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SCIENTIFIC REPORT OF EFSA

Technical specifications on harmonised epidemiological indicators for public health hazards to be covered by meat inspection of swine[1]

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ABSTRACT

In this report harmonised epidemiological indicators are proposed for food-borne biological hazards to public health that are related to pigs and pork and that can be addressed within meat inspection. These hazards include *Salmonella*, *Yersinia enterocolitica*, *Toxoplasma gondii*, *Trichinella*, *Cysticercus* (*Taenia solium*) and mycobacteria. An epidemiological indicator is defined as the prevalence or incidence of the hazard at a certain stage of the food chain or an indirect measure of the hazards that correlates to human health risk caused by the hazard. The indicators can be used by the European Commission and the Member States to consider when adaptations in meat inspection methods may be relevant and to carry out risk analysis to support such decisions. It is foreseen that the indicators will be used in the pork safety assurance framework proposed by the EFSA Scientific Opinion, particularly to help categorise farms/herds and slaughterhouses according to the risk related to the hazards as well as setting appropriate targets for final chilled carcases. Depending on the purpose and the epidemiological situation risk managers should decide on the most appropriate indicator(s) to use, either alone or in combinations, at national, regional, slaughterhouse or farm/herd level. It is recommended that risk managers should define the harmonised requirements for the controlled housing conditions of farms. Member States are invited to organise training regarding the implementation of the indicators and the reporting of data generated by the implementation in accordance with Directive 2003/99/EC. The proposed indicators should be regularly reviewed in light of new information and the data generated by their implementation. For some hazards further research is needed on the risk factors and the role of pork as a source of human infection.

KEY WORDS

Meat inspection, biological hazard, epidemiological indicators, *Salmonella*, *Yersinia*, *Toxoplasma*, *Trichinella*, *Cysticercus*, mycobacteria

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SUMMARY

The European Commission has requested that European Food Safety Authority provide technical assistance on harmonised epidemiological criteria (indicators) for specific public health hazards in food and animals to be used by risk managers when they consider that the current methods for meat inspection do not adequately address the relevant risks. It is related to the mandate from the Commission for a Scientific Opinion on the public health hazards to be covered by inspection of meat. The first Opinion and this report concern the meat inspection of swine (pigs) and they were published in October 2011.

In this report, harmonised epidemiological indicators are proposed for food-borne biological hazards to public health that are related to pigs and pork and that can be addressed within meat inspection. These hazards include *Salmonella, Yersinia enterocolitica*, *Toxoplasma gondii*, *Trichinella*, *Cysticercus* (*Taenia solium*) and mycobacteria. An epidemiological indicator is understood to mean the prevalence or incidence of the hazard at a certain stage of the food chain or an indirect measure of the hazards (such as audits) that correlates to a human health risk caused by the hazard. The epidemiological indicators can be used by the European Commission and the Member States to consider when adaptations to meat inspection methods may be relevant, and to enable the Member States to carry out risk analysis to support any such decisions. It is foreseen that the epidemiological indicators may be used in the pork safety assurance framework proposed by the European Food Safety Authority Scientific Opinion on the public health hazards to be covered be inspection of meat (swine), particularly to help categorise farms/herds and slaughterhouses according to the risk related to particular hazards, as well as setting appropriate targets for final chilled carcases.

The risk managers should decide on the most appropriate use of the epidemiological indicators at the European Union and national levels. Depending on the purpose and the epidemiological situation of the country the indicators may be applied at national, regional, slaughterhouse or farm/herd level. The indicators can be used alone or in combinations. The indicators may be used to classify the countries, regions or farms according to the infection status related to the hazards. Some indicators may also be used to evaluate the measures taken in the slaughterhouses to control a specific hazard.

Most of the epidemiological indicators are proposed for subpopulations of pigs or pig carcases at the farm or slaughterhouse level. Some indicators include auditing of the farms for controlled housing conditions or auditing the transport of slaughter pigs, lairage conditions or slaughter methods. In the case of rare biological hazards in European Union pig production, epidemiological indicators are suggested as an aid in surveillance for possible emergence of such hazards. In the case of some of the biological hazards addressed it is accepted that there is a need for more research to clarify the factors that place pigs at risk of infection and the role of pork as a source of human infections.

Comparable data from the European Union Member States were available for only a few of the proposed epidemiological indicators. This was the case of some of the indicators relating to *Trichinella* and *Salmonella*. For each epidemiological indicator addressed the key elements of minimum monitoring or inspection requirements are defined. This includes the animal population to be targeted, the stage of the food chain where the sampling should take place, sampling strategy, type and details of the specimen to be taken, diagnostic or analytical method to be used, and a case definition. A general description is provided on how to choose the sampling strategy for the different types of indicators.

It is recommended that the European Commission and the Member States define the harmonised requirements for controlled housing conditions, transport to slaughter, and holding animal in the lairage that are referred to in the epidemiological indicators. In addition, the Member States are invited to support further studies to clarify the factors placing pigs at risk of *Yersinia enterocolitica* infection and the role of pork as a source of human toxoplasmosis.

The implementation of the proposed epidemiological indicators will generate additional data that will provide a more precise picture of the epidemiological situation in the European Union and this data may be used to update the indicators, when appropriate. It is recommended that the Member States report the data generated from monitoring these indicators in accordance with and using the framework prescribed in Directive 2003/99/EC. The proposed indicators should be reviewed regularly in light of new information and the data generated by their implementation. The European Commission and the Member States are invited to organise training to ensure harmonised implementation of the minimum monitoring and inspection requirements of the epidemiological indicators.

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BACKGROUND AS PROVIDED BY THE COMMISSION

Requests for technical assistance defining harmonised human health epidemiological criteria to carry out risk analysis within the scope of meat inspection

During their meeting on 6 November 2008, Chief Veterinary Officers (CVO) of the Member States agreed on conclusions on modernisation of sanitary inspection in slaughterhouses based on the recommendations issued during a seminar organised by the French Presidency from 7 to 11 July 2008. Inter alia, it was concluded that "*EFSA and the European Centre for Disease Prevention and Control (ECDC) should define animal and human health epidemiological criteria required for the Member States to carry out their own risk analysis to be able, if appropriate, to adapt the general inspection methods within the framework provided by the legislation*". The CVO conclusions have been considered in the Commission Report on the experience gained from the application of the Hygiene Regulations, adopted on 28 July 2009. Council conclusions on the Commission report were adopted on 20 November 2009 inviting the Commission to prepare concrete proposals allowing the effective implementation of modernised sanitary inspection in slaughterhouses while making full use of the principle of the 'risk-based approach'.

In accordance with Article 9(2) of 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/EC and repealing Council Directive 92/117/EEC[4], EFSA shall examine and publish a summary report on the trends and sources of zoonoses, zoonotic agents and microbiological resistance in the European Union based on reports transmitted by the Member States. In addition, EFSA has prepared several scientific reports on (harmonised) monitoring of food-borne infections. Prevalence data from the zoonoses monitoring are considered as relevant epidemiological criteria to carry out a risk analysis, however, such data may be limited in certain Member States or not sufficiently harmonised to compare the situation between Member States. It is, therefore, appropriate to lay down harmonised human health epidemiological criteria and their minimum requirements. Such criteria should provide a tool to be used by risk managers in case they consider the current methods for meat inspection disproportionate to the risk.

In accordance with Article 20 of Regulation (EC) No 854/2004 of the European Parliament and of the Council laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption[5], the Commission shall consult EFSA on certain matters falling within the scope of the Regulation whenever necessary.

[4] 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 L 325, 12.12.2003, p. 31–40.

[5] 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, p. 206–320.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

The scope of this mandate is to request technical assistance on harmonised epidemiological criteria for specific public health hazards in food and animals to be used by risk managers in case they consider the current methods for meat inspection address the relevant risk not adequate.

Where possible, such epidemiological criteria should be based on monitoring activities already laid down in European Union provisions, in particular in Regulation (EC) No 882/2004[6], Regulation (EC) No 2160/2003[7], Regulation (EC) No 852/2004[8], Regulation (EC) No 853/2004[9], Regulation (EC) No 854/2004 and their implementing acts.

The following species or groups of species should be considered, taking into account the following order of priority identified in consultation of the Member States: domestic swine, poultry, bovine animals over six weeks old, bovine animals under six weeks old, domestic sheep and goats, farmed game and domestic solipeds.

In particular, EFSA is requested within the scope described above to:

1. Define harmonised epidemiological criteria for specific hazards already covered by current meat inspection (trichinellosis, tuberculosis, cysticercosis, …) and for possible additional hazards identified in a scientific opinion on the hazards to be covered by inspection of meat (see Annex 1), which can be used to consider adaptations of meat inspection methodology (e.g. prevalence, status of infection).
2. Provide a summary of comparable data from Member States based on the above defined harmonised epidemiological criteria, if existing, e.g. from ongoing monitoring in humans, food or animals.
3. Recommend methodologies and minimum monitoring/inspection requirements to provide comparable data on such harmonised epidemiological criteria, in particular if comparable data are missing. These criteria should also be achievable in small Member States.

[6] Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. OJ L 165, 30.4.2004, p. 1–141.

[7] Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of *Salmonella* and other specified food-borne zoonotic agents. OJ L 325, 12.12.2003, p. 1–15.

[8] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–54.

[9] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.

TECHNICAL SPECIFICATIONS

1. INTRODUCTION

There are a number of food-borne diseases affecting humans that can be related to consumption of pork and traced back to swine (pigs). These hazards include parasites, bacteria and some viruses. According to the European Union (EU) Summary Report on Zoonoses and Food-borne Outbreaks in 2009 (EFSA and ECDC, 2011), 7.8 % of the reported verified food-borne outbreaks in the EU were caused by pork and products thereof, which followed eggs and egg products (17.3 %) and mixed and buffet meals (8.1 %) as the most important food vehicle categories. Within the food-borne outbreaks linked to consumption of pork and products thereof, 39.5 % were caused by *Trichinella*, 22.4 % by *Clostridium* spp., 9.8 % by *Salmonella*, and 6.6 % by *Staphylococcus* spp. The relevant hazards related to pork vary among the Member States (MSs) in accordance with the epidemiological situation and food consumption habits.

Meat inspection offers an opportunity to control some of these food-borne hazards, and in fact *Trichinella* and *Cysticercus* are directly targeted through the current meat inspection procedures for pigs (Regulation (EC) No 853/2004). However, most of the other biological hazards related to pigs and pork are not specifically addressed by the meat inspection system in place in the EU.

It is possible to use the data on prevalence and incidence of the biological hazards in animals, meat and humans as one aspect of the criteria when determining and ranking the human health importance of the hazards to be covered by meat inspection. These epidemiological criteria or indicators may be used by the risk managers when considering adaptations of current meat inspection methods for pigs. In the case of pigs, relevant prevalence data that could be used when designing the epidemiological indicators have been collected from the EU MSs within the framework of the annual reporting in accordance with Directive 2003/99/EC on the monitoring of zoonoses. Also, the EU-wide baseline surveys on *Salmonella* in slaughter and breeding pigs provide for fully harmonised datasets from the MSs (EFSA, 2008, 2009b). Data on incidence of food-borne diseases in humans are collected by the European Centre for Disease Prevention and Control (ECDC) based on Decision 2119/98/EC on setting up a network for the epidemiological surveillance and control of communicable diseases in the EU[10].

The Scientific Opinion of EFSA on the public health hazards to be covered by inspection of meat (swine) (EFSA, 2011) proposes a new generic framework for pork (carcase) safety assurance for biological hazards. It is foreseen that the harmonised epidemiological indicators will be used as part of this framework. Therefore, this report should be read in parallel with that Opinion.

2. DEFINITIONS

For the purpose of this report, the following definitions will apply:

Audit - a systematic and independent examination to determine whether arrangements, activities and related results comply with the requirements set for controlled housing conditions, transport, lairage and slaughter methods and whether these arrangements and activities are implemented effectively and are suitable to achieve the desired objectives.

Backyard pigs - pigs raised in small numbers in households in order to produce meat mainly for private consumption.

[10] Decision No 2119/98/EC of the European Parliament and of the Council of 24 September 1998 setting up a network for the epidemiological surveillance and control of communicable diseases in the Community. OJ L 268, 3.10.1998, p. 1–7.

Biosecurity - implementation of measures that reduce the risk of introduction and spread of zoonotic agents. It requires the adoption of a set of attitudes and behaviours by people to reduce risk in all activities involving domestic, farmed and wild animals and their products.

Breeding pigs - pigs (sow or boar) of at least six months of age kept for breeding purposes.

Carcase - the body of an animal after slaughter and dressing (Regulation (EC) No 853/2004).

Controlled housing conditions - a type of animal husbandry where pigs are kept at all times and for their whole life under conditions controlled by the food business operator with regard to feeding, housing and biosecurity of the holding.

Fattening pigs - pigs farmed not for reproductive purposes, but intended to be taken to a slaughterhouse for the production of meat and meat products.

Free-range pigs – pigs that have free access to the outside for all or most of their production life. They receive their nutritional needs from prepared feed or from pasture or forage depending on the season.

Indoor pigs – pigs raised and kept within buildings without outdoor access.

Harmonised epidemiological indicator (HEI) - prevalence or incidence of the hazard at a certain stage of the food chain or an indirect measure of the hazards (such as audits of farms) that correlates to a human health risk caused by the hazard.

Outdoor pigs - pigs that are raised outdoors or have an access to outdoors.

Pigs – domestic animals of the subspecies *Sus scrofa domesticus*.

Pork – meat of the subspecies *Sus scrofa domesticus*.

Risk factor - a variable associated with an increased risk of disease or infection.

Separation of head – removal of the head containing the tongue and throat from the pig carcase before splitting the carcase. Removal can be total or partial. In the latter case the head is still connected to the carcase, but in such a way that during the splitting of the carcase the splitting equipment has no contact with the head.

Slaughterhouse - establishment used for slaughtering and dressing animals, the meat of which is intended for human consumption (Regulation (EC) No 853/2004).

Wild boar – wild and farmed animals of the species *Sus scrofa*.

3. APPROACH APPLIED TO SELECT THE EPIDEMIOLOGICAL INDICATORS

3.1. Harmonised epidemiological indicators

In this report, the term 'epidemiological indicator' is used instead of 'epidemiological criterion' for the sake of clarity. A harmonised epidemiological indicator is, in this context, understood to mean the prevalence or incidence of the hazard at a certain stage of the food chain that correlates to a human health risk caused by the hazard. Indirect indicators of the hazards, such as audits of farms or transport, are also covered.

