

SCIENTIFIC REPORT submitted to EFSA

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

Prepared by: Mike A. Taylor^(a), Jaap Boes⁽ⁱ⁾, Pascal Boireau^(d), Franck Boué^(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), Lucy Snow^(b),
Georgios Theodoropoulos^(h), Isabelle Vallée^(d), Maria M. Vieira-Pinto^(l),
Irene-A. Zimmer^(a)

ABSTRACT

The current disease situation and national monitoring of *Sarcocystis* in the European Union Member States is reviewed to identify the relevance of the parasite for public health considering specific needs in the European countries. Two species, *S. suis* and *S. bovihominis*, are recognised to have zoonotic significance and to be relevant to the Member States. Due to a lack of data from the Member States, the impact on human health is unclear as well the situation in animal populations. It can be assumed that the zoonotic *Sarcocystis* species are circulating in most European food animal populations, though seemingly without major impact on public health. Limitations are also related to the commonly used detection method, visual inspection at the slaughterhouse, which does not allow differentiation between the two zoonotic species from the non-zoonotic ones. Consequently a harmonised scheme for monitoring *Sarcocystis* cannot be justified by a public health perspective without further evidence of impact on public health.

- ^(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) Nacionālais 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

Sarcocystis, *S. bovihominis*, *S. suis*, harmonised monitoring, pig

¹ Question No EFSA-Q-2009-01074. Accepted for publication on 10 December 2009.

SUMMARY

Sarcocystis spp. are protozoan parasites of livestock, which infect mammals, including man, birds and lower vertebrates. The life cycle for all species requires more than one host for completion. Sexual stages occur in the predator (e.g. dogs, cats and man) following ingestion of bradyzoite cysts (asexual stage) in the muscle of infected intermediate hosts, and oocysts are passed in the faeces. The parasite derives its name from the obligatory intramuscular cyst stage (sarcocyst) present in the intermediate (prey) host and the nomenclature for *Sarcocystis* species incorporates those of the intermediate and final host e.g. *Sarcocystis bovihominis*. Most *Sarcocystis* species infecting man and domestic animals are species-specific for their intermediate hosts and family-specific for their final host.

Whilst a number of *Sarcocystis* species occur in domestic animals, about 130 species have been reported to date, most are not of zoonotic significance. Sarcocystosis in humans is caused by ingestion of contaminated raw pork or beef. Only two zoonotic species relevant to the European Union Member States have been identified, *S. sui hominis* and *S. bovi hominis*. Both species are known to cause unspecific gastro-intestinal symptoms, though *S. sui hominis* to a more severe degree and potentially including circulatory problems. At this stage, the impact on humans is unclear due to a lack of public health data from the Member States. Studies on human sarcocystosis published in scientific literature date back to over 15 years and it is questionable how accurately the results, often gathered from selected target groups, reflect the current situation across the whole population in Europe. Similarly unknown is the situation in animal populations, where some infections are being picked up during meat inspections as gross visible lesions leading to local, or more rarely, whole carcass condemnation. A few scientific studies have been carried out and published, but again, questions as to the prevalence of the zoonotic species and comparability of the results remain.

Not considered relevant to European Union Member States, but mentioned here for completeness are several yet unidentified species of *Sarcocystis* that have been involved in human muscular sarcocystosis. For those species, humans act as an accidental intermediate host, in which cysts are present in muscle tissue. Most cases are reported from or associated with the Far East and epidemiological cycles involving pythons and monkeys have been suggested putatively, however, at this point the life cycle and definitive hosts are not known. The reason for those species not being considered relevant to European Union Member States are their seemingly geographical limitations and the fact that humans act as dead end hosts, meaning that even infected individuals entering European Union Member States would not contribute to the spread of these agents.

It can be assumed that *Sarcocystis* spp, including the zoonotic species, are circulating in most European food animal populations, though seemingly without major impact on public health. Current detection methods and reporting of *Sarcocystis* spp. are variable within the European Union and mostly those species causing economic impact leading to carcass condemnation in meat inspection are detected. Current detection methods are limited in sensitivity and cannot differentiate the two zoonotic species from the non-zoonotic ones. The definitive confirmatory method is by electron microscopy, which is prohibitively expensive for routine diagnosis. Consequentially, a unified scheme for monitoring *Sarcocystis* cannot be justified at this stage based on public health needs without further evidence of clinically significant human cases being directly linked to this parasite.

The recommendation is therefore to seek clarification of the relevance of these two species (*S. sui hominis* and *S. bovi hominis*) to/public health. If a need for surveillance in animals should be identified, new, improved and specific diagnostic tests should be developed for monitoring pigs and cattle at slaughterhouses and reporting should be introduced in due course. Preference should be given to monitoring *S. sui hominis* in pigs, because of its potential to cause more severe symptoms in humans compared to *S. bovi hominis* in cattle. Visual inspection at the slaughterhouse will identify macroscopic lesions, not necessarily caused by the two zoonotic species, but does not allow species differentiation.

Species differentiation can be carried out using various methods that are not suitable for high throughput routine inspections.

It is questionable whether reporting of the non-zoonotic *Sarcocystis* species is required within the European Union and those current procedures for local downgrading or condemnation of infected carcasses should rest with individual slaughterhouses and countries without the need for central recording.

