe? In: Craig P, Pawlowski Z (Eds): Cestode Zoonoses: *Echinococcosis* and *Cysticercosis*, IOS Press, Amsterdam, pp 65-80.

Romig T, 2003. Epidemiology of echinococcosis. Current Concepts in Clinical Surgery. Langenbeck's Archives of Surgery. 388, 209, 217 pp..

Sakai, H., Nonaka, N., Yagi, K., Oku, Y., Kamiya, M., 1998a. Coproantigen detection in a survey of Echinococcus multilocularis infection among red foxes, Vulpes vulpes Schrencki, in Hokkaïdo, Japan, Journal of Veterinary Medical Science, 60, pp. 639-641.

Schantz PM, Chai J, Eckert J, Jenkins DJ, Macpherson CNL, Thakur A. 1995. Epidemiology and control of hydatid disease. In: Thompson RCA, Lymbery AJ (eds) *Echinococcus* and hydatid disease. CAB International, Wallingford, pp. 233-331.

Schweiger A, Ammann RW, Candinas D, Clavien PA, Eckert J, Gottstein B, Halkic N, Muellhaupt B, Prinz BM, Reichen J, Tarr PE, Torgerson PR, Deplazes P, 2007. Human alveolar echinococcosis after fox population increase, Switzerland. Emerging Infectious Diseases, Jun, 13, 6, pp. 878-82.

Smith GC, Gangadharan B, Taylor Z, Laurenson MK, Bradshaw H, Hide G, Hughes JM, Dinkel A, Romig T, Craig PS, 2003. Veterinary Parasitology, 118, pp. 133-142.

Štefanic´ S, Shaikenov BS, Deplazes P, Dinkel A, Torgerson PR,Mathis A, 2004. Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* ("sheep strain") in naturally infected dogs. Parasitol Res, 92, pp. 347–351.

Takumi K, de Vries A, Chu ML, Mulder J, Teunis P, van der Giessen J, 2008. Evidence for an increasing presence of *Echinococcus multilocularis* in foxes in The Netherlands. International Journal for Parasitology, Apr, 38, 5, pp. 571-8.

Takumi K, van der Giessen J, 2005. Transmission dynamics of *Echinococcus multilocularis*; its reproduction number, persistence in an area of low rodent prevalence, and effectiveness of control. Parasitology, 131, pp. 1-8.

Thiess A, Schuster R, Nöckler K, Mix H, 2001. Helminth findings in indigenous raccoon dogs Nyctereutes procyonoides (Gray, 1843), Berliner und Münchener Tierärztliche Wochenschrift, 114, pp. 273-6.

Thompson RC, Eckert J, 1983. Observations on *Echinococcus multilocularis* in the definitive host. Z Parasitenkd. 69, 3, pp. 335-45.

Thompson RCA, DP McManus, 2001. Aetiology: parasites and life-cyles, pp. 1-19. In: J. Eckert, M. A. Gemmell, F.-X. Meslin, and Z. S. Pawlowski (ed.), WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. (OIE) World Organisation for Animal Health, Paris, France.

Thompson, RCA, McManus DP, 2002. Towards a taxonomic revision of the genus *Echinococcus.* Trends in Parasitology 18, pp. 452-457.

Thompson RC, Deplazes P, Eckert J, 2003. Journal of Parasitology, 89, pp. 1086-8.

Thompson RC, Kapel C, Hobbs R, Deplazes P, 2006. Journal of Parasitology, 132, pp. 709-716.

Trachsel D, Deplazes P, Mathis A., 2007. Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mithochondrial DNA. Parasitology, 134, Pt 6, pp. 911-20.

Van der Giessen JW, Rombout YB, Franchimont JH, Limper LP, Homan WL, 1999. Detection of *Echinococcus multilocularis* in foxes in The Netherlands. Veterinary Parasitology, 82, 1, pp. 49-57.

Worbes H, Schacht KH, Eckert J, 1989. *Echinococcus multilocularis* in a swamp beaver (Myocaster coypus), Angew Parasitol., 30, pp. 161-5.

Xiao N, Qiu J, Nakao M, Li T, Yang W, Chen X, Schantz PM, Craig PS, Ito A, 2006. *Echinococcus shiquicus*, a new species from the Qinghai-Tibet plateau region of China: discovery and epidemiological implications. Parasitology International, 55 Suppl, S233, 6 pp.