The purpose of the harmonised epidemiological indicators proposed in this report is to enable the European Commission (EC) and the MSs to consider whether adaptations to meat inspection methods may be made at the MS level and to enable the MSs to carry out a risk analysis (or components thereof) to support decisions on any such adaptations of meat inspection methods. For those hazards identified in the complimentary EFSA Scientific Opinion (EFSA, 2011) as the most relevant in the context of meat inspection, the epidemiological indicators provide information to be used in the pork carcase safety assurance framework proposed by the Opinion. This applies particularly in the process of classification of the farms/herds and slaughterhouses according to risk related to a particular hazard as well as the setting of related targets for final carcases. The indicators, either alone or in combination, may be used by risk managers at the national, regional, slaughterhouse or farm/herd level depending on the purpose.

The principles applied in the identification of the appropriate indicators in this reports are as follows:

- For each biological hazard, the prevalence of the agent at key points in the food chain, broken down by risk factors that may be used for risk-based sampling (e.g. type of production system, age of animals) is considered. The key points are those at which risk is first created, primarily on-farm, but also possibly points at which the hazard can enter the food chain (e.g. during transport and slaughter) and where the hazard reservoir occurs (e.g. wildlife);
- The key epidemiological indicator for a given hazard will almost always be the prevalence in the animal population or in the food;
- The identification of a range of risk factors is not, in itself, adequate. The estimation of the impact of these risk factors on public health is required in order to consider the need to amend the meat inspection methods. This is most easily measured by estimating the prevalence of the agent in the populations subject to different levels of exposure to the risk factor.

In this report the following approach is applied to select the harmonised epidemiological indicators (the first Terms of Reference (ToR)):

- The hazard and, when appropriate, its life cycle is described. The current epidemiological situation within the EU, both as regards to animals and humans, is evaluated and the role of pork as the source of human infections is discussed for each hazard.
- For each hazard, the main food chain related to pigs and the risk and risk-reducing factors along the chain, as well as the meat inspection and other risk mitigation strategies are presented. This description includes an identification of possible epidemiological indicators.
- The possible epidemiological indicators are evaluated against selected criteria (i.e. their quality, appropriateness, data availability and feasibility) using a scoring system. The epidemiological indicators that received the highest scores are selected.

Following the selection of the harmonised epidemiological indicators, the available data from the annual reporting in accordance with the Directive 2003/99/EC, as well as from the EU-wide baseline surveys were reviewed for comparable data from the MSs. This comparable data is presented in chapter 6 (the second ToR).

In the cases where no comparable data is available, harmonised monitoring requirements are proposed for each selected epidemiological indicator (the third ToR). These include the definition of the animal population to be targeted, the stage of the food chain where the sampling should take place, type and details of the specimen to be taken, diagnostic or analytical method to be used and a case definition. A general description is provided on how to choose the sampling strategy for each case.

In addition, a case study on the use of the selected harmonised epidemiological indicators for *Trichinella* is presented in Annex 4.

3.2. The biological hazards addressed

The first ToR of the mandate for technical assistance from the EC asks for the harmonised epidemiological indicators to be defined for specific hazards already covered by current meat inspection (such as trichinellosis, tuberculosis, cysticercosis …). In the case of meat inspection of pigs these hazards are *Trichinella*, *Cysticercus* (*Taenia solium*) and mycobacteria.

In addition, according to the first ToR the epidemiological indicators should be defined for possible additional hazards identified in a Scientific Opinion on the hazards to be covered by inspection of meat. The Scientific Opinion on the public health hazards to be covered by inspection of meat (swine) (EFSA, 2011) identifies *Salmonella*, *Yersinia enterocolitica* and *Toxoplasma gondii* as such hazards to be covered by the generic framework for pork (carcase) safety assurance.

4. EPIDEMIOLOGICAL INDICATORS FOR THE BIOLOGICAL HAZARDS

4.1. *Salmonella*

4.1.1. Pathogenesis

Salmonella has long been recognised as an important zoonotic pathogen of economic significance in animals and humans. The genus *Salmonella* is currently divided into two species*: S. enterica* and *S. bongori*. *S. enterica* is further divided into six sub-species and most *Salmonella* belong to the subspecies *S. enterica* subsp. *enterica*. Members of this subspecies have usually been named based on where the serovar or serotype was first isolated. In the following text, the organisms are identified by genus followed by serovar (e.g. *S.* Typhimurium). More than 2,500 serovars of zoonotic *Salmonella* exist and the prevalence of the different serovars changes over time.

Human salmonellosis is usually characterised by the acute onset of diarrhoea, abdominal pain, nausea, and sometimes vomiting, after an incubation period of 12-72 hours. Symptoms are often mild and most infections are self-limiting, lasting a few days. However, in some patients, the infection may be more serious and the associated dehydration can be life threatening. In these cases, as well as when *Salmonella* causes bloodstream infection, effective antimicrobials are essential for treatment. Salmonellosis has also been associated with long-term and sometimes chronic sequelae e.g. reactive arthritis.

The common reservoir of *Salmonella* is the intestinal tract of a wide range of domestic and wild animals which results in a variety of foodstuffs, of both food of animal and plant origin, as sources of human infections. Transmission often occurs when organisms are introduced in food preparation areas and are allowed to multiply in food (e.g. due to inadequate storage temperatures, inadequate cooking or cross contamination of ready-to-eat food). The organism may also be transmitted through direct contact with infected animals or between humans, or from faecally contaminated environments.

In the EU, *S.* Enteritidis and *S.* Typhimurium are the serovars most frequently associated with human illness. Human *S.* Enteritidis cases are most commonly associated with the consumption of contaminated eggs and poultry meat, while *S.* Typhimurium cases are mostly associated with the consumption of contaminated pig, poultry and bovine meat.

In animals, subclinical infections are common. The organism may easily spread between animals in a herd or flock without detection and animals may become intermittent or persistent carriers. Fever and diarrhoea due to *Salmonella* infection are less common in pigs than in cattle, sheep and horses, whereas goats and poultry usually show no signs of infection (EFSA and ECDC, 2011).

4.1.2. Current situation and trends in the EU

Salmonella are responsible for many cases of human illness and in most developed countries, including the EU, they are the second most common cause of bacterial gastro-intestinal illness (EFSA and ECDC, 2011). A total of 108,614 confirmed cases of human salmonellosis were reported in EU in 2009 and the number of cases decreased by 17.4 %, compared to 2008 continuing the statistically significant decreasing trend for the fifth consecutive year. It is assumed that the observed reduction of salmonellosis cases is mainly attributed to successful implementation of national *Salmonella* control programmes in fowl populations, but other control measures along the food chain may have also contributed to the reduction. In foodstuffs, *Salmonella* was most often detected in fresh broiler, turkey and pig meat, on average at levels of 5.4 %, 8.7 % and 0.7 %, respectively. *Salmonella* was rarely detected in other foodstuffs, such as dairy products, fruit and vegetables (EFSA and ECDC, 2011). Within the EU-wide baseline survey on *Salmonella* in slaughter pigs that was carried out in 2006-2007 (EFSA, 2008), the EU observed prevalence of *Salmonella* in the lymph nodes of slaughter pigs at the slaughterhouse level was 10.3 % among the 24 participating MSs. In the same survey, the EU observed prevalence of *Salmonella* on the carcases was 8.3 % within the 13 MSs providing the

information. In the EU-wide baseline survey on *Salmonella* in breeding holdings of pigs in 2008 (EFSA, 2009b) where 24 MSs participated, 31.8 % of the holdings were found to be contaminated with *Salmonella*. However, the MS specific *Salmonella* prevalence varied widely in all the baseline surveys (Tables 11-13).

4.1.3. Pork as a source of infection for humans

There are several routes of transmission for salmonellosis, but the majority of human infections are transmitted to humans through consumption of contaminated food of animal origin. Contaminated pork and derived products, have been implicated in a number of human salmonellosis cases. *S.* Typhimurium is the predominant serotype isolated from humans in Europe and pigs are an important reservoir of this particular serotype (Boyen et al., 2008).

The Biological Hazard Panel has assessed the public health risks from *Salmonella* in pigs and the impact of possible control measures. The assessment suggested that pigs and pork may be responsible for 10 % to 20 % of all human cases of salmonellosis in the EU in 2009, but with differences between countries, and that controlling *Salmonella* more effectively within the pig population would have a direct impact on reducing the number of human cases (EFSA, 2010b).

4.1.4. Risk and risk-reducing factors

Risk factors related to *Salmonella* infections in pigs are summarised by relevant Scientific Opinions from the Panels on Animal Health and Welfare and on Biological Hazards (EFSA, 2005b, 2006b, 2010b). One of the main risk factors for *Salmonella* farm positivity is the introduction of subclinically infected pigs. Also optimal hygiene and management routines are of major importance to minimise the risk of *Salmonella* spread within a farm. These include all-in/all-out systems, rodent control, no access of pets and birds, visitor hygiene, and no close contact with other production animals. The control of *Salmonella* contamination of feed is essential because of the high potential for spread to a large number of farms. Certain types of feed (e.g. pelleted feed versus non-pelleted feed) were associated with an increased risk of *Salmonella*, while others (such as wet feed, and the use of whey) were associated with a reduced risk. Moreover, duration and condition of transport and lairage can significantly increase the risk of *Salmonella* shedding from pigs. High hygiene standards at all slaughtering steps are also essential to control the risk of *Salmonella* contamination within the slaughterhouse.

Berends et al. (1997) showed that there was a strong correlation between the number of live animals that carry *Salmonella* in their faeces and the number of contaminated carcases at the end of the slaughter line. Furthermore they found that about 70 % of all carcase contamination resulted from the animals themselves being carriers, and 30 % because other animals were carriers (i.e. cross-contamination).

4.1.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected for *Salmonella* in pigs (Table 1).

Table 1: Harmonised epidemiological indicators for *Salmonella* in pigs

Indicators (animal/ food category/other)	Food chain stage	Analytical/ diagnostic method	Specimen
HEI 1 *Salmonella* in breeding pigs	Farm	Microbiology (detection and serotyping)	Pooled faeces sample
HEI 2 *Salmonella* in fattening pigs prior to slaughter	Farm	Microbiology (detection and serotyping)	Pooled faeces sample
HEI 3 Controlled housing conditions at farm (both for breeding pigs and fattening pigs)	Farm	Auditing	Not applicable
HEI 4 Transport and lairage conditions (both for breeding pigs and fattening pigs)	Transport and slaughterhouse	Auditing of time, mixing of batches and reuse of pens in lairage	Not applicable
HEI 5 *Salmonella* in fattening pigs – in-coming to slaughter process (evisceration stage)	Slaughterhouse	Microbiology (detection and serotyping)	Ileal contents
HEI 6 *Salmonella* in fattening pigs – carcases after slaughter process before chilling	Slaughterhouse	Microbiology (detection and serotyping)	Carcase swabs
HEI 7 *Salmonella* in fattening pigs – carcases after slaughter process and after chilling	Slaughterhouse	Microbiology (detection and serotyping)	Carcase swabs

The scheme describing the food chain and related risk and risk-reducing factors as well as the evaluation of possible epidemiological indicators is presented in Annex 2.

Microbiological testing of either faeces, ileal content or carcase swabs are the analytical methods proposed for those HEIs related to sampling of pigs or their carcases for *Salmonella* infection or contamination. Microbiological analysis and typing of *Salmonella* spp. will provide data on specific new zoonotic serovars such as monophasic variants of *S.* Typhimurium and new emerging serovars which may go undetected if only serological surveillance systems were in place. Particular *Salmonella* clones of special public health significance (e.g. clones with high virulence or resistance towards antimicrobials deemed critically important for treatment of human infections, but not necessarily related to particular serovars) may be identified. However, this requires all MSs to implement harmonised and standardised methods for identifying such clones.

Testing of ileo-caecal lymph nodes for *Salmonella* is not included because sampling of ileal content is easier in practice and ileal content is a more sensitive indicator of *Salmonella* infection during transport and lairage than the lymph nodes (De Busser et al., 2011).

Serological testing of serum or meat juice samples for detection of *Salmonella* antibodies is a useful tool to complement the assessment of farm management practices and biosecurity levels related to controlled housing conditions on farms (HEI 3). However, the use of serological testing of serum or

meat juice is not proposed as an epidemiological indicator to determine the prevalence of *Salmonella* in either pigs or farms, as currently, the implementation of serological monitoring in pigs by the MSs is partly discouraging (Scott Hurd et al., 2008). The number of samples typically collected may only provide weak evidence of changing *Salmonella* status at an individual farm level (Snary et al., 2010) and it is estimated that in the serological monitoring the number of slaughter pigs from low risk herds by far exceeds the number of pigs from high risk herds due to the low surveillance system sensitivity. Furthermore, serological testing does not provide information on *Salmonella* serovars and clones.

HEI 1 focuses on evaluating the risk of introducing pigs infected with *Salmonella* onto breeding or fattening farms.

HEI 2 focuses on the provision of information on the occurrence of the *Salmonella* and the serovars present on the farm producing fattening pigs. Monitoring of trends in the *Salmonella* status of the farm will be enabled by regular sampling of fattening pigs from the same farm.

HEI 3 focuses on classifying farms on the basis of controlled housing conditions, including bio-security and management practices, in farms by applying auditing techniques. Serological testing of pigs (meat juice) may provide useful additional information on the *Salmonella* status of the farm when combined with auditing biosecurity and management practices.

HEI 4 focuses on the transport and lairage conditions of the pigs. In particular specific aspects such as transport time, mixing of pig batches and reuse of pens in lairage are covered. HEI 4 combined with HEI 2 and HEI 5 will provide information on the influence of transport and lairage conditions on *Salmonella* carriage of the pigs.

HEI 5 focuses on providing an indicator of the *Salmonella* status of pigs entering the slaughter process. The chosen sample specimen will take account of the on-farm conditions, the time spent during transport and transport conditions, the mixing of pig batches from different sources, and the condition and practices in the lairage. The related serotyping results or more detailed typing of isolates will give reliable information of *Salmonella* infections of pigs occurring during transport and lairage. For example, variable number of tandem repeat (VNTR) analysis has been used to differentiate between *Salmonella* strains that have been followed from farm to slaughterhouse and strains which have been isolated in a lairage and a slaughterhouse but not on farms, indicating that reservoirs of infection beyond the farm gate also contribute to carcase contamination risk (Kirchner et al., 2011).