TABLE OF CONTENTS

Abstract	1
Summary	2
Table of Contents	4
Background	6
Terms of reference.....	6
Acknowledgements	7
Introduction and Objectives	8
Objectives.....	9
Objective 1. Identify current disease situation in the Member States and current national level of monitoring and reporting information	9
1.1 Rationale	9
1.2 Approach.....	9
1.3 Results.....	9
Objective 2. Identify animal species and/or foodstuffs which could be affected and specify which should be monitored	11
2.1 Identify parasite species to be monitored.....	11
2.1.1 Rationale.....	11
2.1.2 Approach	11
2.1.3 Results	12
2.2 Identify relevant animal species and/or foodstuffs to be monitored.....	13
2.2.1 Rationale.....	13
2.2.2 Approach	13
2.2.3 Results	13
Objective 3. Identify most suitable diagnostic and analytical methods to be used	14
3.1 Rationale	14
3.2 Approach.....	14
3.3 Results.....	14
Objective 4. Define sample size, collection procedure, specimen types and sampling techniques.....	15
Objective 5. Propose harmonised monitoring and reporting schemes	16
5.1 Harmonised monitoring	16
5.2 Reporting	16
5.2.1 Description of surveillance programme.....	16
5.2.2 Individual sample information.....	16
5.2.3 Population data	17
Objective 6. Propose information to be analysed by the Commission and EFSA for detecting trends.....	18
6.1 Descriptive analyses.....	18
6.2 Monitoring trends over time	18
6.3 Spatial analysis	18

References	19
Appendices	22
A. <i>Sarcocystis</i> zoonotic species risk assessment.....	22
B. <i>Sarcocystis</i> , relevant animals and foodstuffs to be monitored	23
C. <i>Sarcocystis</i> , Summary of analytical methods.....	24
D. <i>Sarcocystis</i> , Summary of country responses	25
Abbreviations	27

BACKGROUND

In the European Food Safety Authority's (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.

EFSA's 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 (EFSA, 2006a). 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 Community Summary Report.

TERMS OF REFERENCE

The objective of the call is to obtain proposals for projects, which will develop harmonised monitoring and reporting schemes for *Sarcocystis* 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 the 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 the 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 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;
- Nacionālais Diagnostikas Centrs Pārtikas un Veterinārā Dienesta (NDC FVS), Latvia;
- Agence Française de Sécurité Sanitaire des Aliments (AFSSA), France;
- Istituto Superiore di Sanità (ISS), Italy;
- Rijksinstituut voor Volksgezondheid en Milieu (RIVM), The Netherlands;
- Bundesinstitut für Risikobewertung (BfR), Germany;

and in co-operation with:

- Agricultural University of Athens (AUA), Greece;
- Prince Leopold Institute of Tropical Medicine Antwerp (ITG), Belgium;
- 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;
- 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 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 *Sarcocystis* in animals and foodstuffs in the European Union

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

INTRODUCTION AND OBJECTIVES

INTRODUCTION

Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, forms the basis for data on zoonoses being collected throughout the MSs and reported to the European Commission on an annual basis. These data are collected and examined by the European Food Safety Authority (EFSA), who, in collaboration with the European Centre for Disease Control (ECDC) and assisted by the Zoonoses Collaboration Centre (ZCC), produce an annual report, The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union, which is then published in the EFSA Journal. The report is aimed at detection of sources and trends within the EU MSs and to aide the long-term goal of protecting human health.

Sarcocystis is not included in list A of Annex I of the Directive 2003/99/EC, which determines which agents have to be monitored on a mandatory basis, but could be included within 'other zoonoses' in the list of agents, that have to be monitored depending on the epidemiological situation in a country (list B). It is not mentioned in either of the Scientific Opinions of the Scientific Panel on BIOHAZ and of the Scientific Panel on AHAW (EFSA, 2006a and EFSA, 2007a). However, Regulation (EC) No 854/2004 (EC, 2004) covers *Sarcocystis* under the general term 'zoonotic diseases'. Comments on data collection and data submission can be found in the Manual for Reporting on Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Food-borne Outbreaks in the framework of Directive 2003/99/EC and on some other pathogenic microbial agents for information derived from the reporting year 2006 (EFSA, 2007b). In 2004 and 2005 Belgium was the only country to report the occurrence of *Sarcocystis* in cattle. Supporting information on the species detected and age of cattle were provided for 2005. In 2006, only Luxembourg provided data on *Sarcocystis*, reporting the total number of cattle examined and the number of *Sarcocystis*-positives. No further specifications of the agent or age of animals were given.

Sarcocystis spp. was to be reviewed as part of this project as there seemed to be uncertainty as to its zoonotic relevance, its occurrence and the public health needs within the MSs. The objective of this project is to develop a harmonised scheme for the monitoring and reporting of *Sarcocystis* in animals and/or foodstuffs in the EU. The schemes were to include specifics about the sampling, diagnostic methods and the collecting and analysing of information. 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 at 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 the MSs. The rationale behind this was to identify public health needs in the MSs, and to create a basis for formulating the sampling plans. Other milestones assessed the agent and its 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 surveillance. Existing analytical methods were summarised and assessed regarding their feasibility in sampling schemes that are for use throughout the EU.

The milestones/objectives, approach, underlying rationale and results are described in detail hereafter.

OBJECTIVES

Objective 1. Identify current disease situation in the 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 to all EU MSs. Consideration should also be paid to testing schemes currently carried out in MSs. The table was designed to gather data needed to assess the 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 established contacts to National Competent Authorities, networks within the project team (network of National Reference Laboratories for Parasites) or contacts provided via EFSA (reporting officers). The spreadsheets sought to collect information on confirmed human cases and the current disease situation relevant to animal populations, as well as for supporting information on sampling and testing carried out in the MSs. Where answers were not received, literature searches were used to fill the gaps. A summary table was compiled to give a brief overview over the current disease and testing situation in the different MSs and can be found in Appendix IV.

1.3 Results

Human sarcocystosis is not a notifiable disease in the MSs and consequentially, no information on human sarcocystosis has been received from any MS. A literature search revealed only a few articles on human intestinal sarcocystosis in Europe. In fact most available data seem to have been collected more than 15 years ago, and a review can be found in Dubey et al. (1989) and Fayer (2004). Human intestinal sarcocystosis is not uncommon in Europe and prevalence data ranges from of 1.6% to 10.4% have been reported (Dubey et al., 1989). These results are based on microscopic examination of faecal samples, a technique that does not distinguish between *S. suis* and *S. bovihominis*. The incidence worldwide is estimated to be between 6% and 10 % in the human population (CFSPH, 2005), though it is unclear what this estimate is based on and how accurately it reflects the situation in the EU MSs.