APPENDICES

A. SUMMARY OF COUNTRY RESPONSES

MS	Institute contacted	Response	Data available?	Comment
A	Institute for Veterinary Investigation. Innsbruck	No	No	No data available from literature.
BE	Institute of Tropical Medicine Veterinary Department	Y	Y	Data collected during meat inspection. Every animal from livestock. For wildlife foxes and wild boar.
BG	National Veterinary Service, Bulgaria	No	No	No data available from literature.
CY	Department of Veterinary Services, Ministry of Agriculture, Natural Resources and Environment, Republic Of Cyprus	Y	Y	Bovine goat sheep carcasses examined in the slaughterhouses. No data on wildlife.
CZ	Department of Veterinary Hygiene, Public Health and Ecology	Y	Y	Some data on foxes.
DK	Danish Meat Association	Y	No	Routine inspection in slaughterhouse, data on cattle and pigs.
EE	Veterinary and Food Board	Y	No	Current surveillance and sampling of macroscopic lesions performed during official meat inspection. Data on moose and wild boar.
FI	Finnish Food Safety Authority	Y	Y	CoproELISA, PCR, visual examination of organs at meat inspection of intermediate hosts, microscopic examination of cysts and adult parasites.
FR	Agence française de securite sanitaire des aliments (AFSSA) et Direction Générale de l'Alimentation (DGAl)	Y	Y only *multilocularis*	Data from foxes only if sampling management.
DE	Federal Institute for Risk Assessment	Y	Y	Routine inspection in slaughterhouse. Data from livestock production, from wildlife and domestic animals.
GR	Ministry of Rural Development and Food	Y	Y	Data for cattle, goat, pig, sheep for *E. Granulosus*.
HU	Laboratories for Parasitology, Fish and Bee Diseases, Veterinary Diagnostic Directorate, Central Agricultural Office	Y	Y	Some data available from sheep, cattle, pig.
IE	Central Meat Control Laboratory	Y	Y	Animal information for cattle. Data for cattle, goat, pig, sheep for *E.* granulosus. Some data for foxes.
IT	Istituto Superiore di Sanità	Y	Y	Routine inspection in slaughterhouse, data on sheep.

A. SUMMARY OF COUNTRY RESPONSES (CONTD.)

MS	Institute contacted	Response	Data available?	Comment
LV	State Veterinary Medicine Diagnostic Centre	Y	Y	Inspection in slaughterhouse, cattle, goats, pigs, sheep for *E. Granulosus*. For *E. multilocularis,* the number of foxes are limited.
LT	State Food and Veterinary service of Lithuania	Y	Y	Animal information for cattle. Data for cattle, goats, pigs, sheep for *E. granulosus*. Some data for foxes.
LU	Institute of Tropical Medicine Veterinary Department	Y	Y	Data from cattle and some from foxes.
MT		N	N	
NL	National Institute for Public Health and the Environment (RIVM)	Y	Y	Current surveillance is performed during official meat inspection (only for macroscopic lesions). Data for livestock production (*E. granulosus*) and for wildlife (*E. multilocularis*) only if programme of surveillance.
PL	National Public Health Institute	N	N	No data available from literature.
PT	Universidade de Trás-os-Montes e Alto Douro (UTAD)	Y	Y	Current surveillance is performed during official meat inspection (only for macroscopic lesions). Data for livestock production and only cervidae and wild boar for wildlife.
SE	National Veterinary Institute Dept. of Parasitology	N	N	No data available from literature.
RO	Animal Health Division VS-MOA	N	N	No data available from literature.
SK	Veterinary and Food Administration of the Slovak	N	N	No data available from literature.
SL	National Veterinary Institute University Ljubljana	N	N	No data available from literature.
ES	Laboratorio Central de Sanidad Animal del M.A.P.A.	N	N	No data available from literature.
UK	Meat Hygiene Service/Food Standards Agency	Y	Y	Current surveillance is performed during official meat inspection (only for macroscopic lesions). Data for livestock production and from farmed deer.