HEI 6 focuses on providing an indicator of the process hygiene on a slaughter line by measuring the presence of *Salmonella* on pig carcase pre-chilling. Sampling is performed prior to chilling rather than after chilling as it is easier to recover and cultivate *Salmonella* bacteria at this point. Active attachments to the carcase and bacterial stress during chilling are two factors that mitigate against sampling post-chilling. By combining the results (especially the obtained serovars) from HEI 5 and HEI 6 it is possible to assess the ability of the slaughter process to influence *Salmonella* contamination of the carcases. The Scientific Opinion (EFSA, 2011) notes that there is a general recognition in the scientific literature that indicator microorganisms are much better suited for use in process hygiene assessment, than pathogenic microorganisms (Bolton et al., 2000; Koutsoumanis and Sofos, 2004; Blagojevic et al., 2011). This is due to the facts that pathogens occur in animals/on carcasses relatively rarely, are affected also by on farm factors, are difficult to count/quantify and require more laborious handling in better equipped laboratories. Pathogen testing is much more valuable for the purposes of consumer exposure assessment and pathogen reduction programmes; so is more related to setting of targets for slaughterhouses.

HEI 7 focuses on providing an indicator of the *Salmonella* status of the carcases after the entire slaughter process (including chilling) has been completed. The microbial levels found at this point in the process reflect the *Salmonella* contamination level entering the food chain from the slaughterhouse. The data derived from monitoring of HEI 7 may be used to set *Salmonella* targets for slaughterhouses as referred to in the Scientific Opinion (EFSA, 2011).

The proposed HEIs give different types of information on the risk of *Salmonella* infection in pigs or contamination of the carcases and risk managers should choose the HEIs to be applied and then also interpret the available information in the appropriate way. The indicators may be used alone or in different combinations.

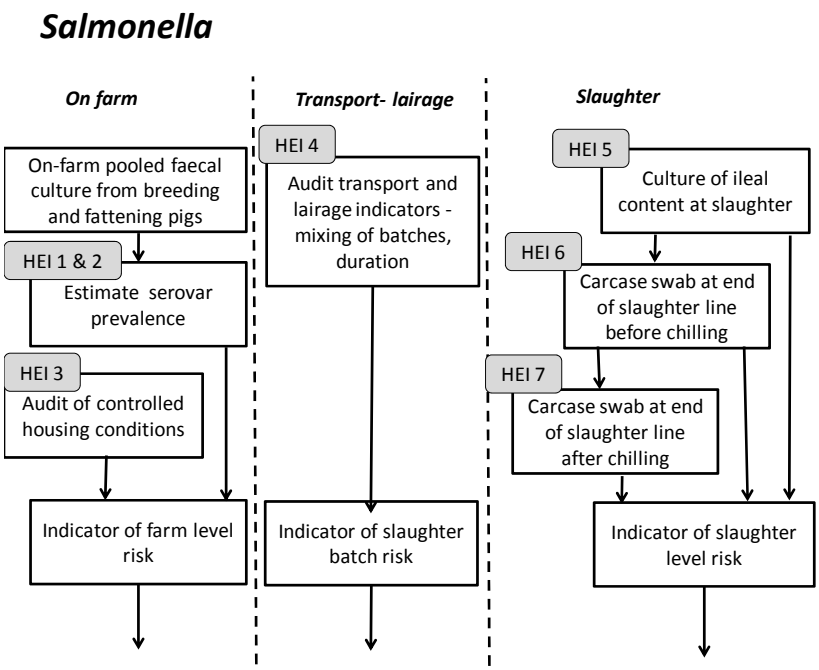


Figure 1: Schematic diagramme illustrating the harmonised epidemiological indicators for *Salmonella* in pigs.

4.1.6. Harmonised monitoring requirements

Animal population

At farm:

- Breeding pigs.
- Fattening pigs.

At slaughterhouse:

- Fattening pigs.

Farms are subject to an audit of the production system standards to define the biosecurity and controlled housing conditions. This covers both farms with breeding and fattening pigs.

Transport of animals to the slaughterhouse and the lairage conditions at the slaughterhouse are subject to an audit of time between the loading of pigs to their slaughter, mixing of pigs from different batches and the reuse of the pens at lairage.

Stage of the food chain

- The farm for breeding and fattening pigs.
- The farm for controlled housing conditions.
- Transport and slaughterhouse for transport and lairage conditions.
- The slaughterhouse for fattening pigs.

Sampling

- HEI 1 and HEI 2
    - Target population: Breeding pigs on farm (HEI 1) and fattening pigs at farm prior to slaughter (HEI 2).
    - Epidemiological unit: The farm.
    - Sampling strategy:
        - On farms with a large number of breeding/ fattening pigs, a representative sample (random or systematic) of all breeding/ fattening pigs. On small farms, in order to achieve the required precision, it may be necessary to use a census of all breeding/ fattening pigs.
    - Sample size: Adequate to assess whether the prevalence is above a threshold defined by the risk manager (calculated as described in Annex 3).
    - Survey interval:
        - Repeated at a frequency (to be determined by risk managers) adequate to characterise the farm risk (in terms of the range of serotypes present).
- HEI 3
    - Target population: All farms claiming to meet the controlled housing conditions
    - Epidemiological unit: The farm.
    - Sampling strategy: Census (all farms claiming to meet the controlled housing conditions should be audited).
    - Audit interval:
        - Repeated at a frequency (to be determined by risk managers) adequate to maintain confidence that farms continue to meet the definition of officially recognised controlled housing.
- HEI 4
    - Target population: All batches of slaughter pigs.
    - Epidemiological unit: The slaughter batch.
    - Sampling strategy: Census (all batches) or representative sample.
    - Audit interval: Audit for every batch or repeated at a frequency (to be determined by risk managers) adequate to characterise the transport, mixing and lairage risks (in terms of the range of serotypes present).
- HEI 5
    - Target population: Carcases of fattening pigs arriving at the evisceration stage of the slaughter line.

- Epidemiological unit: The slaughter batch at the slaughterhouse.
- Sampling strategy:
  - Representative sample (random or systematic).
- Sample size: Adequate to assess the *Salmonella* status of the in-coming pigs in the slaughterhouse, or to assess the difference in prevalence before and after processing (calculated as described in Annex 3).
- Survey interval:
  - Initial survey,
  - Repeated at a frequency (to be determined by risk managers) adequate to characterise the slaughterhouse risk (required particularly when procedures in the slaughterhouse change).

- HEI 6

  - Target population: Carcases of fattening pigs after the slaughter process, prior to chilling.
  - Epidemiological unit: The slaughter batch at the slaughterhouse.
  - Sampling strategy:
    - Representative sample (random or systematic).
  - Sample size: Adequate to assess the *Salmonella* status of the carcases after processing (before chilling), or to assess the difference in prevalence before and after processing (calculated as described in Annex 3).
  - Survey interval:
    - Initial survey,
    - Repeated at a frequency (to be determined by risk managers) adequate to characterise the slaughterhouse risk (required particularly when procedures in the slaughterhouse change).

- HEI 7

  - Target population: Carcases of fattening pigs after the slaughter process, and after chilling.
  - Epidemiological unit: The slaughter batch at the slaughterhouse.
  - Sampling strategy:
    - Representative sample (random or systematic).
  - Sample size: Adequate to assess the *Salmonella* status of the carcases leaving the slaughter process.
  - Survey interval:
    - Initial survey,
    - Repeated at a frequency (to be determined by risk managers) adequate to characterise the prevalence of *Salmonella* positive pork carcases entering the food chain.

Type and details of sample

- Pooled faeces samples at the farm from breeding and fattening pigs (e.g. as foreseen in the EU baseline survey on breeding pigs (EFSA, 2007d)).

- Ileal content samples at the slaughterhouse, directly after evisceration:

  - the ileal contents are pushed together,

  - both ends now containing the ileal content are firmly tied off and closed, and

  - this part of the ileum is removed from the intestinal package. At the laboratory the necessary amount of the ileal content is aseptically collected for analysis.

- Carcase surface samples of pig carcases at the slaughterhouse (e.g. as foreseen in the EU baseline survey on slaughter pigs (EFSA, 2008)).

- Questionnaire-based audit of farm procedures including specific conditions for *Salmonella*.

- Questionnaire-based audit of transport, mixing of batches and lairage including specific conditions for *Salmonella.*

Diagnostic/analytical methods

- Microbiological sampling: ISO 6579 Annex D (ISO, 2007); Detection and serotyping.

Case definition

- Finding of *Salmonella* in a sample.
- Farms found not complying with the controlled housing conditions.
- Transport and lairage found not complying with the agreed conditions.

4.2. *Yersinia*

4.2.1. Pathogenesis

The bacterial genus *Yersinia* comprises three main species that are known to cause human infections: *Yersinia enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis* (plague). The last major human outbreak of *Y. pestis* in Europe was in 1720, and today it is believed to no longer exist in Europe. *Y. pseudotuberculosis* and specific types of *Y. enterocolitica* cause food-borne enteric infections in humans.

Yersiniosis caused by *Y. enterocolitica* most often causes diarrhoea, at times bloody, and occurs mostly in young children. Symptoms typically develop four to seven days after exposure and last an average of one to three weeks. In older children and adults, right-sided abdominal pain and fever may be the predominant symptoms and can often be confused with appendicitis. Other symptoms such as a rash, joint pain and/or bacteraemia may occur. Infection is most often acquired by eating contaminated food, particularly raw or undercooked pig meat. The bacterium is able to grow at +4 °C and makes contaminated refrigerated food a probable source of infection. Untreated water can also transmit the organism.

Yersiniosis caused by *Y. pseudotuberculosis* shows many similarities with the disease pattern of *Y. enterocolitica*. Infections are caused by the ingestion of the bacteria from raw vegetables, fruit or other foodstuffs via water or direct contact with infected animals.

Y. enterocolitica is closely related to a large array of *Yersinia* spp. without any reported public health significance. Within *Y. enterocolitica*, the majority of isolates from food and environmental sources are non-pathogenic types. It is, therefore, crucial that investigations discriminate between which strains are pathogenic for humans. Biotyping of the isolates is essential to determine the pathogenicity to humans, and this method is ideally complemented by serotyping. Pathogenicity can also be determined by PCR methods. In Europe, the majority of human pathogenic *Y. enterocolitica* belong to biotype 4 (serotype O:3) or, less commonly, biotype 2 (serotype O:9, O:5,27). Pigs are considered to be the primary reservoir for the human pathogenic types of *Y. enterocolitica*, mainly for biotype 4 (serotype O: 3). Biotype 2 (serotype O: 9) has also been isolated from other animal species, such as cattle, sheep and goats. Clinical disease in animal reservoirs is uncommon (EFSA and ECDC, 2011).

4.2.2. Current situation and trends in the EU

Human enteropathogenic *Yersinia* belongs to the zoonotic bacteria and humans are mostly infected by contaminated foodstuffs. The most important species is *Y. enterocolitica* and to a much less extent *Y. pseudotuberculosis*. Within *Y. enterocolitica* biotypes 1B, 2, 3, 4 and 5 are pathogenic for human, whereas biotype 1A is considered apathogenic for humans (EFSA, 2007c). The number of notified confirmed human cases of *Yersinia* infections in the EU was 7,595 or 1.65 cases/100,000 inhabitants in 2009, making it the third most often reported zoonotic disease in the EU. Since 2004 a constant decrease in the number of human cases has been observed. Most of the human infections are caused by *Y. enterocolitica*. Among animals and food, findings of *Y. enterocolitica* were mainly reported from pigs and pig meat. On average, 4.8 % of pig meat units were found positive for *Y. enterocolitica* in the reporting MSs group and a high prevalence was reported by two MSs in slaughter batches of pigs (EFSA and ECDC, 2011).

4.2.3. Pork as a source of infection for humans

Pigs are considered the most important reservoir for *Y. enterocolitica* infections in humans. For instance a case-control conducted in Belgium demonstrated a correlation between the consumption of raw minced pork and the prevalence of human yersiniosis (Tauxe et al., 1987). Moreover genetic typing of *Y. enterocolitica* strains indicated that a large number of human strains were undistinguishable from strains present in pig tonsils (Fredriksson-Ahomaa et al., 2001b, 2006).

Slaughter of infected pigs may lead to the contamination of carcases and offal of these pigs, and also to cross-contamination of carcases and offal of subsequently slaughtered pigs (Fredriksson-Ahomaa et al., 2001a; Laukkanen et al., 2009). Important sources for (cross) contamination are the intestinal content and the tonsils. Results from a prevalence study carried out by Van Damme and De Zutter (2011) following individual pigs during slaughter indicated that the medial throat region was the most contaminated site of the carcase (32.8 %), followed by the breast region (17.2 %), medial site just before the sacrum (9.4 %) and the pelvic duct (8.3 %). The percentage of positive tonsils and rectum content of the examined pigs was 57.2 % and 20.0 %, respectively. This study also indicated that cross-contamination between carcases may occur. The number of *Y. enterocolitica* on the different carcase sites was low. At retail pig tongues are frequently contaminated with *Y. enterocolitica* whereas minced pork is less contaminated (Fredriksson-Ahomaa et al., 1999).

4.2.4. Risks and risk-reducing factors

Risk and risk-reducing factors related to *Yersinia* infections of pigs are summarized by a relevant Scientific Opinion from the Panel on Biological Hazards (EFSA, 2007c). Slaughter techniques and slaughter hygiene may influence the contamination rate of carcases and edible offal. Faecal contamination can be considerably reduced by sealing off the rectum with a plastic bag immediately after it has been freed. Since the oral cavity is frequently contaminated, handling the head during slaughter (removal of the tongue, splitting of the carcase and post mortem inspection) may lead to the spreading of the contamination present in this part of the carcase. During cutting, further processing and distribution of fresh pork and offal, *Yersinia* contamination can be spread further.