Less than 100 cases of muscular sarcocystosis in humans have been reported worldwide (Fayer, 2004). Most cases have been found in or are linked to tropical and subtropical environments. A study in Malaysia reported 20% seroprevalence in humans, which is considered representative for the geographical area of Asia and South East Asia (Arness et al., 1999; CFSPH, 2005; Dubey et al., 1989; Fayer, 2004). The *Sarcocystis* species involved are still unknown, but at least 7 structurally distinct types have been reported. Morphological similarities between the species isolated from human muscle and species found in macaques suggested that other primates may be the true intermediate host for some of the agent species, whilst the python has been proposed as a definite host in Malaysia (Dubey et al., 1989; Arness, 1999; Fayer 2004). The clinical significance in humans is still unknown; most cases seem to go unnoticed, whereas very few cases have been reported to trigger long-term effects (Arness et al., 1999).

Animal situation: slaughterhouse inspection is carried out in all MSs according to Regulation (EC) No 854/2004. This will detect macroscopic lesions and will either lead to condemnation of the whole carcass, in the case of generalised sarcocystosis, or, in the case of light or localised infection, rejection of the affected parts. Only a few MSs record these results centrally or are working towards a central recording system. Slaughterhouse inspection does not allow detection of microscopic lesions or species differentiation or identification.

Prevalence studies carried out between 1966 and 1974 in Austria, Germany, Poland, Spain and The Netherlands, all cited by Heydorn (1977), found 67%, 80%, 90.2% 96.6% and 93.3% of cattle infected respectively. Own studies carried out by Heydorn in Germany (Berlin area) between 1972 and 1977 found all cattle over two years of age infected with *Sarcocystis* (Heydorn, 1977). Similar studies have been cited by Vangeel (2007) concluding that for most regions where studies have been carried out, i.e. regions of New Zealand (Böttner et al., 1987), Belgium (Bosschere et al., 2001 and Vercruysse, 1989), France (Fortier et al., 1993), Iraq (Latif et al., 1999), The Netherlands (van Knapen, 1987), and Ethiopia (Woldemeskel, 1996), the prevalence in adult bovines was close to 100%. However, attention needs to be called to the limited number of animals used in some surveys and the different analytical methods employed, which did not always differentiate the species (due to lack of test sensitivity and specificity).

The estimated prevalence of *Sarcocystis* in pigs in central Europe is approximately 35% for breeding animals and approximately 10% for fattening pigs (Dauguschies, 2006). Recently a cross-sectional study on the seroprevalence in breeding sows has been carried out in the German federal state of Hesse and resulted in 29% of the tested animals found to be positive (Damriyasa et al., 2004). Species specification was not possible in this study because of cross-reactivity of the antigen used in the test, though it was suspected that *S. suihominis* is probably more common. In a prevalence study in the Netherlands in 1993, *Sarcocystis* was reported in dairy cattle (100%), 89% in adult sheep and 43% in sheep below 10 months of age, but no positives were found in fattening pigs, sows and veal calves investigated by artificial digestion of oesophagus and diaphragm tissue (van Knapen et al., 1993). Older studies, carried out between 1963 and 1974, reviewed in Heydorn (1977) and 1978 to 1994 (cited in Damriyasa et al., 2004) report infections of 2.8% - 60% in Germany, 1.1% - 95% in Poland, 7.4% - 32% in Austria, 25% in Hungary, 10.5% in Bulgaria, 1.8% - 3.5% in Denmark, 18% in Iowa (USA), and 16% in Japan. Where results were differentiated, the reported prevalence in older animals (sows and boars) was higher compared to fattening pigs. However, the differences and wide percentage ranges were not only considered the result of using various detection methods but also significantly influenced by differences in husbandry and hygiene factors.

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

2.1.1 Rationale

In the Call for Proposals (CFP/EFSA/Zoonoses/2007/01), the Manual for Reporting on Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Food-borne Outbreaks in the framework of Directive 2003/99/EC (EFSA, 2007b), the Reports on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004 (EFSA, 2005) and The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2005 (EFSA, 2006b), *Sarcocystis* is either referred to as *Sarcocystis* spp. or it is not further specified. We considered it important to clarify which species are relevant in the context of public health, i.e. which are the zoonotic species and what is their impact on human health. The effect on human health needs to be considered when addressing the feasibility of sampling schemes especially in the light of the economic impact that those sampling schemes could have on individual MSs. A clear definition of the species in question was also required for addressing analytical methods, as methods may differ from species to species and different analytical techniques may be required for species differentiation.

2.1.2 Approach

Literature (scientific publications, textbooks, official websites such as the World Organisation for Animal Health (OIE), World Health Organisation (WHO) and European Centre for Disease Prevention and Control (ECDC) on *Sarcocystis* was reviewed and the information/existing knowledge on zoonotic species summarised. 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 '*Sarcocystis* Zoonotic species Risk Assessment' in Appendix I.

The species were run through the following criteria:

Criterion 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.

Criterion 2: Pathogenicity (+ - +++)

This qualitative assessment was based on clinical symptoms reported in humans. Because of the subjective nature, dose dependence and inconsistency of clinical symptoms in individuals and subgroups, a qualitative scale that would reflect the severity of symptoms commonly cited in the majority of patients was used. The following categories were used:

- (+) mild: clinical symptoms so mild that disease often unnoticed or not addressed by the individual;
- (++) moderate: noticeable gastrointestinal symptoms of short duration;
- (+++) severe: gastrointestinal symptoms including vomiting and diarrhoea, circulatory problems, drowsiness and dyspnoea.

Criterion 3: Geographical distribution

Parasite species can occur in geographically confined areas, where they are adapted to certain climatic conditions and/or to the availability of certain host species. Introduction of species currently not circulating in EU MSs is theoretically possible. Here the likelihood of introduction and consequentially establishment, once introduced, was assessed. This depended mostly on the epidemiology of the agent and the role of humans as intermediate or final/dead end host or 'vector'.