B. *ECHINOCOCCUS GRANULOSUS* STRAINS REPORTED FROM EU MSS

Country	Genotypes
Belgium	G4
Bulgaria	G1, G2, G3
Estonia	G7
Finland	G10
France	G1, G2, G3
Great Britain	G1, G4
Ireland	G4
Italy	G1, G2, G3, G4, G5, G7
Latvia	G7
Lithuania	G7
Netherlands	G5
Portugal	G1, G2, G3
Poland	G7, G9
Romania	G1, G2, G3, G7
Slovak Republic	G7
Spain	G1, G2, G3, G4, G7
Sweden	G10
Switzerland	G4, G5

Note: From published papers

C. ZOONOTIC SPECIES RISK ASSESSMENT

Non-European species

Species	Strain	Zoonotic (Y/N)	Final host (FH)	Intermediate host (IH)	Geographical distribution	Present in MSs (Y/N)	Likelihood of introduction (H/M/L)	Likelihood of establishment in Europe (H/M/L) *Intermediate/final hosts present/ life cycle sustainable?*	Human Susceptibility (H/M/L)	Pathogenicity (humans)* *E.g. severity of symptoms/ morbidity*	Mortality rate (%)	Long term effects	Economic impact of disease	Risk groups?	Monitoring in Europe recommended? (Y/N)	Comments	References
E. granulosus	G1	Y	Domestic dog, wild canids	Primarily ungulates	Worldwide	Y	Already circulating	_____	H	Hydatite cyst Infection by metacestode stage, developement of cysts more than 10 month post infection.	Low	Yes	Moderate-Low Public health could be moderate	People consuming vegetable soil and contact with infected dog.	**Y**	Natural circulation in some European countries. Widely spread in North Africa.	Bowles J, Blair D, Mc Manus D, 1995. Eckert J, Deplazes P, 2004. Thompson A, Mc Manus D, 2002. Mc Manus D, Thompson R, 2003. Moro P, Schantz PJ, 2008. Casulli et al., 2008 (in press).
	G2	Y	Dog, fox	Sheep, goats	Tasmania, Argentina	Y	Already circulating	_____	H	Hydatite cyst Infection by metacestode stage, developement of cysts more than 10 month post infection.	Unknown	Yes	Low	People consuming vegetable soil and contact with infected dog.	**Y**	Recent observation of G2 in Italy.	Eckert J, Deplazes P, 2004. Casulli et al., 2008 (in press).
	G3	No	Dog	Cattle, buffalo, sheep	Asia, Europe	Y	Already circulating	____	No	N/A		Yes	Low	People consuming vegetable soil and contact with infected dog.	**Y**		Eckert J, Deplazes P, 2004. Casulli et al., 2008 (in press).
E. equinus	G4	No	Dog	Horses and other equines	Asia, Europe, Middle East, South Africa	Y	Already circulating	_____	No	N/A		____	Low	People consuming vegetable soil and contact with infected dog.	**Y**		Bowles J, Blair D, Mc Manus D. 1995. Thompson A, Mc Manus D, 2002. Mc Manus D, Thompson R, 2003. Casulli et al., 2008 (in press).
E. ortleppi	G5	Y	Dog	Cattle	Europe, South Africa and South America Eurasia	Y	Already circulating	____	H	Morbidity	Unknown	Yes	Yes	People consuming vegetable soil and contact with infected dog.	**Y**	Recommended in cattle the IH to monitor spread and risk of infection.	Bowles J, Blair D, Mc Manus D, 1995. Thompson A, Mc Manus D, 2002. Mc Manus D, Thompson R, 2003. Casulli et al., 2008 (in press).
E. canandensis	G6	Y	Dog	Camel, goats	Middle EastAfrica, China, Argentina	N	L	L	H	Morbidity	Unknown	Yes	Very low	_____	**N**		Bowles J, Blair D, Mc Manus D, 1995. Thompson A, Mc Manus D, 2002. Mc Manus D, Thompson R, 2003. Casulli et al., 2008 (in press).
	G7	Y	Dog	Pig	Europe, Eurasia, South America	Y	Already circulating	_____	H	Morbidity	Unknown	Yes	Yes	People consuming vegetable soil and contact with infected dog.	**Y**		Bowles J, Blair D, Mc Manus D, 1995. Thompson A, Mc Manus D, 2002. Mc Manus D, Thompson R, 2003.
	G8	Y	Wolf	Cervid: moose reindeer	North America, and Eurasia	N	L	L	H	Pulmonary localisation	Unknown	Yes	Very low	_____	**N**	The risk is low due to the fact that the FH is the wolf and the IH corresponds to Nordic species. In Europe monitoring will be a luxury.	Bowles J, Blair D, Mc Manus D, 1995. Thompson A, Mc Manus D, 2002. Mc Manus D, Thompson R, 2003. Casulli et al., 2008 (in press). Moks et al., 2008.
	G9	Unknown	Lion	Zebra, bush pig, buffalo, antelope	Africa	N	L	L	Unknown			Yes	Very low	_____	**N**	Small area distribution	Eckert J, Deplazes P, 2004.
	G10	Y	Wolf	Reindeer, fennoscandian	Eurasia	Y	Already circulating	_____	Unknown			Yes	Yes	People consuming vegetable soil and contact with infected wolf.	**N**	New strain- small distribution area .The risk is low due to the fact that the FH is the wolf and the IH corresponds to Nordic species.	Lavikainen A, Lehtinen MJ, Meri T, Hirvelä-Koski V, Meri S, 2003.