Knowledge of risk and risk-protective factors contributing to the infection of pigs is rather limited to date. The prevalence of *Y. enterocolitica* in sows seems to be low (Korte et al. 2004; Gürtler et al., 2005). During rearing pigs become colonized after the first two months (Gürtler et al., 2005; Nesbakken et al., 2006). Initially *Y. enterocolitica* is presented in both faeces and tonsils. During aging of pigs the percentage of colonized pigs declines whereas the decrease in the number of positive faeces samples is more pronounced than in tonsils (Nesbakken et al., 2006). Consequently at slaughter age, *Y. enterocolitica* is mostly found in the tonsils and to a lesser extent in rectal material. This pathogen had also been found in other parts of the intestines and in the submaxillary and mesenteric lymph nodes but at a much lower percentage (Nesbakken et al., 2003). In most colonized tonsils high numbers of *Y. enterocolitica* have been detected (Van Damme et al., 2010). Additional results obtained from a single slaughterhouse in France, where 900 individual pigs from 45 batches were sampled, demonstrated that 19.8 % of the individual pigs were positive for pathogenic *Y. enterocolitica* on their tonsils, which corresponded to 80 % of the batches testing positive (Fondrevez et al., 2010). Infection of pigs leads to the production of antibodies. At slaughter age the percentage of pigs having a serological response is much higher than the percentage of pigs carrying *Y enterocolitica* (Nesbakken et al., 2006; von Altrock et al., 2006).

Farming systems (e.g. conventional and organic) seem to have no influence on the prevalence of positive farms, but the prevalence within farms is higher on farms applying the conventional production compared to the organic production system (Nowak et al., 2006; Laukkanen et al., 2009). Within farms the prevalence is very variable, indicating the influence of specific farm factors (Laukkanen et al., 2009). Based on antibodies data in pigs at slaughter, Skjerve et al. (1998) concluded that more finishing farms than farrow-to finish farms were infected with *Y. enterocolitica*.

4.2.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected for *Yersinia enterocolitica* in pigs (Table 2).

Table 2: Harmonised epidemiological indicators for *Yersinia enterocolitica* in pigs.

Indicators (animal/ food category/other)	Food chain stage	Analytical/ diagnostic method	Specimen
HEI 1 *Yersinia enterocolitica* in fattening pigs - in-coming to slaughter process (evisceration stage)	Slaughterhouse	Microbiology (detection and biotyping)	Tonsils or rectal content
HEI 2 Slaughter method: separation of head	Slaughterhouse	Auditing	Not applicable
HEI 3 *Yersinia enterocolitica* in fattening pigs – carcases after slaughter process before chilling	Slaughterhouse	Microbiology (detection and biotyping)	Carcase swabs
HEI 4 *Yersinia enterocolitica* in fattening pigs – carcases after slaughter process and after chilling	Slaughterhouse	Microbiology (detection and biotyping)	Carcase swabs

The scheme describing the food chain and related risk and risk-reducing factors as well as the evaluation of possible epidemiological indicators is presented in Annex 2.

No useful harmonized indicator for *Y. enterocolitica* can be used at the farm level at present. In order to determine the infection status of pigs at the farm level, tonsil samples would be the best. However, for animal welfare reasons, taking such samples routinely from pigs cannot be justified. On the other hand, examination of faeces leads to considerable underestimation of the number of positive pigs at the farm level (Nesbakken et al., 2006). Furthermore, the available data from the literature show that the presence of antibodies can not be directly linked to the presence of *Y. enterocolitica* in pigs to be slaughtered. Consequently, serological testing of slaughter pigs is not a good harmonized epidemiological indicator to detect infected pigs.

The prevalence of human pathogenic *Y. enterocolitica* is higher in fattening pigs at slaughter age than in sows. Therefore, fattening pigs is the group of interest to estimate the entrance of pathogenic *Y. enterocolitica* into the pig meat chain (HEI 1, HEI 3 and HEI 4).

Available data show that the highest prevalence of *Y. enterocolitica* on pork can be found at the slaughterhouse on pig carcases. Further along the chain, the prevalence in pork seems to be much lower. Therefore, focusing on the initial contamination of pig carcases at the slaughterhouse seems to be the most efficient way to collect data on the presence of *Yersinia* in the meat chain. Moreover at this stage the effect of both the status of the slaughtered pigs and the slaughter practice and hygiene applied in the slaughterhouses on carcase contamination can be evaluated. Such data can be used to classify pig slaughterhouses and may contribute to the development of control measures preventing the contamination of pig carcases.

HEI 1 is an indicator of the *Y. enterocolitica* infection status of pigs entering the slaughter process. Depending on the applied slaughter process, either tonsils or rectal content can be sampled. Although no data concerning the impact from both sources on the spread of the *Y. enterocolitica* contamination on pig carcases are published, it can be suggested that according to the possible sources for contamination during slaughter (faecal and tonsils/oral cavity contamination, or only faecal

contamination) different sites on the carcases have to be selected. When separation of the head occurs, contamination of the carcase originating from the tonsils and oral cavity can be expected to be very limited, thus only faecal contamination needs to be taken into account. In the case where separation of the head is not applied, faecal contamination and especially contamination of the tonsils/oral cavity has to be considered. Therefore, the status of the slaughtered pigs is preferentially based on the presence of *Y. enterocolitica* in faecal content and tonsils, respectively.

The purpose of HEI 2 is to indicate the risk and type of *Y. enterocolitica* contamination in the slaughterhouse. The removal of the head would considerably reduce the contamination originating from the tonsils and oral cavity. This practice would result in faeces being the main source of carcase contamination. The Scientific Opinion (EFSA, 2011) observes that *Y. enterocolitica*-risk-reduction hygiene measures include separation of the head from the carcase before head opening/splitting and tongue separation to prevent cross-contamination with *Y. enterocolitica* 'residing' in pig tonsils/lymph nodes/mouth, and its further handling separately from the slaughter line.

HEI 3 indicates the *Y. enterocolitica* contamination load of pig carcases after the slaughter process but prior to chilling. By combining the results from HEI 1 and HEI 3 it is possible to assess the capability of the slaughter line process to control *Y. enterocolitica* contamination of the carcases.

HEI 4 is an indicator of the *Y. enterocolitica* status of the carcases after the entire slaughter process, including chilling of the carcases. This will reflect the *Y. enterocolitica* contamination level entering the food chain from the slaughterhouse. The data derived from implementation of HEI 4 may be used in setting the targets recommended by the Scientific Opinion (EFSA, 2011).

The Scientific Opinion (EFSA, 2011) notes that there is a general recognition in the scientific literature that indicator microorganisms are much better suited for use in process hygiene assessment, than pathogenic microorganisms (Bolton et al., 2000; Koutsoumanis and Sofos, 2004; Blagojevic et al., 2011). This is due to the facts that pathogens occur in animals/on carcasses relatively rarely, are affected also by on farm factors, are difficult to count/quantify and require more laborious handling in better equipped laboratories. Pathogen testing is much more valuable for the purposes of consumer exposure assessment and pathogen reduction programmes; so is more related to setting of targets for slaughterhouses.

The proposed HEIs give different types of information on the risk of *Y. enterocolitica* infection in pigs or contamination of the carcases and risk managers should choose the HEIs to be applied and then also interpret the available information in the appropriate way. The indicators may be used alone or in different combinations.

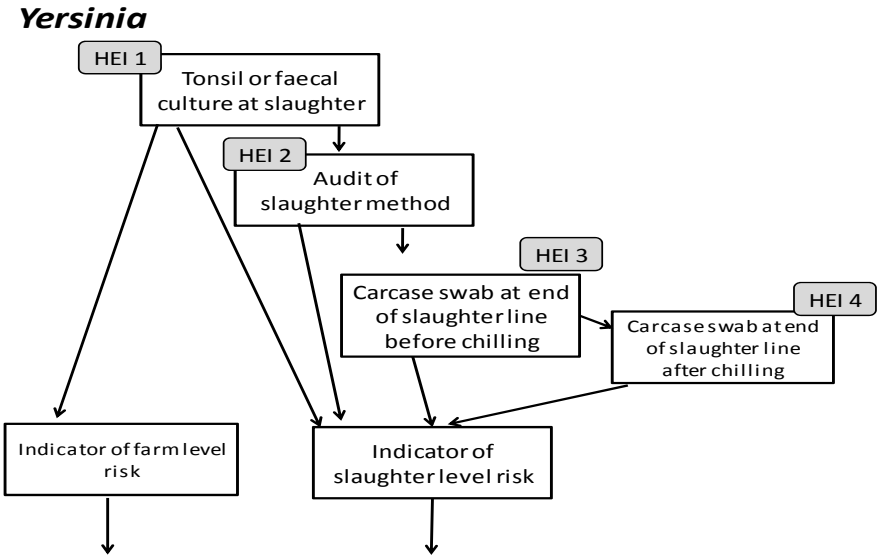


Figure 2: Schematic diagramme illustrating the harmonised epidemiological indicators for *Y. enterocolitica* in pigs.

4.2.6. Harmonised monitoring requirements

Animal population

At slaughterhouse:

- Fattening pigs.

The slaughter process at the slaughterhouse is subject to an audit regarding the separation of the head.

Stage of the food chain

- The slaughterhouse for fattening pigs.
- The slaughterhouse for the slaughter process.

Sampling

- HEI 1
  - Target population: Carcases of fattening pigs arriving at the evisceration stage of the slaughter line.
  - Epidemiological unit: The slaughter batch at the slaughterhouse.
  - Sampling strategy:
    - Representative sample (random or systematic).
  - Sample size: Calculated to enable assessment of the *Y. enterocolitica* status of the in-coming pigs in the slaughterhouse, or the change in prevalence of the pathogen between the start and end of the slaughter process (as described in Annex 3).
  - Survey interval:

  - Initial survey,
  - Repeated at a frequency (to be determined by risk managers) adequate to characterise the slaughterhouse risk (required particularly when procedures in the slaughterhouse change).

- HEI 2

  - Target population: Carcases of fattening pigs.
  - Epidemiological unit: The slaughter batch at the slaughterhouse.
  - Sampling strategy: Representative sample (random or systematic, as described in Annex 3).
  - Audit interval:
    - Initial audit,
    - Follow-up audit to provide assurance of no change in status.

- HEI 3

  - Target population: Carcases of fattening pigs after slaughter and prior to chilling.
  - Epidemiological unit: The slaughter batch at the slaughterhouse.
  - Sampling strategy:
    - Representative sample (random or systematic).
  - Sample size: Calculated to enable assessment of the *Y. enterocolitica* status of the carcasses after processing (before chilling), or the change in prevalence of the pathogen between the start and end of the slaughter process (as described in Annex 3).
  - Survey interval:
    - Initial survey,
    - Repeated at a frequency (to be determined by risk managers) adequate to characterise the slaughterhouse risk (required particularly when procedures in the slaughterhouse change).

- HEI 4

  - Target population: Carcases of fattening pigs after the slaughter process, including chilling.
  - Epidemiological unit: The slaughter batch at the slaughterhouse.
  - Sampling strategy:
    - Representative sample (random or systematic).
  - Sample size: Adequate to assess the *Y. enterocolitica* status of the carcases leaving the slaughter process.
  - Survey interval:
    - Initial survey,
    - Repeated at a frequency (to be determined by risk managers) adequate to characterise the slaughterhouse risk (required particularly when procedures in the slaughterhouse change).

Type and details of sample

- At the stage of evisceration either tonsils or faeces are sampled. With separation of the head, faeces should be sampled, whereas when the tongue is removed during evisceration, tonsils should be sampled.

- For carcase surface sampling, swabs have to be taken from the selected carcases before chilling. Sampling sites have to be sites on the carcases with the possibility of a higher risk for

contamination. This risk will depend on the possible source of contamination. In the case where the tongue is removed during slaughter, both the faecal content and the tonsils/oral cavity may be a source for carcase contamination. However when separation of the head is applied during slaughter only the contamination from faecal content needs to be considered. As a consequence, the following sampling sites are proposed:

- removal of tongues during evisceration: pelvic ductus, belly, brisket and medial masticatory muscle. In cases where the splitting equipment comes into contact with the tonsils/oral cavity the medial side just before the sacrum may also be considered.

- separation of the head: pelvic ductus, belly and brisket.

Where possible at least an area of 100 cm^2 has to be sampled.

- Questionnaire- or inspection-based audit of procedures for separation of the head during slaughter.

Since the proposed type and details of samples are largely based on different hypothesis, more research is needed to collect additional scientific data. Based on such information the present proposal on type and details of sample may have to be modified.

Diagnostic/analytical methods

- The analytical method to be used depends on the sample type to be analysed.

- For the analysis of carcase swabs the cold enrichment has to be applied, since the ISO method for the detection of *Y. enterocolitica* has been proven to be less efficient.

- Tonsils and faecal material are an important source for the contamination of the carcases during slaughter. When the pigs become contaminated with *Y. enterocolitica* during transport, lairage and slaughter, generally only low numbers of *Y. enterocolitica* are found. On the other hand, highly colonized pigs have a high risk as a source of contamination. Therefore detection of pigs with highly colonized rectal content and tonsils is crucial. To detect such pigs direct inoculation of a homogenate of rectal content or tonsillar tissue is sufficient (detection limit of 10 cfu/g tissue). The method is rapid (1 day) and cheap in comparison with methods based on enrichment of the samples. Moreover, for most samples, quantification of *Y. enterocolitica* is possible.

- All isolated *Y. enterocolitica* should be biotyped. Strains belonging to biotypes 1B, 2, 3, 4, 5 are also preferentially serotyped.

Case definition

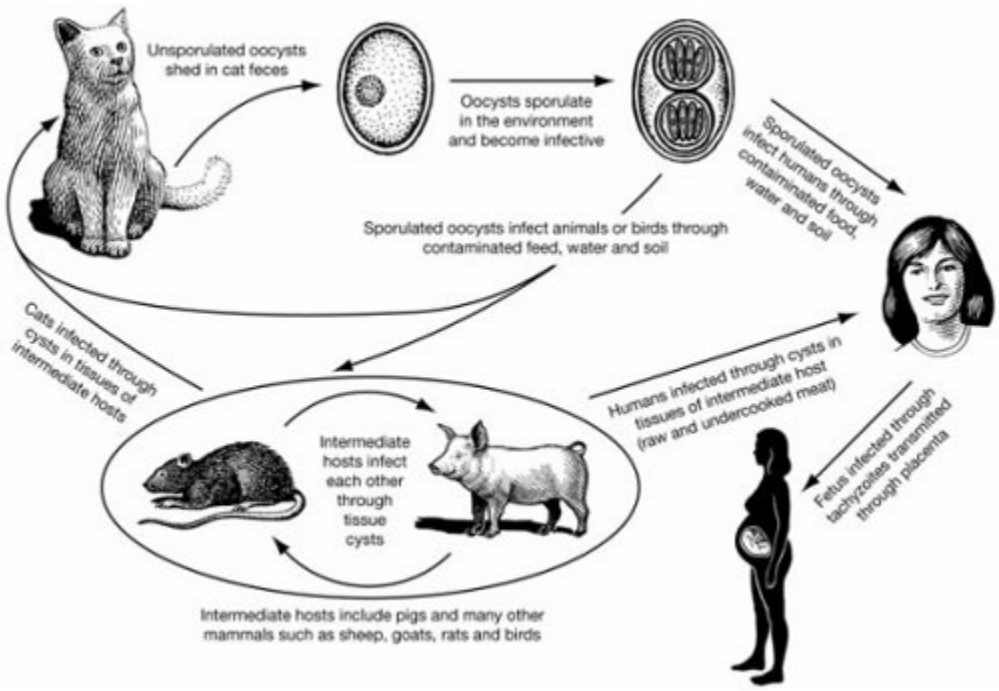
- Finding of at least one pathogenic *Y. enterocolitica* strain (i.e. a strain belonging to biotypes 1B, 2, 3, 4 and 5).
- No separation of head is applied during the slaughter process.