Criterion 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

Over 100 species of *Sarcocystis* are presently known (Vangeel, 2007). Of these, only two identified species (*S. suis*, *S. bovihominis*) and a yet unknown complex/group, previously referred to as *S. lindemanni*, have been reported as zoonotic.

Several species may be involved in human muscular sarcocystosis. There are still many unknowns in the life cycle and definitive hosts for any species that form sarcocysts in human muscles (Arness et al., 1999). Symptoms in humans range from acute self-limiting to chronic or moderately severe (vasculitis, myositis), though most cases appear to go unnoticed or are only mild (Arness et al. 1999; Fayer 2004). Most reported cases were considered to be incidental observations, detected in tropical and subtropical areas (Southeast Asia). A study carried out in Malaysia found a prevalence of almost 20% (Thomas and Dissanaike, 1978), and most reported cases were acquired in the Far East. Risk groups are hard to identify because of so many unknowns but are suspected to include travellers and military personnel exposed to risk factors e.g. contaminated food/water. For muscular sarcocystosis, humans act as a dead end host, which makes the agents unlikely to become part of an epidemiological chain, even if introduced into non-endemic areas. These species are therefore not considered relevant to be monitored in Europe.

Clinical symptoms in humans caused by *S. bovihominis* are transient and are generally described as mild and non-specific gastrointestinal symptoms such as abdominal pain, nausea and diarrhoea. Several infection studies on human volunteers have been carried out and even with what was considered a high infection dose, not likely to occur in naturally infected meat, only mild symptoms were reported and no medical intervention sought (Rommel and Heydorn, 1972; Dubey et al., 1989; Fayer, 2004; Pena et al., 2001; Vangeel et al., 2007; Chen et al., 1999). These observations together with a lack of data on human sarcocystosis in Europe raised the question as to the pathogenic potential of this parasite and the extent of the problem it realistically causes. Therefore it was concluded that further research and data on the prevalence of *S. bovihominis* in humans is needed to obtain better insight into the epidemiology and the scale of the problem this agent actually poses and to form a sound basis for the development of monitoring schemes.

Similar conclusions were drawn for *S. suis*, the only difference being that this species has the potential to cause severe symptoms in humans, which can affect the gastrointestinal tract (nausea, vomiting, abdominal pain and diarrhoea) and can include circulatory problems (tachycardia), drowsiness and dyspnoea. Again, these symptoms had been observed during infection studies carried out on volunteers (Heydorn, 1977; Kimmig et al., 1979; Li et al., 2007). Because of its potential of causing severe clinical symptoms in humans *S. suis* should be prioritised over *S. bovihominis*, though again a clarification of the public health impact is needed as sufficient justification for recommending test development and systematic monitoring.

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, have an impact on the human food chain or are suitable for surveillance in a public health context. The aim here 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

Only pigs and wild boar were reported as intermediate hosts for *S. suis*, whilst cattle are the intermediate host for *S. bovihominis*. All those species are part of the human food chain and could be considered in monitoring schemes for the direct protection of human health (Dubey and Odening, 2001; Taylor et al., 2007). See spreadsheet 'Relevant animals and foodstuffs to be monitored' in Appendix II.

Foodstuffs: No other foodstuffs other than carcasses are relevant for the monitoring purposes.

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. measuring of national prevalence to a certain level, the result 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) were compiled in a table and test specifics (sensitivity, specificity), listed as far as available. Also considered were the expenditure and complexity of the test methods. The costs were roughly estimated, where possible, bearing in mind that they vary 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. Apart from visual inspection the only muscle incisions required by slaughterhouse inspection of cattle are incisions into the internal and external masseters (not applicable to animals under six weeks of age) and a lengthwise incision of the heart in cattle of all ages. For pigs only the lengthwise incision of the heart is required.

Meat inspection in the EU is performed visually at the slaughterhouse according to the Regulation (EC) No 854/2004.

Visual inspection at the slaughterhouse will identify macroscopic lesions, not necessarily caused by the two zoonotic species, but does not allow species differentiation. Species differentiation can be carried out using various methods. One method is based on microscopy of hematoxylin and eosin-stained histological section and identification of distinctive physical features. However, there is a lack of sensitivity due to the limitation of the size of the muscle section that can be examined and the physical features can vary with the age of the sarcocyst, the host cell type and the fixation methods (Fayer, 2004) and often morphological distinction requires transmission electron microscopy. Molecular techniques have been employed for detection of *Sarcocystis*, isolated by digestion of the tissue (Vangeel et al., 2007; Gonzalez et al., 2006) or used in combination with histological sections (Pritt et al., 2008). Either method seems unsuitable for high throughput routine inspection. A summary of analytical methods can be found in Appendix III.

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

These recommendations are **an example only** of what could be carried out **once the need for monitoring has been established**. It is based on the example monitoring of *S. suis*, as we considered this species more important, based on the potential impact of human health, i.e. the severity of clinical symptom, as explained in detail in objective 2.1.

Animal population to be monitored: as pigs and wild boar have been identified as the only intermediate host, and both of them are part of the human food chain, these are the species which should be monitored.

Selection of slaughterhouses: All slaughterhouses slaughtering pigs or wild boar for human consumption.

Selection of animals: All animals destined for human consumption.

Objective 5. Propose harmonised monitoring and reporting schemes

5.1 Harmonised monitoring

Sample sizes are not relevant if all slaughter animals are to be tested in slaughterhouse. Attention should be focused on improved reporting of the results.

Results of all positive animals should be reported to a competent authority and collated for submission to the EU. Ideally this competent authority would be the same for *Trichinella*, *Echinococcus* and *Cysticercus* in order to minimise the amount of population data that needs to be reported.

5.2 Reporting

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

1. description of surveillance programme; and
2. individual data for each positive animal.

MSs are encouraged to use information Food Chain Information (FCI) where possible as collection of information on origin of carcasses is mandatory under Regulation (EC) No 854/2004. For the purposes of reporting, only aggregate level data (5.2.1.) need to be reported to the EU.