* depending on amount of intake

C. ZOONOTIC SPECIES RISK ASSESSMENT (CONTD.)

Non-European species

Species	Strain	Zoonotic (Y/N)	Final host (FH)	Intermediate host (IH)	Geographical distribution	Present in MSs (Y/N)	Likelihood of introduction (H/M/L)	Likelihood of establishment in Europe (H/M/L) *Intermediate/final hosts present/ life cycle sustainable?*	Human Susceptibility (H/M/L)	Pathogenicity (humans)* *E.g. severity of symptoms/ morbidity*	Mortality rate (%)	Long term effects	Economic impact of disease	Risk groups?	Monitoring in Europe recommended? (Y/N)	Comments	References
E. multilocularis		Y	Primarly foxes, also other canids and cats	Rodents, other small mammals	North America, Northern and Central Eurasia	Y	Already circulating	_____	H	Infection by metacestode stage. Alveolar structure compose of small vesicules principaly in liver. Metastase,in distant organs such as lungs, brain, bones.	Low	Yes	Very low. Public health could be high	People consuming vegetable soil or fruit, Hunters, dog owner.	**Y**	Natural circulation in European countries. Widely spread central Europe, Expansion of the endemic areas.	Bowles J, Blair D, Mc Manus D, 1995. Eckert J, Deplazes P, 2004. Thompson A, Mc Manus D, 2002. Moro P, Schantz P J, 2008.
E. vogeli		Y	Bush dog, domestic dog	Rodents: paca and agouti	Central and South America	N	L	L	H	Polycystic, principally in liver and in some case in lungs.		Yes		_____	**N**		Eckert J, Deplazes P, 2004. D'Alessandro A, Rausch L,2008. Thompson A, Mc Manus D, 2002.
E. oligarthus		Y	Wild felidae: ocelot, jaguar, cougar, puma….	Rodents: agouti, spiny rat, paca	Central and South America	N	L	L	H	Low number of human cases. Orbital location with large fluid cyst and cardiac location.		Yes		_____	**N**		Eckert J, Deplazes P, 2004. D'Alessandro A, Rausch L, 2008. Thompson A, Mc Manus D, 2002.
E. shiquicus		Unknown	Tibetan fox	Rodents: ochotona curzoniae	Tibet	N	L	L	Unknown			Low		_____	**N**		Xiao N, Qiu J, Nakao M, Li T, Yang W, Chen X, Schantz PM, Craig PS, Ito A, 2006.