4.3. *Toxoplasma*

4.3.1. Life cycle

Toxoplasmosis is a common infection in animals and humans. It is caused by an obligate intracellular protozoan parasite, *Toxoplasma gondii*. Many species of warm-blooded animals can act as intermediate hosts and, seemingly, most animal species may be carriers of tissue cysts of this parasite. Cats and wild felids are the only definitive hosts that may pass oocysts with their faeces and these needs to sporulate in the environment before becoming infective. The infection may be acquired by humans through the consumption of raw or undercooked meat contaminated with parasite cysts, or food and water contaminated with sporulated oocysts or from handling contaminated soil or cat litter trays. In humans, the majority of infections are asymptomatic or cause mild flu-like symptoms. However, toxoplasmosis can be life threatening, especially for immunocompromised individuals. If acquired during pregnancy, toxoplasmosis can cause abortion or congenital malformation affecting the brain, eyes or other organs of the foetus. In animals, *T. gondii* is an important cause of abortion in sheep and goats, yet it may be controlled by proper management practices and vaccination. The parasite is most frequently reported in cats, dogs, sheep, goats and pigs (EFSA and ECDC, 2011). Severe clinical toxoplasmosis in pigs is considered rare (Dubey, 2010).

T. gondii primarily exists in three forms: oocysts, tachyzoites, and bradyzoites. Oocysts are only produced in the definitive host, members of the family *Felidae*. After oocysts are passed in faeces they sporulate (2-5 days) in the environment and become infective. Once ingested, the oocysts can infect humans and other intermediate hosts. They develop into tachyzoites, which are the rapidly multiplying trophozoite form of *T. gondii*. They divide rapidly in cells, causing tissue destruction and spreading of infection. Tachyzoites in pregnant women are capable of infecting the foetus. Eventually tachyzoites localize to muscle tissues and the central nervous system where they convert to bradyzoites and form tissue cysts. This is thought to be a response to the host immune reaction. Ingestion of cysts in contaminated meat is also a source of infection, as bradyzoites transform back into tachyzoites upon entering a new host. (Modified from http://www.stanford.edu/group/parasites/ParaSites2006/Toxoplasmosis/lifecycle.html).



http://www.omafra.gov.on.ca/english/livestock/swine/facts/04-055.htm

Figure 3: Life cycle of *Toxoplasma*

4.3.2. Current situation and trends in the EU

The epidemiological situation of toxoplasmosis in the EU is summarised in the EU and Community Summary Reports on zoonoses and food-borne outbreaks (EFSA, 2010a; EFSA and ECDC, 2011). Seventeen MSs reported data on human toxoplasmosis in 2009. In total, 1,259 confirmed cases in humans were reported with an EU notification rate of 0.65/100,000 population. Most cases were reported among women aged 24-44 years, most likely as a result of toxoplasmosis screening in pregnant women. In 2009, 18 MSs provided information on *Toxoplasma* in animals. The highest proportions of positive samples were reported in sheep and goats (24.4 %), dogs (15.5 %), and cats (11.0 %), while 5.3 % of the tested bovine animals and 0.4 % of pigs were positive. However, in years 2007 and 2008, 11.4 % and 4.9 % of the tested pigs, respectively, were reported *Toxoplasma* positive. According to the literature, the prevalence of toxoplasmosis in women in the United Kingdom was 8 % (Tenter et al., 2000), whereas in France and Belgium 67 % of the human population was reported to be infected (Pozio, 2003). The prevalence of toxoplasmosis in intensively bred pigs, in countries such as the Netherlands and Germany has been reported to be between 1-10 % (Tenter et al., 2000), whereas if biosecurity standards are low the prevalence could increase up to 51-55 % (Dubey et al., 2005).

4.3.3. Pork as a source of infection for humans

The three major sources of *Toxoplasma* infection for humans are ingestion of food or water contaminated with *Toxoplasma* oocysts from cat faeces, ingestion of infected meat containing tissue cysts, and congenitally from an infected mother to her foetus (Miró et al., 2008). According to the Scientific Opinion from the Panel on Biological Hazards (EFSA, 2007b), when incidences of prenatal infections are compared with seroprevalences in women of childbearing age, it would appear that only a small percentage of infections with *Toxoplasma* in adult human populations are acquired vertically (congenitally). This raises the question of how humans acquire the infection postnatally. Not all possible routes of infection are epidemiologically important, and sources of infection may vary greatly among different ethnic groups and geographical locations. In general, it is believed that the majority of horizontal transmissions to humans are caused either by ingestion of tissue cysts in infected meat, meat-derived products or offal (viscera), or by ingestion of soil, water or food contaminated with sporulated oocysts derived from the environment or, less frequently, directly from feline faeces. However, the relative importance and frequency of horizontal transmissions via tissue cysts versus oocysts in a given population is unknown. In the EU, no comprehensive source attribution studies have been made to investigate the main sources of human toxoplasmosis cases.

Epidemiological studies points to consumption of raw or undercooked minced meat products, mutton and pork to be an important risk factor for infection during pregnancy (Kapperud et al., 1996; Tenter et al., 2000). In the USA, the proportion of human cases that are food-borne has been estimated to be around 50 % (Mead et al., 1999; Schlundt et al., 2004). Whereas Tenter et al. (2000) and Schlundt et al. (2004) estimated that the percentage of meat-borne cases was approximately 30 % to 63 %, depending on eating habits with a higher risk if undercooked meat was consumed, however, raw pork as a source of infection for humans is not known.

The small size of the *Toxoplasma* cysts in meat (100 µm) means that these cysts are impossible to detect in carcases with the current post mortem meat inspection practices. Therefore, toxoplasmosis in pigs is difficult to identify by current meat inspection practices.

4.3.4. Risks and risk-reducing factors

Risk factors related to *Toxoplasma* infections in pigs are summarised by a Scientific Opinion from the Panel on Biological Hazards (EFSA, 2007b). The extent of *Toxoplasma* infection in pigs depends on the farming system with outdoor access leading to a higher prevalence. Specifically, the risk of detecting *Toxoplasma* antibodies has been found to be statistically higher in free-range pig farms than in intensive farms. Organic farms and free-ranging pigs have increased opportunities for contact with

Toxoplasma compared with animals reared indoors, under the assumption that biosecurity measures are implemented and correctly enforced in the latter situation. Furthermore, in several EU countries, seropositivities appeared to increase in older pigs, such as sows, which are usually kept on farms with more extensive management and which are frequently more exposed to the environment than fattening pigs.

The risk of toxoplasmosis in pigs has also been associated with the presence of cats (Assadi-Rad et al., 1995; Villari et al., 2009; García-Bocanegra et al., 2010), the size of the farm – with small farms being more likely to be seropositve (Assadi-Rad et al., 1995; Villari et al., 2009), the level of cleaning and disinfection (Veronesi et al., 2011), and to the use of water from private sources, especially wells (Villari et al., 2009).

4.3.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected for *Toxoplasma* in pigs (Table 3).

Table 3: Harmonised epidemiological indicators for *Toxoplasma* in pigs

Indicators (animal/ food category/other)	Food chain stage	Analytical/ diagnostic method	Specimen
HEI 1 Farms with officially recognised controlled housing conditions (including control of cats and boots)	Farm	Auditing	Not applicable
HEI 2 *Toxoplasma* in breeding pigs from officially recognised controlled housing conditions	Slaughterhouse	Serology	Blood
HEI 3 *Toxoplasma* in all pigs from non-officially recognised controlled housing conditions	Slaughterhouse	Serology	Blood

The scheme describing the food chain and related risk and risk-reducing factors as well as the evaluation of possible epidemiological indicators is presented in Annex 2.

Based on the existing information, it is reasonable to assume that pigs raised in controlled housing conditions in the absence of cats are the least likely to be infected with *T. gondii*. Therefore, testing of fattening pigs under these housing conditions is not selected as an indicator due to expected low prevalence and the high sample size needed. For these reasons, HEI 1 requires the auditing of housing conditions standards at the farm instead.

However, since the length of exposure to possible sources of *Toxoplasma* infection over time (i.e. the age of the pigs) is also a risk factor in the controlled housing conditions, HEI 2 provides an indication of the prevalence in breeding pigs from such housing conditions by serological testing.

Pigs from non-controlled housing conditions will have a greater risk of exposure to *Toxoplasma*. Hence, HEI 3 anticipates serological testing of both breeding and fattening pigs from non-controlled housing farms.

Both HEI 2 and HEI 3 reflect the *Toxoplasma* infection status of the farm where the pigs originate from. The categorisation of *Toxoplasma* infected and non-infected farms may turn out to be

challenging and it may require a continuous testing of pigs, since the *Toxoplasma* status of the farm may change rapidly because of breaches in biosecurity and the possibility of cats entering the holdings.

Serological tests appear to be the most feasible method for testing pigs. The serological tests have a few disadvantages that will require attention, such as a lack of standardisation of techniques, as well as the tests and antigens that have not been officially validated. There is a need to make standard serum samples available. Also, a positive result does not indicate that the muscle sample contains viable parasites.

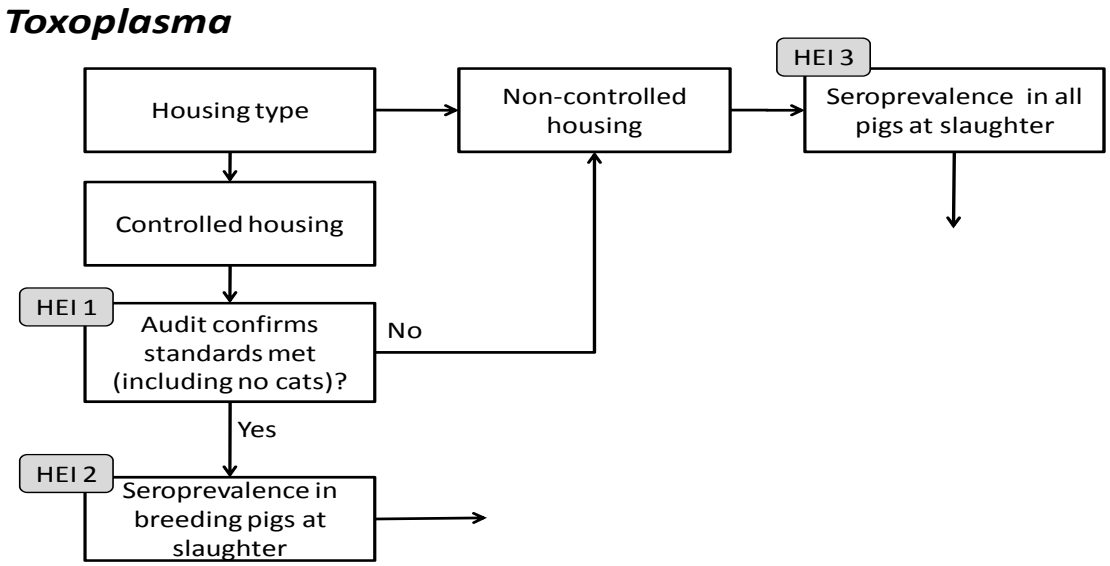


Figure 4: Schematic diagramme illustrating the harmonised epidemiological indicators for *Toxoplasma gondii* in pigs.

4.3.6. Harmonised monitoring requirements

Animal population

At slaughterhouse:

- Breeding pigs from officially recognised controlled housing conditions.
- All pigs from non-officially recognised controlled housing conditions.

Farms are subject to an audit for the controlled housing conditions.

Stage of the food chain

- The farm for an audit for controlled housing conditions.
- The slaughterhouse for breeding pigs from officially recognised controlled housing conditions and for pigs from non-officially recognised controlled housing conditions.

Sampling

- HEI 1
    - Target population: All farms claiming to meet the definition of officially recognised controlled housing for *Toxoplasma*.
    - Epidemiological unit: The farm.
    - Sampling strategy: Census (all farms claiming to meet the controlled housing conditions should be audited).
    - Audit interval:
        - Repeated at a frequency (to be determined by risk managers) adequate to maintain confidence that farms continue to meet the definition of officially recognised controlled housing.
- HEI 2 and HEI 3
    - Target population: Breeding pigs from officially recognised controlled housing (HEI 2) and pigs raised in housing that is not officially recognised as being controlled housing (HEI 3) at slaughter.
    - Epidemiological unit: The farm.
    - Sampling strategy: Random or systematic to achieve a representative sample in the slaughterhouse.
    - Sample size: Calculated with the objective of determining if the prevalence of *Toxoplasma* is less than the threshold prevalence determined by risk managers (as described in Annex 3).
    - Survey interval:
        - Initial one-off survey to determine prevalence in pigs,
        - If prevalence is below threshold repeated at an interval to provide ongoing assurance that the prevalence continues to be acceptable.

Type and details of samples

- From pigs blood samples are collected at slaughtering and the blood is stored at room temperature to allow the blood to clot, then serum is separated and stored at -20 °C until the serological test. The pooling of samples should not be carried out.
- At the farm: questionnaire-based audit of farm procedures including specific conditions for *Toxoplasma* to be agreed by the risk managers.

Diagnostic/analytical methods

- Detection of antibodies to *T. gondii* by blood serum test.
- Tests proposed are based on the ELISA format:
    - ELISA using formalin-fixed whole tachyzoites as antigen (Gamble et al., 2005).
    - ELISA using SAG1 (P30) antigen: commercial kits use native antigen. Recombinant SAG1 antigen is also now available (Chen et al., 2001; Kimbita et al., 2001).

- ELISA using a mixture of recombinant antigens (Holec-Gasior et al., 2010).

The above tests are not officially validated at the EU level.