5.2.1 Description of surveillance programme

- MS name
- Region name (if applicable)
- Animal production type (for each of fattening pigs, breeding pigs, i.e. sows and boars, and wild boar)
 - Number of animals tested
 - Number of positive animals
 - Percentage positive for *Sarcocystis* spp.

5.2.2 Individual sample information

- Species and production type (as defined in FCI)
- Date of analysis
- Status* (positive/negative – see case definition)
- Parasite species
- Analysis method used

5.2.3 Population data

- Total number of animals slaughtered in each MS (if known) for:
 - Fattening pigs
 - Breeding pigs (sows and boars)
 - Wild boar

Data should be submitted on an annual basis through EFSA's website on standardised forms with validation rules to ensure data is consistent.

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

The following provide suggestions for analysis of reported data but will be dependent on the quantity and quality of data reported by the MSs. Consideration should also be given to recommendations from the EFSA working group on the statistical analysis of temporal and spatial trends in zoonotic agents in animals and food, which is due to publish a report shortly.

6.1 Descriptive analyses

Suggestions for descriptive analysis include:

- tables showing the proportion of positive samples in each MS for pigs by production type and for wild boar.
- a description of the prevalence of *Sarcocystis* in different pig groups should also be presented (fattening pigs, sows and boars).

If all animals are tested, Community level prevalence estimates can be obtained by combining the MS level data (i.e. weighting or other adjustment not required).

6.2 Monitoring trends over time

For determining linear trends using binary data approaches such as the use of a logistic model is recommended when the number of years exceeds two. Different models may be used for fattening pigs, sows and boars and wild boar to look for trends within production types.

A similar approach can be used to determine trends for each MS. At Community level multilevel models (e.g. GEE or random effects) can be applied to all EU data to determine the overall trend within the EU and in the case of a random effects models, additional parameters for trends within MSs.

6.3 Spatial analysis

Unless MS report at the regional level (e.g. NUTS 1 or 2) choropleth maps to show the prevalence of *Sarcocystis* at the MS level in pigs and wild boar.

Further spatial analysis is not recommended for this parasite.

REFERENCES

- Arness MK, Brown, JD, Dubey JP, Neafie RC, Granstrom DE, 1999. An outbreak of acute eosinophilic myositis attributed to human *Sarcocystis* parasitism. *American Journal of Tropical Medicine and Hygiene*, 61(4), pp. 548-553.
- Beaver PC, Gadgil RK, Morera P, 1979. *Sarcocystis* in Man: a Review and Report of Five Cases. *American Journal of Tropical Medicine and Hygiene*, 28(5), pp. 819-844.
- BfR (Federal Institute for Risk Assessment, Germany), 2008. Stellungnahme Nr. 026/2008: Ungenügend erhitztes Schweinefleisch könnte Sarkosporidien enthalten. (Opinion no. 026/2008: insufficiently cooked pork could contain sarcocysts). (In German).
- Böhmler G, Brüggemann M, Djuren M, Höxter K, Monazahian M, 2008. Sarkosporidien in Thüringer Mett als Auslöser einer lebensmittelassoziierten Gruppenerkrankung: eine bisher unterschätzte Gefahr, *Journal für Verbraucherschutz und Lebensmittelsicherheit*, 3, pp. 4-10. (In German).
- Böttner A, Charleston WAG, Pomroy WE, Rommel M, 1987. The prevalence and identity of *Sarcocystis* in beef cattle in New Zealand. *Veterinary Parasitology*, 24, pp. 157-168.
- De Bosschere H, Ducatelle R, 2001. Inverse correlation between myositis eosinophilia and number of *Sarcocystis* cystozoites in heart tissue of cattle. *Flemish Veterinary Journal*, 70, pp. 118-123.
- Chen X, Zuo Y, Zuo W, 1999. Observation on the clinical symptoms and sporocysts excretion in human volunteers experimentally infected with *Sarcocystis hominis*. Source: *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi*, Chinese Journal of Parasitology and Parasitic Diseases, 17(1), pp. 25-27. (In Chinese).
- CFSPH (Center for Food Security and Public Health) 2005. Sarcocystosis. <http://www.cfsph.iastate.edu/Factsheets/pdfs/sarcocystosis.pdf>. Last update: May 2005.
- Damriyasa IM, Bauer C, Edelhofer R, Failing K, Lind P, Petersen E, Schares G, Tenter AM, Volmer R, Zahner H, 2004. Cross-sectional survey in pig breeding farms in Hesse, Germany: seroprevalence and risk factors of infections with *Toxoplasma gondii*, *Sarcocystis* spp. and *Neospora caninum* in sows. *Veterinary Parasitology*, 126, pp. 271-286.
- Dauguschies A, 2006. Protozoeninfektionen des Schweines. In: Schnieder T. (Ed), *Veterinärmedizinische Parasitologie*. Parey Verlag, Berlin, pp. 359-368. (In German).
- Dubey JP, Odening K, 2001. Toxoplasmosis and related infections. In: Samuel, WM, Pybus, MJ, Kocan, A.A. (Eds.), *Parasitic Diseases of Wild Mammals*, 2nd edition. Manson Publishing Ltd, London. ISBN 1-84076-009-5.
- Dubey JP, Speer CA, Fayer R, 1989. *Sarcocystis* of animals and man. CRC Press, Inc., Boca Raton, Fla.
- 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.
- EFSA, 2005. Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004. *The EFSA Journal*, 2005, p. 310. Available online at: www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620772157.htm