* depending on amount of intake

D. *ECHINOCOCCUS GRANULOSUS* – RELEVANT ANIMALS AND/OR FOODSTUFFS TO BE MONITORED

Animal species or foodstuff	Role in infection chain (DH/PH/SH/IH/DEH/ RH)*	Part of human food chain/diet (Y/N)	Known as source of human infection/linked to outbreaks (Y/N)	Suspected source of infection/outbreaks (Y/N)	Relevant to be monitored (Y/N)	Rationale for monitoring/application of result	References
Sheep	IH	Y	N	N	Y	The monitoring system is easy and cheap. In Mediterranean countries dogs have access to offal of infected sheep and spread the disease to humans.	Eckert J and Deplazes P, 2004. Christodoulopoulos G, Theodoropoulos G, Petrakos G, 2008.
Goats	IH	Y	N	N	Y	The monitoring system is easy and cheap. In Mediterranean countries dogs have access to offal of infected sheep and spread the disease to humans.	Mc Manus D and Thompson R, 2003. Christodoulopoulos G, Theodoropoulos G, Petrakos G, 2008.
Cattle	IH	Y	N	N	Y	The monitoring system is easy and cheap.	Eckert J and Deplazes P, 2004.
Pigs	IH	Y	N	N	Y	The monitoring system is easy and cheap.	Mc Manus D, Thompson R, 2003.
Horses	IH	Y	N	N	N		Bowles J, Blair D, Mc Manus D, 1995.
Camels	IH	N	N	N	N		Maillard S, Benchikh-Elfegoun MC, Knapp J, Bart JM, Koskei P, Gottstein B, Piarroux R, 2007.
Reindeer	IH	Y	N	N	N		Lavikainen A, Lehtinen MJ, Meri T, Hirvelä-Koski V, Meri S,
Deer	IH	Y	N	N	N		Lavikainen A, Lehtinen MJ, Meri T, Hirvelä-Koski V, Meri S,
Moose	IH	Y	N	N	N		Moks E, Jõgisalu I, Valdmann H, Saarma U, 2008.
Wild boar	IH	Y	N	N	N		Martín-Hernando MP, González LM, Ruiz-Fons F, Garate T, Gortazar C, 2008 (in press).
Dog	DH/PH	N	Y	Y	N	Human health problem: contamination of owner and family.	Eckert J, 1997.
Wild canids (fox…)	DH/PH Secondary importance?	N	Y	Y	N	Human health problem: contamination of owner and family.	Smith GC, Gangadharan B, Taylor Z, Laurenson MK, Bradshaw H, Hide G, Hughes JM, Dinkel A, Romig T, Craig PS, 2003.

*DH = definitive or final host in which an organism undergoes its sexual phase of reproduction.
*PH = Primary host. Animal that maintains an infection in its endemic area.
*SH = Secondary Host. Species that is additionally involved in the life cycle of an agent, especially outside typical endemic areas.
*IH = Intermediate Host. Animal in which the infectious agent undergoes some development, frequently with asexual reproduction.
*DEH = Dead-end host or incidental host. Host that usually does not transmit an infectious agent to other animals.
*RH = Reservoir Host. Host in which an infectious agent normally lives and multiplies, therefore a common source of infection (frequently a primary host).

E. *ECHINOCOCCUS MULTILOCULARIS* – RELEVANT ANIMALS AND/OR FOODSTUFFS TO BE MONITORED

Animal species or foodstuff	Role in infection chain (DH/PH/SH/IH/DEH/RH)*	Part of human food chain/ diet (Y/N)	Known as source of human infection/ linked to outbreaks (Y/N)	Suspected source of infection/outbreaks (Y/N)	Relevant to be monitored (Y/N)	Rationale for monitoring/application of result	References
Foxes	DH/PH	N	Y	Y	Y	Indicator animal for determining prevalence in wildlife. Determination of contaminated areas. Human health problem: contamination of hunter.	Eckert J, Conraths F, Tackman K, 2000. Romig T, 2002. Malczewski A, Gawor J, Malczewska M, 2008. Berke O, Romig T, von Keyserlingk M, 2008.
Dogs	DH (IH) secondary importance?	N	Y (rare)	Y	N	Human health problem: contamination of owner and familly.	Kapel CM, Torgerson PR, Thompson RC, Deplazes P, 2006. Thompson RC, Kapel C, Hobbs R, Deplazes P, 2006. Petavy A, Deblock S, Prost C, 1990.
Cats	DH secondary importance?	N	Y (rare)	Y	N	Human health problem: contamination of owner and family.	Kapel CM, Torgerson PR, Thompson RC, Deplazes P, 2006. Thompson RC, Deplazes P, Eckert J, 2003.
Racoon dogs	DH/PH	N	Y	Y	Y	Indicator animal for determining prevalence in wildlife. Determination of contaminated areas.	Thiess A, Schuster R, Nöckler K, Mix H, 2001.
Voles	IH	N	N	N	N	————	Hansen F, Jeltsch F, Tackmann K, Staubach C, Thulke HH, 2004.
Musk rats	IH	N	N	N	N	————	Boussinesq M, Bresson S, Liance M, Houin R, 1986. Borgsteede FH, Tibben JH, van der Giessen JW, 2003.
Nutria	IH	N	N	N	N	————	Worbes H, Schacht KH, Eckert J, 1989.
Pig	DEH	Y	N	N	N	————	Deplazes, Eckert, 2001.
Wild boar	DEH	Y	N	N	N	————	Pfister T, Schad V, Schelling U, Lucius R, Frank W, 1993. Boucher JM, Hanosset R, Augot D, Bart JM, Morand M, Piarroux R, Pozet-Bouhier F, Losson B, Cliquet F, 2005.
Non-human primate	DEH	N	N	N	N	————	Bacciarini LN, Gottstein B, Wenker C, Gröne A, 2005.