- Other formats: Bead flow cytometry.
  - New systems using multiplex bead arrays push the potential applications for flow cytometry further. These systems simultaneously measure up to 100 compounds in suspension using very small volumes (Bonetta, 2005).

Case definition

- Findings of *Toxoplasma* antibodies in a blood sample.
- Farms found not complying with the controlled housing conditions.

4.4. *Trichinella*

4.4.1. Life cycle

Trichinellosis (also known as trichinosis) is caused in humans by nematodes (roundworms) of the genus *Trichinella* but animals do not show any clinical signs of the infection. In addition to the classical agent, *T. spiralis*, found worldwide in domestic and wild pigs and seldom in carnivores, three other species of *Trichinella* are now recognized in Europe. *Trichinella spiralis* circulates mainly among domestic and sylvatic pigs and among raccoon dogs, whereas it has been rarely detected in the other carnivores: red fox, wolves, mustelids, and lynx. This species has been detected in 17 MSs. *Trichinella nativa* circulates mainly among carnivores of Nordic MSs. *Trichinella britovi* is the most widespread species infecting mainly carnivores and, to a lesser degree, domestic and sylvatic pigs, and it has been detected in most of the MSs. *Trichinella pseudospiralis*, the only species infecting both mammals and birds, has been detected in 13 MSs (Pozio and Murrell, 2006; Merialdi et al., 2011; International *Trichinella* Reference Centre).

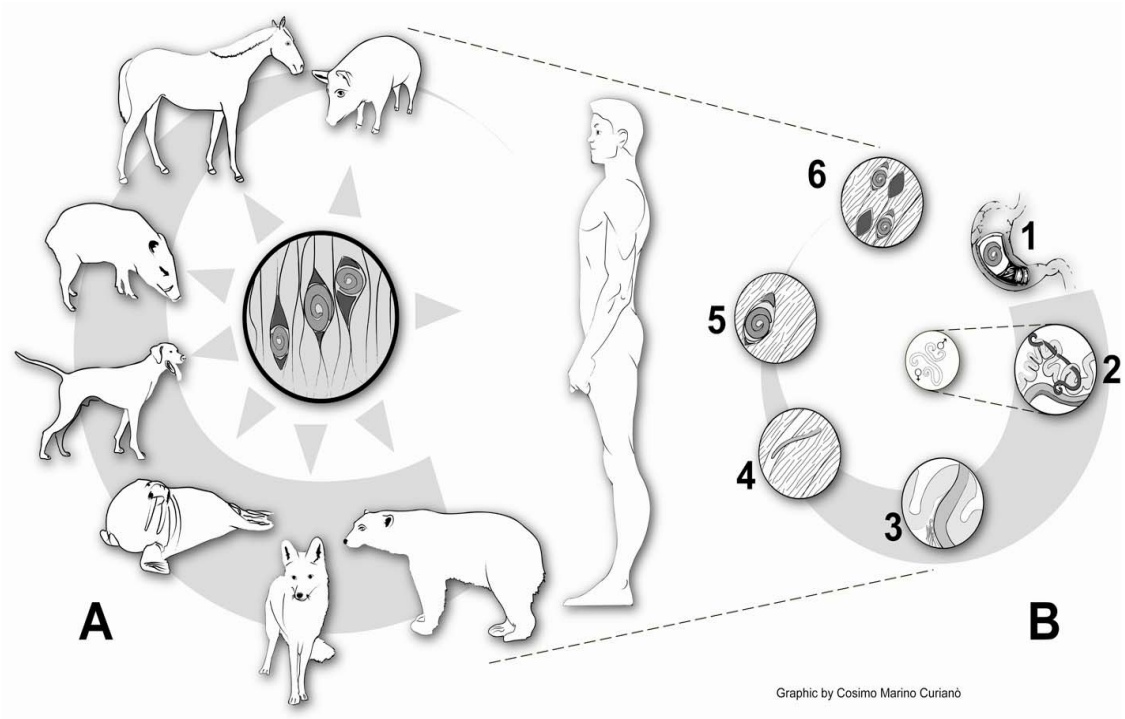


Figure 5: Life cycle of *Trichinella* spp. (Gottstein et al., 2009)

Trichinella spp. life cycle is presented in Figure 5. (A) Main sources of *Trichinella* spp. infections for humans including pigs, horses, wild boars, dogs, walruses, foxes, and bears. (B) *Trichinella* spp. cycle in the host body. In the entrance phase, muscle tissues are digested in the stomach, and larvae are released (1); larvae penetrate the intestinal mucosa of the small intestine and reach the adult stage within 48 hours, and the male and female mate (2); the female worm releases newborn larvae in the lymphatic vessels (3); in the parenteral phase, the newborn larvae reach the striated muscle and actively penetrate into the muscle cell (4); the larvae grow to the infective stage in the nurse cell (the former muscle cell) (5); and, after a period of time (weeks, months, or years), a calcification process occurs (6). (Modified from www.iss.it/site/*Trichinella*/index.asp with permission from the publisher).

The clinical signs of acute trichinellosis in humans are characterised by two phases. The first phase of trichinellosis symptoms may include nausea, diarrhoea, vomiting, fatigue, fever and abdominal discomfort. However, this phase is often asymptomatic. Thereafter, a second phase of symptoms including muscle pains, headaches, fevers, eye swelling, aching joints, chills, cough, itchy skin, diarrhoea or constipation may follow. In more severe cases, difficulties with coordinating movements

as well as heart and breathing problems may occur. A small proportion of cases die from trichinellosis infection. Systematic clinical signs usually appear about 8-15 days after the consumption of contaminated meat (EFSA and ECDC, 2011).

4.4.2. Current situation and trends in the EU

Nematodes of the genus *Trichinella* circulate in wild animals in most EU MSs. The epidemiological situation is summarised in the Community (CSR) and EU Summary Reports (EUSR) on zoonoses and food-borne outbreaks as well as by Alban et al. (EFSA, 2005d, 2006a, 2007a, 2009a, 2010a; EFSA and ECDC, 2011; Alban et al., 2011). In 2009, a total of 748 cases of trichinellosis in humans were notified in the EU. As in previous years, Bulgaria and Romania, accounted for the majority, 89.8 % of cases. *Trichinella* is very rarely detected from pigs in the EU and, from 2007 to 2009, only eight MSs reported *Trichinella* findings from pigs, and most positive pigs were from Romania. In 2009 the prevalence in pigs was 0.0002 % at the EU level. The parasite was more often reported from farmed wild boars, where the overall prevalence was 0.03 % in 2009. Most *Trichinella* findings in MSs have been reported in wildlife, and the reported overall prevalence in hunted wild boars was 0.2 % in 2009 (EFSA and ECDC, 2011). The prevalence of infection in wild animals is highly variable from one country to another according to the environmental conditions, breeding practices, hunters' behaviour, and host species composition. The increasing number of wild boars and red foxes and the spread of the raccoon dog from Eastern to Western Europe may increase the biomass of parasites of the genus *Trichinella* circulating among wild animals (Alban et al., 2011).

During 2004-2009, *Trichinella*-infected pigs were detected in 10 MSs (EFSA, 2005d, 2006a, 2007a, 2009a, 2010a; EFSA and ECDC, 2011; International *Trichinella* Reference Centre) (Table 4). In most MSs *Trichinella* spp. larvae have been detected only in backyard, outdoor or free-ranging pigs. A few MSs have reported positive finding also from pigs reared in controlled housing conditions. However, *Trichinella* spp. larvae have never been detected in pigs reared on farms with officially recognised controlled housing conditions.

4.4.3. Pork as a source of infection for humans

Pork has been traditionally one of the main sources of trichinellosis infections in humans (Pozio and Murrell, 2006). According to the data reported by EU MSs on food-borne outbreaks caused by *Trichinella* during the years 2007-2009, 88 (81 %) from the total of 108 outbreaks were caused by consumption of pork. In the case of outbreaks where more detailed information on the source of infection was provided, the meat, originating from backyard pigs that were not tested for *Trichinella,* was reported as the food vehicle (EFSA, 2009a, 2010a; EFSA and ECDC, 2011). Some of the causes of this epidemiological situation include the reduction of the number of the slaughterhouses in the EU, particularly the small ones, that has made the organisation of the *Trichinella* testing cumbersome for pigs slaughtered at the farm and has resulted in fewer tests. Human trichinellosis caused by the consumption of pork has been documented not only in the MSs that have reported *Trichinella* cases in pigs (Table 5), but also in other MSs. This is mainly due to the importation of non-controlled infected pork (from backyard and/or free-ranging pigs) from EU and third countries or from the ingestion of infected pork abroad and the development of the disease in one of the MS when the infected patient returned home (Murrell and Pozio, in press).

4.4.4. Risk and risk-reducing factors

Risk and risk-reducing factors related to *Trichinella* infections of pigs are summarised by relevant Scientific Opinions from the Panel on Biological Hazards (EFSA, 2005a, 2005b) and by Pozio and Murrell (2006). Infections of pigs occur when there are biosecurity failures, which increase the probability of pigs coming into contact with reservoirs. These include, for example, feeding pigs on food waste that potentially contains pork scraps, or exposure of pigs to carcases of dead pigs or infected wildlife. Pigs raised outdoors have the risk of contact with potentially *Trichinella*-infected wildlife. In pigs raised indoors, the risk of infection is mainly related to the lack of compliance with

rules on the treatment of animal waste. In such farms, infection could also occur due to the breakdown of the biosecurity barriers around the farm, allowing the introduction of infected rodents.

In officially recognised controlled housing conditions where there is no access for wild animals, where pigs are appropriately fed with controlled feed and no pork scraps potentially infected with *Trichinella* are ingested by pigs, these zoonotic parasites do not represent a risk (Table 4).

Table 4: Epidemiological patterns of *Trichinella* infections in pigs in EU MSs

Country	Average number (million heads) of pigs slaughtered per year	Average number (last 5 years) of *Trichinella*-infected pigs per year[(a)]	No. of *Trichinella*-infected pigs officially reported from 2004 to 2009[(b)]	Approximate year of detection of the last *Trichinella* infected pig	Farm origin of infected pigs in the last 10 years
Austria	5.5	0	0	1970	
Belgium	11.1	0	0	1950	
Bulgaria	0.9	20*	90	2011	backyard and free-ranging
Cyprus	0.7	0	0	never	
Czech Rep	3.8	0	0	never	
Denmark	20.7	0	0	1930	
Estonia	0.5	0	0	1999	
Finland	2.4	0	3	2005	backyard
France	25.7	1**	13	2008	free-ranging
Germany	54.8	1**	3	2010	backyard and free-ranging
Greece	1.9	0	0	1984	
Hungary	4.9	1**	0	2009	backyard
Ireland	2.5	0	0	1968	
Italy	13.6	1**	6	2011	free-ranging
Malta	0.1	0	0	never	
Latvia	0.5	0	0	2001	backyard
Lithuania	0.9	10**	43	2011	backyard
Luxembourg	0.1	0	0	never	
Netherlands	14.5	0	0	2005	
Poland	22.3	37**	227	2011	backyard
Portugal	5.9	0	0	1966	
Romania	5.6	700*	2547	2011	backyard, free-ranging, old 'industrial' holdings
Slovakia	1.0	0	4	2008	backyard
Slovenia	0.3	0	0	not available	
Spain	41.3	4**	230	2010	backyard and free-ranging
Sweden	3.0	0	0	1995	
UK	9.4	0	0	1976	
Total	253.9	773 (0.0001 %)***	3,156 (0.0002 %)****		

* no detailed information is available on the pig farm origin; ** infected pigs originating from backyard or organic farms; *** 73 (0.00003 %) positive pigs, excluding Romania, per year; **** 609 positive pigs, excluding Romania, from 2004 to 2009.

(a): Data from the European Union Reference Laboratory for Parasites (http://www.iss.it/crlp)

(b): Data from CSRs and EUSR (EFSA, 2005d, 2006a, 2007a, 2009a, 2010a; EFSA and ECDC, 2011)

Table 5: Trichinellosis cases in humans caused by pork consumption of local pig production or from local hunting activities, documented in EU countries in the last 25 years (1986-2009). Data from Murrell and Pozio (in press)

Country	No. of infections	Source of infection
Bulgaria	5,834	domestic pig, wild boar
Czech Rep	31	domestic pig
Estonia	91	domestic pig, wild boar
France	73	wild boar
Germany	184	domestic pig, wild boar
Greece	3	farmed wild boar
Hungary	165	domestic pig, wild boar
Italy	33	domestic pig
Latvia	636	domestic pig, wild boar
Lithuania	4,636	domestic pig, wild boar
Poland	3,118	domestic pig, wild boar
Romania	30,235	domestic pig
Slovakia	440	domestic pig, wild boar
Slovenia	201	domestic pig
Spain	1,244	domestic pig, wild boar

4.4.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected for *Trichinella* in pigs (Table 6).

Table 6: Harmonised epidemiological indicators for *Trichinella* in pigs

Indicators (animal/ food category/other)	Food chain stage	Analytical/ diagnostic method	Specimen
HEI 1 *Trichinella* in free-range and backyard pigs (both fattening and breeding pigs)	Slaughterhouse	Digestion	Meat
HEI 2 *Trichinella* in pigs from non-officially recognised controlled housing conditions (both fattening and breeding pigs)	Slaughterhouse	Digestion	Meat
HEI 3 Farms with officially recognised controlled housing conditions and *Trichinella* free status[(a)]	Farm	Auditing	Not applicable
HEI 4 *Trichinella* in wildlife (e.g. wild boar, bear, raccoon dog, fox, jackal, wolf, lynx, wild cats, genet, mustelids)	Environment	Digestion	Meat

(a): E.g. according to the Commission Regulation (EC) No 2075/2005[11].

[11] Commission Regulation (EC) No 2075/2005 of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat. OJ L 338, 22.12.2005, p. 60–82.

The scheme describing the food chain and related risk and risk-reducing factors as well as the evaluation of possible epidemiological indicators is presented in Annex 2.

Available data from more than 1.7 billion *Trichinella* tests recorded in the CSRs and EUSR from all MSs since 2000 demonstrate that the risk of *Trichinella* infection is very low (an overall prevalence of 0.0002 % in 2009) (EFSA, 2005d, 2006a, 2007a, 2009a, 2010a; EFSA and ECDC, 2011). Based on this extraordinary number of tests, there has never, in the last 50 years, been a human case of trichinellosis that has been shown to have been caused by consumption of pork raised under conditions that meet the requirements of officially recognised controlled housing.