- EFSA Scientific Panels on Biological Hazards (BIOHAZ) and on Animal Health and Welfare (AHAW), .2006a. 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, available online at:
http://www.efsa.europa.eu/en/science/biohaz/biohaz_opinions/biohazahaw_ej403_zoonoses.html.
- EFSA, 2006b. 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. 3-288. Available online at: www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620767319.htm
- EFSA, Scientific Panels on Biological Hazards (BIOHAZ) and on Animal Health and Welfare (AHAW), 2007a. Scientific Opinion on the Review of the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2005. The EFSA Journal (2007), 600, pp. 1-32. Available online at: www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178672823356.htm
- EFSA, 2007b. Report of the Task Force on Zoonoses Data Collection - Manual for Reporting on Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Food-borne Outbreaks in the framework of Directive 2003/99/EC and on some other pathogenic microbiological agents for information derived from the reporting year 2006. The EFSA Journal (2007), 100, pp. 1-86. Available online at: www.gencat.cat/salut/acsa/Du12/html/ca/dir1316/dn1316/report_manual_2006_en.pdf
- Fayer R, 2004. *Sarcocystis* spp. in Human Infections. Clinical Microbiology Reviews, 17 (4), pp. 894-902.
- Fortier G, Collobert JF, Viel S, Mariau V, 1993. Prévalence de la Sarcosporidiose musculaire bovine dans le Calvados. Rec. Med. Vet. Ec. Alfort 169, pp. 779-781.
- González LM, Villalobos N, Montero E, Morales J, Álamo Sanz R, Muro A, Harrison LJS, Parkhouse RME, Gárate T, 2006. Differential molecular identification of *Taeniid* spp. and *Sarcocystis* spp. cysts isolated from infected pigs and cattle. Veterinary Parasitology, 142, pp. 95-101.
- Heydorn AO, 1977. Sarkosporidien infiziertes Fleisch als mögliche Krankheitsursache für den Menschen. Archiv für Lebensmittelhygiene, 28, pp. 27-31. (In German).
- Kimmig P, Piekarski G, Heydorn AO, 1979. Zu Sarkosporidiose (*Sarcocystis suis hominis*) des Menschen. Immun. Infekt. 7, pp. 170-177. (In German).
- Latif BMA, Al-Delemi JK, Mohammed BS, Al-Bayetti SM, Al-Amiry AM, 1999. Prevalence of *Sarcocystis* spp. In meat-producing animals in Iraq. Veterinary Parasitology, 84, pp. 85-90.
- Li JH, Lin Z, Du JF, Qin YX, 2007. Experimental infection of *Sarcocystis suis hominis* in pig and human volunteer in Guangxi. Source: Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi, Chinese Journal of Parasitology and Parasitic Diseases, 25(6), pp. 466-468. (In Chinese).
- Lunde MN, Fayer R, 1977. Serologic tests for antibody to *Sarcocystis* in cattle. Journal of Parasitology, 63 (2), pp. 222-225.
- Odening K, Wesemeier H-H, Walter, G, Bockhardt I, 1995. On the morphological diagnostics and host specificity of the *Sarcocystis* species of some domesticated and wild Bovini (cattle, banteng and bison). Applied Parasitology, 36, pp. 161-178
- OIE (World Organisation for Animal Health), 2008. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees). Sixth Edition, 2008. Published by the Office International des Epizooties, 12 rue de Prony, 75017 Paris, France. ISBN 978-92-9044-718-4. Available online at: www.oie.int/Eng/Normes/Mmanual/A_summry.htm

- PAOH, 2003. Zoonoses and communicable diseases common to man and animals, 3rd edition, volume III, parasitoses. Acha, P., Szyfres, B. (Eds), Pan American Health Organization, Scientific and Technical Publication No. 580. ISBN 92 75 11993 7.
- Pena HFJ, Ogassawara S, Sinhorini IL, 2001. Occurrence of cattle *Sarcocystis* species in raw kibbe from Arabian food establishments in the city of São Paulo, Brazil, and experimental transmission to humans. *Journal of Parasitology*, 87(6), pp. 1459-1465.
- Pritt B, Trainer T, Simmons-Arnold L, Evans M, Dunams D, Rosenthal BM, 2008. Detection of *Sarcocystis* parasites in retail beef: a regional survey combining histological and genetic detection methods. *Journal of Food Protection*, 71(10), pp. 2144-2147.
- Rommel M, Heydorn AO, 1972. Beiträge zum Lebenszyklus der Sarkosporidien. III. Isospora hominis (RAILLIET und LUCET, 1891) WENYON, 1923, eine Dauerform der Sarkosporidiose des Rindes und des Schweins. *Berliner und Münchner Tierärztliche Wochenschrift*, 8, pp. 143-145. (In German).
- Taylor MA, Coop RL, Wall RL, 2007. *Veterinary Parasitology*, 3rd Edition, Blackwell Publishing Ltd., ISBN: 978-1-4051-1964-1.
- Tenter AM, 1995. Current Research on *Sarcocystis* Species of Domestic Animals. *International Journal for Parasitology*, 25(11), pp. 1311-1330.
- Thomas V, Dissanaiké AS, 1978. Antibodies to *Sarcocystis* in Malaysians. *The Transactions of The Royal Society of Tropical Medicine and Hygiene*, 72 (3), pp. 303-306.
- Vangeel L, Houf K, Chiers K, Vercruyse J, D'Herde K, Ducatelle R, 2007. Molecular-Based Identification of *Sarcocystis hominis* in Belgian Minced Beef. *Journal of Food Protection*, 70 (6), pp. 1523-1526.
- Van Knapen F, Bouwman D, Greve E, 1987. Onderzoek naar het voorkomen van *Sarcocystis* spp. bij Nederlandse runderen met verschillende methoden. *Tijdschr. Diergeneeskd.*, 112, pp. 1095-1100.
- Van Knapen F, Franchimont JH, Kremers AFT, Greve E, Bouwman D, Breedijk CG, van Venrooy L, 1993. Prevalence of *Sarcocystis* spp. In tissue samples of veal calves, dairy cattle, fattening pigs, sows and sheep in the Netherlands. RIVM report 189201004, 1993.
- Vercruyse J, Fransen J, van Goubergen M, 1989. The prevalence and identity of *Sarcocystis* cysts in cattle in Belgium. *Journal of veterinary medicine. B, Infectious diseases and veterinary public health*, 36, pp. 148-153.
- Woldemeskel M, Gebreab F, 1996. Prevalence of sarcocysts in livestock of northwest Ethiopia. *Journal of veterinary medicine. B, Infectious diseases and veterinary public health*, 43, pp. 55-58.
- Wong KT, Pathmanathan R, 1992. High prevalence of human skeletal muscle sarcocystosis in south-east Asia. *The Transactions of The Royal Society of Tropical Medicine and Hygiene*, 86, pp. 631-632.