*DH = definitive or final host in which an organism undergoes its sexual phase of reproduction.
*PH = Primary host. Animal that maintains an infection in its endemic area.
*SH = Secondary Host. Species that is additionally involved in the life cycle of an agent, especially outside typical endemic areas.
*IH = Intermediate Host. Animal in which the infectious agent undergoes some development, frequently with asexual reproduction.
*DEH = Dead end host or incidental host. Host that usually does not transmit an infectious agent to other animals.
*RH = Reservoir Host. Host in which an infectious agent normally lives and multiplies, therefore a common source of infection (frequently a primary host).

F. *ECHINOCOCCUS GRANULOSUS* – SUMMARY OF ANALYTICAL METHODS

Analytical method/technique	Sensitivity	Specificity	Application (sample materials)	Application result	Throughput	Estimated costs	Technical requirements (instruments, etc)	Suitable for QA?	Comments	References
				Individuals/ herd level/ national prevalence etc.	*E.g. number of animals tested per person and day.*	*Will vary from country to country and depend on the throughput. In this context it is meant to give a rough indication to allow comparison between methods, if possible.*		*QA = Quality Assessment*		
Sedimentation and Counting Technique (SCT)	100%	100%	Post mortem diagnosis. Intestine necropsy.	Individuals. Population level. National prevalence.	10???		Stereo microscope.	Y	Time consuming, quality dependent of intestines.	Eckert J, Deplazes P, Craig PS, Gemmel MA, Gottstein B, Heath D, Jenkins DJ, Kamiya M, Lightowlers M, 2001a.
Intestinal Scrapping Technique (IST)	78%	100%	Post mortem diagnosis. Intestine necropsy.	Individuals. Population level. National prevalence.	15-20		Stereo microscope.	Y	Time consuming, quality dependent of intestines.	Hofer S, Gloor S, Muller U, Mathis A, Hegglin D, Deplazes P, 2000.
Shaking in a Vessel Technique (SVT)	96%	100%	Post mortem diagnosis. Intestine necropsy.	Individuals. Population level. National prevalence.	10 (N/A)		Stereo microscope.	Y	Time consuming, quality dependent of intestines.	Duscher G, Prosl H, Joachim A, 2005.
Histology, H& E staining	N/A	N/A	Suspected specimen from internal organs.	Individuals/herd level prevalence.	5		Compound microscope.			OIE, 2005.
Meat inspection carcasses	N/A	N/A	Carcass in slaugterhouse for human consumption.	Individuals level .				Y	Depend on technician experience.	
Coproantigen ELISA (Craig)	77%	N/A	In vivo and post mortem diagnosis. Faecal samples.	Population level. National prevalence.	200 not commercially available		ELISA plate washer. Incubator. ELISA Reader.	Y		Craig PS, Gasser RB, Parada L, Cabrera P, Parietti S, Borgues C, Acuttis A, Agulla J, Snowden K, Paolillo E, 1995. OIE, 2008.
Coproantigen ELISA (chekit)	60-80%	80-95%	In vivo and post mortem diagnosis. Faecal samples.	Population level. National prevalence.	200 not commercially available since 2007		ELISA plate washer. Incubator. ELISA Reader.	Y	Not available.	
Coproantigen ELISA (Allan)	88%	96.5%	In vivo and post mortem diagnosis. Faecal samples.	Population level. National prevalence.	200 not commercially available		ELISA plate washer. Incubator. ELISA Reader.	Y		Allan JC, Craig PS, Garcia Noval J, Mencos F, Liu D, Wang Y, Wen H, Zhou P, Stringer R, Rogan M, et al., 1992. OIE, 2008.
PCR Target : 12S rRNA Stefanic	N/A	100%*	Faecal samples. Liver and organ cysts.	Individuals. Population level. National prevalence.	N/A		Centrifuge, microscope, PCR apparatus, specific primers, Taq polymerase	Y	Diagnosis of *E. granulosus* G1.	Štefanic´ S, Shaikenov BS, Deplazes P, Dinkel A, Torgerson PR,Mathis A, 2004. .
PCR Target : 12S rRNA Abbasi	N/A	100%*	Faecal samples. Liver and organ cysts.	Individuals. Population level. National prevalence.	N/A		Centrifuge, microscope, PCR apparatus, specific primers, Taq polymerase	Y	Diagnosis of *E. granulosus* G1.	Abbasi I, Branzburg A, Campos-Ponce M, Abdel Hafez SK, Raoul F, Craig PS, Hamburger J, 2003.
PCR Target : 12S rRNA Dinkel	N/A	100%*	Faecal samples. Liver and organ cysts.	Individuals. Population level. National prevalence.	N/A		Centrifuge, microscope, PCR apparatus, specific primers, Taq polymerase	Y	Diagnosis of G1 and the group G5/6/7 is performed by a simple PCR, while discrimination between *E. ortleppi* (G5) and G6/7 involves a subsequent semi-nested PCR step.	Dinkel A, Njoroge EM, Zimmermann A, Wälz M, Zeyhle E, Elmahdi IE, Mackenstedt U, Romig T, 2004.
PCR Cest3-Cest5 Target : NAD1 Trachsel	ND	ND	Faecal samples Liver and organs Cyst	Individuals. Population level. National prevalence.	15		Centrifuge, PCR apparatus, specific primers, Taq polymerase	Y		Trachsel D, Deplazes P, Mathis A., 2007.