The lack of *Trichinella* infection in domestic pigs from officially recognised controlled housing conditions in the EU and the sporadic circulation of these parasites, mainly in free-ranging and backyard pigs of less than one quarter of the MSs, determines that, in domestic pigs destined for human consumption, the HEIs are based on a single main risk factor: the type of production system.

HEI 1 and 2 above target domestic pigs of all ages slaughtered for human consumption that do not originate from officially recognised controlled housing conditions. The testing of these pigs takes place in slaughterhouses using the magnetic stirrer method for pooled-sample digestion and would reflect the *Trichinella* status of the farms.

Testing the pigs by serology using an ELISA method is not proposed due to the high number of false positive test results (cross-reactions) caused by the presence of other parasitic infections and the risk of some false negative test results in animals with a low infection level. The use of the western blot method to confirm the ELISA-positive sera is regarded as too expensive. Furthermore, the use of the muscle juice samples instead of serum samples is not a suitable alternative due to the lower concentration (about 10 times less) of antibodies in the muscles than in the serum. However, ELISA tests on pig serum may be useful in monitoring *Trichinella* spp. infection in domestic pigs moved from a lower to a higher status farm i.e. pigs originating from a non-officially recognised *Trichinella*-free farm that are introduced into an officially recognised *Trichinella*-free farm.

Available data demonstrates that the risk of *Trichinella* spp. infection of pigs from officially recognised controlled housing conditions is negligible. For this reason, HEI 3 proposes audits of the housing and biosecurity standards of the farm.

HEI 4 targets wildlife in order to address the risk of transmission of infection from wild animals to pigs. The main animal species covered are wild boars and bears intended for human consumption, since meat from these species is addressed by meat inspection. Other susceptible, carnivore mammal wildlife species can be covered depending on the relevant wildlife population of a country.

The above mentioned HEIs can be used alone or in different combinations depending on the risk manager's decision and the epidemiological situation.

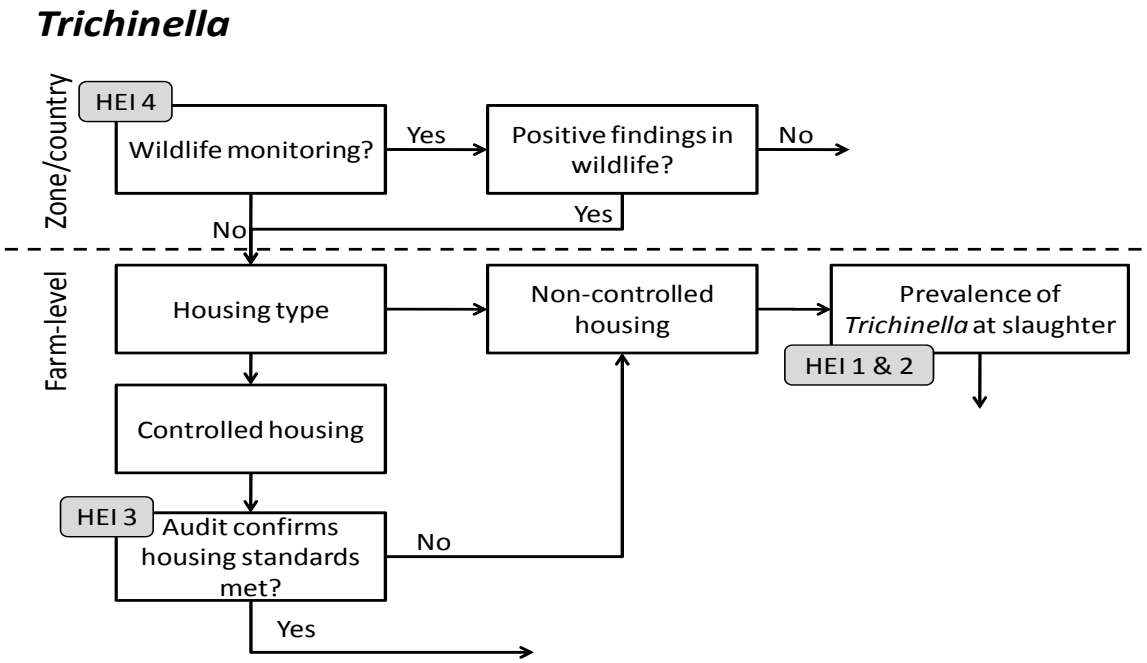


Figure 6: Schematic diagramme illustrating the harmonised epidemiological indicators for *Trichinella* in pigs.

4.4.6. Harmonised monitoring requirements

Animal population

In the environment:

- Wildlife: wild boars, bears and other susceptible wild carnivorous mammals.

At slaughterhouse:

- Free-range and backyard pigs (all ages).
- Indoor pigs from non-officially recognised controlled housing conditions (all ages).

Farms falling under the definition of officially recognised controlled housing conditions are subject to an audit.

Stage of the food chain

- The environment for susceptible wildlife: wild boars, bears and wild carnivorous mammals.
- The farm for auditing officially recognised controlled housing conditions.
- The slaughterhouse for free-range and backyard pigs (both breeding and fattening pigs) and for indoor pigs from non-officially recognised controlled housing conditions (both breeding and fattening pigs).

Sampling

- HEI 1 and HEI 2
    - Target population: Free-range and backyard pigs (HEI 1) and pigs raised in housing that is not officially recognised as being controlled housing (HEI 2) at slaughter (both fattening and breeding pigs).
    - Epidemiological unit: The farm.
    - Sampling strategy: Random or systematic to achieve a representative sample in the slaughterhouse during processing (collection of tissue samples for the digestion test)
    - Sample size: Calculated with the objective of determining if the prevalence of *Trichinella* is less than the threshold prevalence determined by risk managers (as described in Annex 3).
    - Survey interval:
        - Initial one-off survey to determine prevalence in pigs from non-controlled housing,
        - If prevalence is below threshold, repeated at an interval to provide ongoing assurance that the prevalence continues to be acceptable.

- HEI 3
    - Target population: All farms claiming to meet the definition of officially recognised controlled housing.
    - Epidemiological unit: The farm.
    - Sampling strategy: Census (all farms claiming to meet the officially recognised controlled housing should be audited).
    - Audit interval:
        - Repeated at a frequency (to be determined by risk managers) adequate to maintain confidence that farms continue to meet the definition of officially recognised controlled housing.

- HEI 4
    - Target population: Wild boars and other susceptible carnivorous mammals, in a defined geographical area (normally the country, but possibly zones within a country).
    - Sampling strategy: Representative or risk-based wildlife sampling methods as appropriate to the species and country, possibly including:
        - Hunter samples (either passive or structured),
        - Mortality sampling (animals killed by cars or other accidents),
        - Structured sampling (by hunting or trapping),
        - Sentinel herds (groups of identified animals kept in close contact with wildlife).
    - Sample size: Calculated to be adequate to meet a target probability of freedom of *Trichinella* (set by risk managers) on the assumption of a specified design prevalence (also set by risk managers, as described in Annex 3).
    - Survey interval
        - Initial survey when required to demonstrate freedom from *Trichinella* in the wild population,
        - Follow-up surveys to provide ongoing assurance of freedom. The interval will be determined largely by the risk of introduction of new infection (i.e. more frequent where it is hard to control access of wildlife from potentially infected areas, less frequent on islands or other controlled areas).

Type and details of samples

- Muscle samples according to Commission Regulation (EC) No 2075/2005.
- At the farm: questionnaire-based audit of farm procedures including specific conditions for *Trichinella* laid down in Commission Regulation (EC) No 2075/2005.

Diagnostic/analytical methods

- Muscle digestion method according to Commission Regulation (EC) No 2075/2005.
- Preparation of muscle specimens according to Annex 1 of Commission Regulation (EC) No 2075/2005.

Case definition

- Finding of *Trichinella* spp. larvae from a meat sample.
- Farms found not complying with the controlled housing conditions.

4.5. *Cysticercus*

4.5.1. Life cycle

Helminth cestodes cover *Cysticercus cellulosae* (tapeworm: *Taenia solium*), which causes 'Measly Pork' disease. Cysticercosis in pigs is caused by the metacestode larval stage of *Taenia solium* (the pork tapeworm) and *Taenia hydatigena* (non-zoonotic). *T. solium* has humans as definite hosts and domestic pigs as intermediate hosts. Humans can also become infected with the larval stage (cysticercosis) following ingestion of *Taenia* eggs. The lifecycle occurs worldwide in rural areas of developing countries, where pigs have access to human faeces (free roaming pigs, open air defecation, poor sanitation) and where pork is consumed. Human (taeniasis) to human (cysticercosis) infection can occur in areas outside the endemic regions (where the human-pig cycle occurs) and in non-pork consumers (e.g. Muslims, vegetarians) (Murrell et al., 2005).

Life cycle of the pork tapeworm is shown in Figure 7 (Murrell et al., 2005):

- Pigs get infected by eating human stools, or feed or drinking water, contaminated with eggs excreted in faeces from a human tapeworm carrier.
- Eggs, containing the oncosphere larval stage, hatch in the pig's small intestine; the oncosphere penetrates the gut wall and migrates to the muscles and organs.
- Once established, the oncosphere develops into a fluid-filled bladder form called a cysticercus. Intramuscular sites are the most common places for cysticerci to develop in the pig.
- The cysticerci become visible within 2 to 4 weeks post-infection and reach their full size in 60 to 70 days. The cysticerci may remain infective in the pig from a few months up to two years.
- Cysticerci are passed from pigs to humans via raw, undercooked pork or pork that is insufficiently cured to inactivate the parasite. Once in the human intestinal tract, they mature into adult tapeworms. Humans are the only host in which an adult tapeworm develops (definite host).
- The tapeworm attaches to the human intestine by its 'head', called a scolex. The tapeworm grows to a length of 2 to 7 metres in 5 to 12 weeks.
- Most of the tapeworm consists of a chain of 700 to 1,000 segments or proglottids. Each segment at the hind end of the chain (gravid proglottids) is packed with up to 40,000 eggs. These egg-filled segments separate from the chain and are passed in the faeces.

A particular characteristic of the pork tapeworm – in contrast to the beef tapeworm, *T. saginata* – is that humans can also be infected by accidentally ingesting tapeworm eggs from human faeces, and develop cysticercosis. In these cases, the cysticerci develop in the muscles, heart, subcutaneous tissue, eye and brain. Cysticercosis of the brain is called neurocysticercosis (NCC), the most important parasitic infection of the human brain.

The adult pork tapeworm can live for several years, producing thousands of eggs daily. One tapeworm can shed up to 300,000 eggs per day. Most patients harbour only one tapeworm (solitary worm/ *vers solitaire*). Infection with the adult tapeworm (taeniasis) in humans usually results in very mild clinical symptoms or no symptoms at all. Cysticercosis in man can lead to severe neurological conditions, of which epilepsy is the most common (neurocysticercosis).

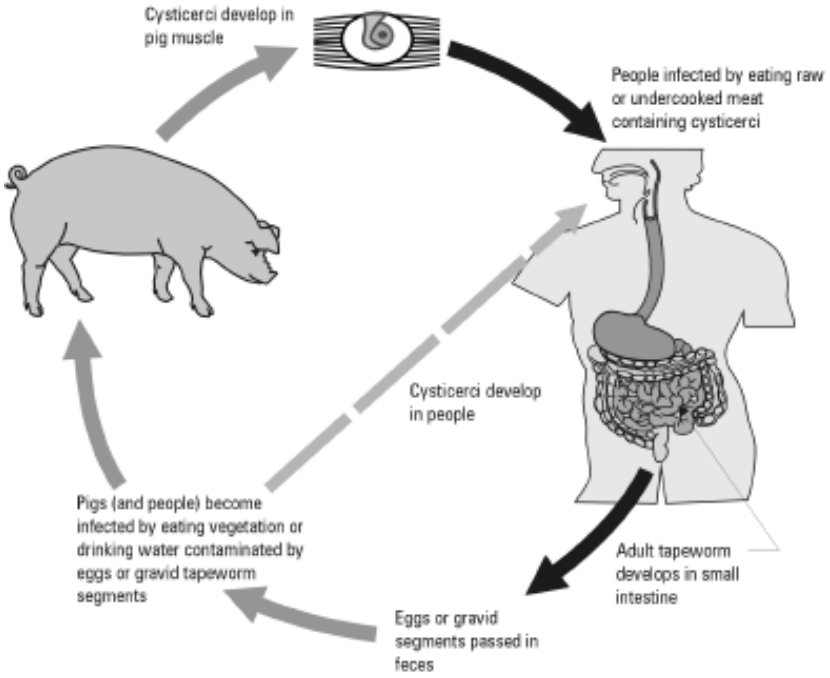


Figure 7: Life cycle of *Cysticercus (Taenia solium)*
(modified from www.dpd.cdc.gov/dpdx/HTML/Cysticercosis.htm)

Pigs usually do not show signs of cysticercus infection. A cysticercus infection (commonly referred to as measly pork or pork measles) is usually only found when the meat is inspected. Cysticerci are found chiefly in the muscles of the swine heart, tongue, head, legs, thigh and neck, but can occur in other parts of the body, such as the brain. A cysticercus is a transparent fluid filled cyst, which contains a single inverted scolex. The cyst can be enclosed by action of the immune system. Degenerated cysts can have a gaseous or calcified appearance.

4.5.2. Current situation and trends in the EU

T. solium has been eradicated in most countries in Europe as a result of improved sanitation, modern pig production and meat inspection, but is still very common in developing countries of Latin America, Africa and Asia where pigs are raised and pork is consumed. In these areas, neurocysticercosis is reportedly responsible for one third of acquired epilepsy cases (Ndimubanzi et al., 2010). Occasional cases of human *T. solium* infections or cysticercosis (neurocysticercosis) are reported in some MSs (Wiegand et al., 1999; Plonka, 2000, 2001; Overbosch et al., 2002; Plonka and Waloch, 2002). Often a history of residing in an endemic country is reported (immigrants, travel) (Esquivel et al., 2005; Chianura et al., 2006). As mentioned earlier, human-to-human transmission might occur, as has been demonstrated in the USA (Wallin and Kurtzke, 2004). Cysticercosis and taeniasis are not notifiable diseases in humans. The incidence of taeniasis can be extrapolated from the sales of taenicidal drugs. However, *T. solium* and *T. saginata* (the beef tapeworm, which is still present in most MSs) are usually not differentiated (eggs of both species are similar; expelled proglottids of the worm have to be stained or subjected to molecular methods for differentiation) (Murrell et al., 2005).