APPENDICES

A. *SARCOCYSTIS* ZOONOTIC SPECIES RISK ASSESSMENT

Species	FH	IH	Zoonotic (Y/N)	Pathog. FH	Pathog. IH	Geographical Distribution	Present in Europe (Y/N)	Likelihood of Est. in non-endemic EU countries (H/M/L)	Econ. impact of human disease (H/M/L)	Monitoring in Europe recommended (Y/N)	Comments	References
<i>Sarcocystis bovihominis</i>	Human	Cattle	Y	+	0	Limited distribution. Thought absent from some EU countries but little information.	Y	M	L	N	Effects on humans usually mild and in many cases asymptomatic. At risk are particularly humans consuming raw or undercooked beef.	BfR, 2008 Fayer, 2004 PAHO, 2003 Taylor et al., 2007 Vangeel et al., 2007
<i>Sarcocystis sui hominis</i>	Human	Pig	Y	++ - +++	+++	Thought worldwide. Has been reported from several European countries, but the exact geographical distribution is unknown.	Y	M	L	Only after clarification of actual impact on human health in the EU and establishment of public health need*.	Human infection can cause cardiac and rheumatoid diseases. At risk are particularly humans consuming raw or undercooked beef. *No consistent epidemiological information is available; furthermore, no useful test is available to detect these parasites at the slaughterhouse.	BfR, 2008 Boehmler et al., 2008 Dauguschies, 2006 Taylor et al., 2007 Fayer, 2004 PAHO, 2003 Li et al., 2007
Several unidentified species	?	Human	Y	?	+- ++	Mainly SE Asia, India, Egypt, Malaysia, Thailand.	N	L	L - M	N	Found in human muscle, mainly in SE Asia. Final host unknown. Most reports of human infection have been considered to be incidental observations, though one study carried out in Malaysia found a prevalence of 21%, and most reported cases were acquired in the Far East. This species is therefore not considered relevant to be monitored in Europe. Because the life cycle and definite hosts are unknown it is difficult to identify risk groups among people travelling to endemic areas.	Arness et al., 1999 Beaver et al., 1979 Fayer 2004 CFSPH, 2005 PAHO, 2003 Wong, Pathmanathan, 1992

Econ. = Economic / Est. = Establishment / FH = Final Host / H/M/L = High/Medium/Low / IH = Intermediate Host / Pathog. = Pathogenicity / Y/N = Yes/No

B. *SARCOCYSTIS*, RELEVANT ANIMALS AND FOODSTUFFS TO BE MONITORED

Animal species or foodstuff	Role in infection chain*	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	Comments	References
Domestic pig	IH	Y	Y	N/A	If public health need can be identified	Direct protection of human health	No consistent epidemiological information is available; furthermore, no useful test is available to detect these parasites at the slaughterhouse.	Taylor et al., 2007 PAHO, 2003
Wild boar	IH	Y	Y	N/A	If public health need can be identified	Direct protection of human health	No consistent epidemiological information is available; furthermore, no useful test is available to detect these parasites at the slaughterhouse.	Dubey and Odening, 2001 Taylor et al., 2007

DEH = Dead-end host or incidental host. Host that usually does not transmit an infectious agent to other animals.

DH = definitive or final host in which an organism undergoes its sexual phase of reproduction.

IH = Intermediate Host. Animal in which the infectious agent undergoes some development, frequently with asexual 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.

RH = Reservoir Host. Host in which an infectious agent normally lives and multiplies, therefore a common source of infection (frequently a primary host).

C. SARCOCYSTIS, SUMMARY OF ANALYTICAL METHODS

Analytical method / technique	Sensitivity	Specificity	Application (sample materials)	Application result	Throughput (samples/day)	Estimated costs (€)*	Technical requirements	Suitable for QA (Y/N)	Comments	References
Meat inspection	N/A	N/A	Carcass	Individual	100-500	1.00	None	N	Gross examination part of routine meat inspection. Only applicable for macrocyst species and no differentiation between pathogenic and non-pathogenic species.	Regulation (EC) No 854/2004 Taylor, personal communication.
Histopathology (LM)	Lack of sensitivity because only a small section of muscle can be examined	N/A	Muscle section (usually HE stained)	Individual	10-20	25.00	Specialist histology facilities	Y	Labour intensive, costly and requires specialist facilities. Species specification possible depending on various factors such as the cyst, host cell types and methods of fixation.	Boehmler et al., 2008 Fayer, 2004 Odening et al., 1995 Tenter, 1995
Muscle Squash (LM)	Lack of sensitivity because only a small section of muscle can be examined	N/A	Muscle sample	Individual	10-50	?	Specialist Laboratory	Y	Species-specific diagnosis of cysts often not possible or requiring electron microscopic methods (see below).	Tenter, 1995
Enzymatic digestion of muscle	N/A	N/A	Muscle sample	Batch	10-100	?	Specialist Laboratory	Y	Specialist facilities required for further identification of cyst after digestion (see below).	BfR, 2008 Taylor 2007
Serology (ELISA)	Not reported	Not reported	Serum sample	Batch	?	?	ELISA reader	Y	Tests only genus-specific due to high cross-reactivity.	Damriyasa et al., 2004 Tenter, 1995
Serology (IHA)	N/A	N/A	Serum sample	Batch		?	Specialist Laboratory	Y	Specialist serological laboratory facilities required.	Lunde and Fayer, 1977
Molecular Identification (PCR)	Not reported	Not reported	Cysts isolated from muscles	Individual	Not reported	Not reported	Specialist Laboratory			Vangeel et al., 2007 Pritt et al., 2008
Electron Microscopy	Lack of sensitivity because only a small section of muscle can be examined	100%	Muscle sample	Individual	1-5	?	Specialist Laboratory	Y	TEM required (laborious, expensive and not readily available). Species specification possible.	Odening et al., 1995 Vangeel et al., 2007

*Will vary from country to country and depend on the throughput. Only rough indication to allow comparison between methods.