* published data, DNA of others parasite can amplified in routine laboratory

G. *ECHINOCOCCUS MULTILOCULARIS* – SUMMARY OF ANALYTICAL METHODS

Analytical method/technique	Sensitivity	Specificity	Application (sample materials)	Application result	Throughput	Estimated costs	Technical requirements (instruments, etc)	Suitable for QA?	Comments	References
				Individuals/ herd level/ national prevalence etc.	*E.g. number of animals tested per person and day.*	*Will vary from country to country and depend on the throughput. Indication only.*		*QA = Quality Assessment*		
Sedimentation and Counting Technique (SCT)	100%	100%	Post mortem diagnosis, necropsy Intestines	Individuals Population level National prevalence	10	arround 30 euros	Stereo microscope	Y	Time consuming, quality dependent of intestines	Eckert J, Deplazes P, Craig PS, Gemmel MA, Gottstein B, Heath D, Jenkins DJ, Kamiya M, Lightowlers M, 2001a.
Intestinal Scrapping Technique (IST)	78%	100%	Post mortem diagnosis, necropsy Intestines	Individuals Population level National prevalence	15-20		Stereo microscope	Y	Time consuming, quality dependent of intestines	Hofer S, Gloor S, Muller U, Mathis A, Hegglin D, Deplazes P, 2000.
Shaking in a Vessel Technique (SVT)	96%	100%	Post mortem diagnosis, intestine necropsy.	Individuals. Population level. National prevalence.	10 (N/A)		Stereo microscope	Y	Time consuming, quality dependent of intestines.	Duscher G, Prosl H, Joachim A, 2005.
Histology, H& E staining	N/A	N/A	Suspected specimen from internal organs.	Individuals/herd level prevalence.	5 ??		Compound microscope			OIE, 2005
Coproantigen ELISA (Deplazes)	80%	95%-99%	In vivo and post mortem diagnosis. Faecal samples.	Population level. National prevalence.	200 not commercially available	3-5 euros	ELISA plate washer. Incubator. ELISA Reader.	Y	Test could only be performed in the laboratory. Detection in prepatency antigen. Routine test for mass screening.	Deplazes P, Alther P, Tanner I, Thompson RCA, Eckert J, 1999.
Coproantigen ELISA (chekit)	60-80%	80%-95%	In vivo and post mortem diagnosis. Faecal samples.	Population level. National prevalence.	200 not commercially available since 2007	10 euros	ELISA plate washer. Incubator. ELISA Reader.	Y	Not available.	
Coproantigen ELISA (Sakai)	87%	70%	In vivo and post mortem diagnosis. Faecal samples.	Population level. National prevalence.	200 not commercially available		ELISA plate washer. Incubator. ELISA Reader.	Y	Test could only be performed in the laboratory.	Sakai H, Nonaka N, Yagi K, Oku Y, Kamiya M, 1998a.
Coproantigen ELISA (Morishima)	95%	N/A	In vivo and post mortem diagnosis. Faecal samples.	Population level. National prevalence.	200 not commercially available		ELISA plate washer. Incubator. ELISA Reader.	Y	Test could only be performed in the laboratory.	Morishima Y, Tsukada H, Nonaka N, Oku Y, Kamiya M, 1999.