ELISA = Enzyme Linked Immunosorbent Assay / HE = Hematoxylin and Eosin / IHA = Indirect Hemagglutination / LM = Light Microscope / N/A = Not Applicable / PCR = Polymerase Chain Reaction / QA = Quality Assurance / TEM = Transmission Electron Microscopy / Y/N = Yes/No

D. SARCOCYSTIS, SUMMARY OF COUNTRY RESPONSES

MS	Information via	Data / Comments
Austria	AGES (Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH. Veterinärmedizinische Untersuchungen Innsbruck)	Routine inspection in slaughterhouses, but data not centrally recorded
Belgium	Prince Leopold Institute for Tropical Medicine (ITG)	Data collected during meat inspection. Cattle infection 56% (Vercruysse et al., 1989).
Bulgaria	Literature search	No recent studies identified but earlier data on cattle available (Meshkov, 1975).
Cyprus	Department of Veterinary Services, Ministry of Agriculture, Natural Resources and Environment, Republic Of Cyprus	No cases of <i>Sarcocystis</i> reported until now. Data on humans not available.
Czech Republic	Department of Veterinary Hygiene, Public Health and Ecology	Data not recorded.
Denmark	Danish Meat Association	Routine inspection in slaughterhouses, but data not centrally recorded.
Estonia	Veterinary and Food Board	Routine inspection in slaughterhouses, but data not centrally recorded.
Finland	Finnish Food Safety Authority	No data available.
France	Ministry of Agriculture	Routine inspection in slaughterhouses, but data not centrally recorded.
Germany	Bundesinstitut für Risikobewertung (BfR - Federal Institute for Risk Assessment)	Routine inspection in slaughterhouses, but data not centrally recorded.
Greece	Provided by the Ministry of Agriculture	Routine inspection in slaughterhouses and data should be reported. No data for <i>Sarcocystis</i> in Greece is recorded by the Ministry. No data on human sarcocystosis is available (official or literature).
Hungary	Central Veterinary Institute, Budapest	Sarcosporidiosis and sarcocystosis are not mandatory reportable infections in Hungary, and almost no published data is available on the epidemiology of these parasites.
Ireland	Central Meat Control Laboratory	No data available.
Italy	Istituto Superiore di Sanità	Routine inspection in slaughterhouses, but data not centrally recorded.

D: SARCOCYSTIS, SUMMARY OF COUNTRY RESPONSES (contd.)

MS	Information via	Data / Comments
Latvia	State Food and Veterinary Service	Routine inspection in slaughterhouses, but data not centrally recorded. No data on human sarcocystosis available.
Lithuania	State Food and Veterinary Service	Routine inspection in slaughterhouses, but data not centrally recorded.
Luxembourg	N/A	Data available via EFSA Community Summary Report.
Malta	Ministry for Rural Affairs and the Environment.	No data available.
The Netherlands	National Institute for Public Health and the Environment (RIVM)	Current surveillance is performed during official meat inspection (only for macroscopic lesions). There are no surveillance strategies to identify invisible sarcocysts. Macroscopic lesions that lead to carcass condemnation will be confirmed by NRL for Parasites (RIVM) and reported to the Official Veterinarian Authority.
Poland	National Public Health Institute	No data available.
Portugal	Universidade de Trás-os-Montes e Alto Douro (official data from 2005).	There are no surveillance strategies to identify invisible sarcocysts. Only the macroscopic lesions that lead to carcass condemnation are reported to the Official Veterinarian Authority on a monthly basis. This information does not include additional information about the age and animal origin.
Romania	Faculty of Veterinary Medicine of Cluj Napoca	No data available.
Slovakia	Reporting officer	No data provided or available via literature.
Slovenia	Reporting officer	No data provided or available via literature.
Spain	Agencia Española de Seguridad Alimentaria y Nutrición	The most part of the requested information it is dispersed over several institutions with different administrations. Organisation of central data collection is currently in progress.
Sweden	National Veterinary Institute (SVA)	No information available.
United Kingdom	Meat Hygiene Service / UK Food Standard Agency	Routine inspection in slaughterhouses. Only the macroscopic lesions that lead to carcass condemnation are reported to the Food Standards Agency.

ABBREVIATIONS

AFSSA	Agence Française de Sécurité Sanitaire des Aliments
AHAW	Animal Health and Welfare
AUA	Agricultural University of Athens
BfR	Bundesinstitut für Risikobewertung
BIOHAZ	Biological Hazards
CFSPH	Center for Food Security and Public Health
CSL	Central Science Laboratory
CSR	Community Summary Report
DEH	Dead-end host or incidental host
DH	definitive or final host
DMA	Danish Meat Association
DTU	National Veterinary Institute
ECDC	European Centre for Disease Control
Econ	Economic
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immunosorbent Assay
Est	Establishment
EU	European Union
FCI	Food Chain Information
FH	Final Host
GEE	Generalised estimating equations
H/M/L	High/Medium/Low
HE	Hematoxylin and Eosin ///
IH	Intermediate Host
IH	Intermediate Host
IHA	Indirect Hemagglutination
ISS	Istituto Superiore di Sanità
ITG	Prince Leopold Institute of Tropical Medicine

LM	Light Microscope
MS	Member State
N/A	Not Applicable
NDC FVS	National Diagnostic Centre of Food and Veterinary Services
NUTS	European Country Classification system
OIE	World Organisation for Animal Health
PAOH	Pan American Health Organization
Pathog	Pathogenicity
PCR	Polymerase Chain Reaction
PH	Primary host
QA	Quality Assurance
RA	Risk Assessment
RH	Reservoir Host
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
SH	Secondary Host
TEM	Transmission Electron Microscopy
UTAD	Universidade de Trás-os-Montes e Alto Douro
VLA	Veterinary Laboratories Agency
Y/N	Yes/No
ZCC	Zoonoses Collaboration Centre