Coproantigen ELISA (Nonaka)	N/A	N/A	In vivo and post mortem diagnosis. Faecal samples.	Population level. National prevalence.	200 not commercially available		ELISA plate washer. Incubator. ELISA Reader.	Y	Test could only be performed in the laboratory.	Nonaka N, Tsukada H, Abe N, Oku Y, Kamiya M, 1998.
PCR Target : RNAsn U1 Monnier	82%	96%*	Faecal samples. Liver and organ cysts.	Individuals. Population level. National prevalence.	15		Centrifuge, PCR apparatus, specific primers, Taq polymerase.	Y	Cross-reaction with DNA from other parsite. Detect DNA from Taenid Eggs	Monnier P, Cliquet F, Aubert M, Bretagne S, 1996.
Microscopy/PCR Target : RNAsn U1 Mathis	94%	100%*	Faecal samples. Liver and organ cysts.	Individuals. Population level. National prevalence.	15	"arround 100 euros"	Centrifuge, PCR apparatus, specific primers, Taq polymerase.	Y	Cross-reaction with DNA from other parsite. Detect DNA from Taenid Eggs	Mathis A, Deplazes P, Eckert J, 1996.
PCR H15-H17 Target: 12S rRNA Dinkel	89%	100%*	Faecal samples. Liver and organ cysts.	Individuals. Population level. National prevalence.	15		Centrifuge, PCR apparatus, specific primers, Taq polymerase.	Y	Cross-reaction with DNA from other parsite. Detect DNA from Taenid Eggs	Dinkel A, Nickisch-Rosenegk MV, Bilger B, Merli M, Lucius R, Romig T, 1998.
PCR Target : 12S rRNA Van der Giessen	ND	100%*	Faecal samples. Liver and organ cysts.	Individuals. Population level. National prevalence.	15		Centrifuge, PCR apparatus, specific primers, Taq polymerase.	Y	Cross-reaction with DNA from other parsite. Detect DNA from Taenid Eggs	Van Der Giessen JW, Rombout YB, Franchimont JH, Limper LP, Homan WL, 1999.
PCR Cest1-Cest2 Target : NAD1 Trachsel	ND	ND	Faecal samples. Liver and organ cysts.	Individuals. Population level. National prevalence.	15		Centrifuge, PCR apparatus, specific primers, Taq polymerase.	Y	Cross-reaction with DNA from other parsite. Detect DNA from Taenid Eggs	Trachsel D, Deplazes P, Mathis A, 2007.

* published data, DNA of others parasite can amplified in routine laboratory

ABBREVIATIONS

AE	Alveolar echinococcosis
AE	Alveolar echinococcosis
CE	Cystic echinococcosis
CRL	Community Reference Laboratory
CSR	Community Summary Report
DEH	Dead end or incidental host
DH	Definitive or final host
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EG	*Echinococcus granulosus*
EM	*Echinococcus multilocularis*
EU	European Union
FCI	Food Chain Information
GEE	Generalised Estimating Equations
IH	Intermediate host
MS	Member State
NRL	National Reference Laboratory
NUTS	European Country Classification system
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PH	Primary host
QA	Quality Assurance
RA	Risk Assessment
RH	Reservoir host
Sh	Secondary host
WHO	World Health Organisation
ZCC	Zoonoses Collaboration Centre