In the CSRs and EUSR on zoonoses (EFSA, 2010a; EFSA and ECDC, 2011) only a few MSs have provided information on cysticercosis in pigs. None on these countries reported findings of cysticerci caused by *T. solium*, but two countries reported a few findings of unspecified cysticerci and cysticerci due to *T. hydatigena.*

T. hydatigena is a tapeworm of dogs (final host), and ruminants (goats, sheep, cattle) and pigs (intermediate hosts). It is not a zoonosis. The parasite may cause some losses in the livestock industry as a result of downgrading of carcases and disease caused by the migrating juvenile worms. The large cysticerci establish in the abdominal cavity, often next to or partially embedded in the liver. The infection occurs in conditions where infected offal is fed to dogs.

4.5.3. Pork as a source of infection for humans

Pork is the only source for acquiring *T. solium* taeniasis in humans. Other tapeworm species are acquired by eating beef (*T. saginata*) or fish (*Diphyllobothrium latum*). Infection with *T. solium* in wild boars has been reported (Solaymani-Mohammadi et al., 2003), but as humans are the only final host, a sylvatic cycle is very unlikely.

4.5.4. Risk and risk-reducing factors

Infection of taeniasis in humans is associated with consumption of raw or undercooked pork from pigs that are free roaming and/or have had access to human faeces. The lack of meat inspection is another risk factor, although only heavily infected carcases will be detected at slaughter, and lightly infected carcases might pass inspection unnoticed (Dorny et al., 2004). All pigs are subjected to visual inspection of the carcase and the organs at slaughter under the current meat inspection rules (Regulation (EC) No 853/2004).

The lifecycle can only occur in conditions where pigs have access to human faeces (outdoor free range, defecation in the pigsty), are fed with contaminated feed or drink from contaminated water, and where open defecation occurs. Cases in pigs are usually clustered around a tapeworm carrier.

Raising pigs indoors with proper sanitation has proved to be effective and sustainable. This has been responsible for complete elimination of *T. solium* in Western Europe, where the parasite used to be common until the early 20th century and it has not re-emerged (Murrell et al., 2005).

4.5.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicator has been selected for *Cysticercus* (*Taenia solium*) in pigs (Table 7 and Figure 8).

Table 7: Harmonised epidemiological indicators for *Cysticercus* in pigs

Indicators (animal/ food category/other)	Food chain stage	Analytical/ diagnostic method	Specimen
HEI 1 *Cysticercus* cysts in pigs (both fattening and breeding pigs)	Slaughterhouse	Visual meat inspection + PCR for confirmation.	Meat

The scheme describing the food chain and related risk and risk-reducing factors as well as the evaluation of possible epidemiological indicators is presented in Annex 2.

The key question in the selection of the HEI was whether or not active transmission of porcine cysticercosis is still occurring in EU MSs. Classical meat inspection should indicate whether there is active transmission. Only the presence of the parasite in pigs indicates active infection; human cases of cysticercosis/taeniasis often are imported from endemic countries. Abnormally high numbers of human cysticercosis cases may indicate active transmission but can also be due to infection from a tapeworm carrier to family members or neighbours (Schantz et al., 1992). Although the sensitivity of meat inspection is low in mild infections (20-25 %) (Dorny et al., 2004), massively infected pigs are

easily detected at slaughter. In endemic areas, both heavily infected and lightly infected carcases may occur (Dorny et al., 2004).

Due to apparent absence or very low prevalence of cysticerci due to *T. solium* in pigs in the EU, a single HEI covering surveillance of all slaughtered pigs is proposed. Such surveillance would enable the detection of any emergence of the parasite in pig populations. The HEI is based on visual inspection of the pig carcases at slaughter and confirmation of the presence of the parasite in suspicious lesions/cysts by molecular analysis.

Serology, aimed at the detection of specific antibodies or circulating antigen, is not recommended because it is unlikely that active transmission of *T. solium* still occurs in MSs and the sensitivity and specificity of the serological tests have limitations. Most tests do not differentiate infections with *T. solium* from *T. hydatigena*. Therefore, both the positive and negative predictive values of these tests in a situation of very low prevalence/absence of infection are low.

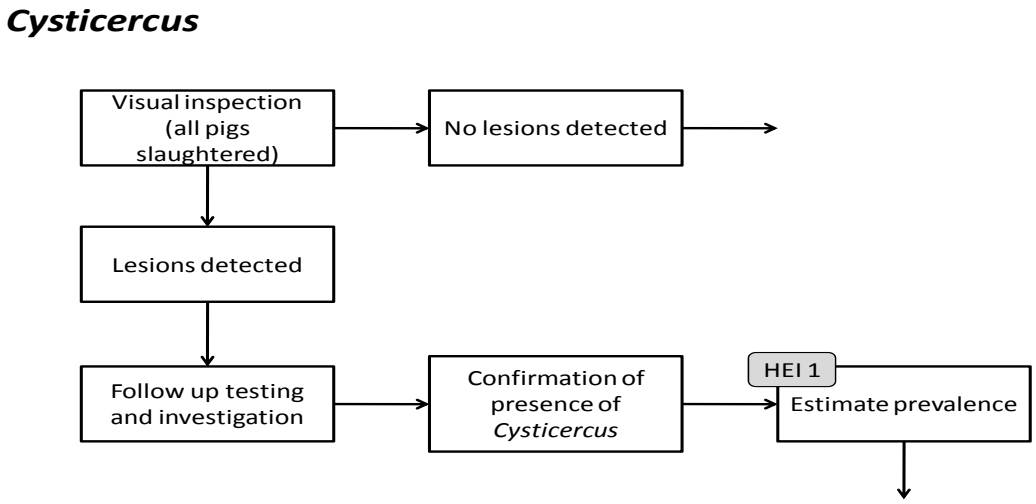


Figure 8: Schematic diagramme illustrating the harmonised epidemiological indicators for *Cysticercus* (*Taenia solium*) in pigs

4.5.6. Harmonised monitoring requirements

Animal population

- All pigs at slaughter.

Stage of the food chain

- At the slaughterhouse.

Sampling

- HEI 1
    - Target population: All pigs.
    - Epidemiological unit: The farm.
    - Sampling strategy:
        - Initial visual inspection: Census – all pigs visually inspected,
        - Suspect lesions: All suspect lesions followed up with further investigation.
    - Survey interval:
        - Ongoing inspection as part of routine meat inspection,
        - Periodic (e.g. annual) assessment of prevalence to be compared to threshold prevalence values determined by risk managers. If the prevalence exceeds the threshold, modifications to meat inspection may be introduced.

Type and details of samples

- Suspected lesion/cyst (viable, degenerated or calcified), isolated from host tissue. The sample is to be stored in a 70 % ethanol solution at room temperature.

Diagnostic/analytical methods

- Report whether small cysticerci are found visually in the muscles or brain (*T. solium*), or large cysticerci (*Cysticercus tenuicollis*, several centimetres diameter) in the abdominal cavity or in/on the liver. MSs should differentiate between *T. solium* and *T. hydatigena*. Suspected *T. solium* cysts can be fixed in ethanol until analysis by a molecular method.

- Suspected lesions have to be confirmed by molecular methods (PCR-RFLP or multiplex PCR to identify *Taenia* species).

- Diagnostic/analytical method to be used: PCR (cox-1 gene, HDP2, mitochondrial 12S rDNA fragment) - multiplex-PCR or PCR-RFLP (Rodriguez-Hidalgo et al., 2002; Yamasaki et al., 2004; González et al., 2010).

- Preparation of specimen in the laboratory: DNA extraction (Boom extraction or commercial kit).

Case definition

- Findings of suspected lesion/cyst from which *T. solium* DNA can be amplified by a molecular method.

4.6. Mycobacteria

4.6.1. Pathogenesis

Tuberculosis is a serious disease of humans and animals caused by the bacterial species of the family *Mycobacteriaceae*, more specifically by species of the *Mycobacterium tuberculosis* complex (MTC). This group includes *Mycobacterium bovis* responsible for bovine tuberculosis. This agent is also capable of infecting a wide range of warm-blooded animals, including humans and pigs. In humans, infection with *M. bovis* causes a disease that is very similar to infections with *M. tuberculosis*, the primary agent of human tuberculosis. Furthermore, the recently defined *M. caprae* also causes tuberculosis among animals, and to a limited extent in humans.

The main transmission routes of *M. bovis* to humans are through contaminated food (especially raw milk and raw milk products) or through direct contact with infected animals. Several wildlife animal species, such as deer, wild boars, badgers and the European bison, might contribute to the spread and/or maintenance of *M. bovis* infection in cattle (EFSA and ECDC, 2011).

Other mycobacteria occasionally produce disease clinically indistinguishable from tuberculosis. *Mycobacterium avium* complex (MAC) was recognised as the most common opportunistic bacterial infection in patients with acquired immunodeficiency syndrome (AIDS) (Cook, 2010). MAC includes eight mycobacteria species and several subspecies with different degrees of pathogenicity, host preference and environmental distribution (Álvarez et al., 2011). *Mycobacterium avium* subsp. *avium* (MAA) is a potential zoonotic pathogen that belongs to MAC. Pigs may be a reservoir of MAA, although other sources have not been excluded (Komijn et al., 1999).

In humans, lymphadenitis due to non-tuberculous mycobacteria (NTM) primarily affects children and is caused by a variety of NTM, although *M. avium* predominates (van Ingen et al., 2010). In addition, other mycobacteria (e.g. *M. kansasii*, *M. xenopi*, *M. malmoense, M. avium* subsp. *hominisuis*) can cause NTM infections (Cook, 2010). Although *M. avium* subsp. *hominisuis* can infect a wide variety of animals, the pig is its primary animal host species, causing granulomatous lesions mainly in lymph nodes of the digestive tract, which can reduce the value of carcases (Álvarez et al., 2011).

4.6.2. Current situation and trends in the EU

In the CSRs and EUSR on zoonoses in 2004-2009 (EFSA, 2005d, 2006a, 2007a, 2009a, 2010a; EFSA and ECDC, 2011), 11 MSs have reported some findings of mycobacteria from pigs at slaughter. The most frequently isolated species were *M. bovis* (40 % of the isolates), *M. avium* complex (11 %), *M. caprae*, (0.4 %), and *M. tuberculosis* (0.1 %).

Bovine tuberculosis eradication programmes have had a direct influence on reducing the infection rate in pigs because of decreased exposure to *M. bovis.* In 2009, 0.45 % of cattle herds were reported as being infected or positive for *M. bovis* in the EU (EFSA and ECDC, 2011). Thus, in the EU, the current risk of *M. bovis* infection of pigs comes from wildlife (Naranjo et al., 2008). Indeed, reports of wild boars infected with *M. bovis* have increased in recent years in several MSs, e.g. in Spain, Italy, Portugal, and France (Zanella et al., 2008; Zanetti et al., 2008; Santos et al., 2009; Boadella et al., 2011), or in the case of the United Kingdom (Foyle et al., 2010) are incidental findings. In addition, *M. bovis* infected badgers in enzootic areas are of great concern in some MSs such as the United Kingdom.

In Europe, studies focusing on the prevalence of granulomatous lymphadenitis cases in pig carcases are not numerous, and most of them are quite old (Desmecht, 1978; Gill et al., 1981, 1982). More recently, Komijn et al. (2007) detected that prevalence of granulomatous lesions in lymph nodes of slaughter pigs was 0.75% in two slaughterhouses in the Netherlands. However, these lesions were associated with the isolation of *Rhodococcus equi*. Deshaies and Desrosiers (2001) reported a prevalence of 0.33 % for granulomatous lymphadenitis among slaughter pigs in Quebec in Canada.

4.6.3. Pork as a source of infection for humans

The genus *Mycobacterium* includes several species that cause tuberculous infections in humans and other animals. In animals latent infections are more common than clinical infections (Boschiroli and Thorel, 2010). *M. bovis* is the cause of a serious zoonotic diseases and it is present in numerous countries (Humblet et al., 2010).

According to the Scientific Opinion (EFSA, 2011) there is currently no evidence of pork-related transmission of mycobacteria to humans (Brown and Tollison, 1979; Offermann et al., 1999; Waddell et al., 2008), as human infection occurs *via* other foods (i.e. milk) or *via* animal environment (direct contact/inhalation).

4.6.4. Risk and risk-reducing factors

Pigs are susceptible to MAC, *M. bovis* and *M. tuberculosis* infections. Infection with any of these mycobacteria can give rise to generalized tuberculosis in pigs and can present a serious risk to public health (mainly an occupational disease). The main infection route for pigs is through the digestive system, by ingesting milk or milk products, contaminated wastes of cooking or slaughterhouses, faeces of tuberculous poultry or cattle. The primary infection complex is observed in retropharyngeal and submandibular, or intestinal and mesenteric lymph nodes. Lesions are mostly often limited to the primary complex. Chronic lesions are not confined to a unique organ, like in cattle. The disease is less frequent in young animals than in adult pigs, but the trend to generalization is greater in young animals (Boschiroli and Thorel, 2010).

Considering the current status of MSs regarding *M. bovis*, the main risk factor for domestic pigs regarding mycobacteria infection is the contact with wildlife (outdoor herds and free-ranging pig herds including farmed wild boars). The main risk-reducing factor consists in applying correct biosecurity measures (e.g. use of fences).

4.6.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicator has been selected for mycobacteria in pigs (Table 8).

Table 8: Harmonised epidemiological indicators for mycobacteria in pigs

Indicators (animal/ food category/other)	Food chain stage	Analytical /diagnostic method	Specimen
HEI 1 Human pathogenic mycobacteria in pigs at slaughter	Slaughterhouse	Visual meat inspection + Microbiology[(a)]	Suspected lesions

(a): Detection of the agent from lesions detected through visual inspection.

The scheme describing the food chain and related risk and risk-reducing factors, as well as the evaluation of possible epidemiological indicators, is presented in Annex 2.

Due to very low prevalence of zoonotic mycobacteria in pigs in the EU, a single HEI covering surveillance of all slaughtered pigs at the slaughterhouse is proposed. This would enable surveillance for detection of emergence of mycobacteria infections in pig populations. The HEI is based on visual inspection of pig carcases at slaughter and confirmation of the presence of the bacteria in suspicious lesions by microbiological testing.

Considering some limitations of the serological testing, such as lack of sensitivity, specificity and the poor detection of more advanced clinical cases, serological testing was not proposed in the HEI.

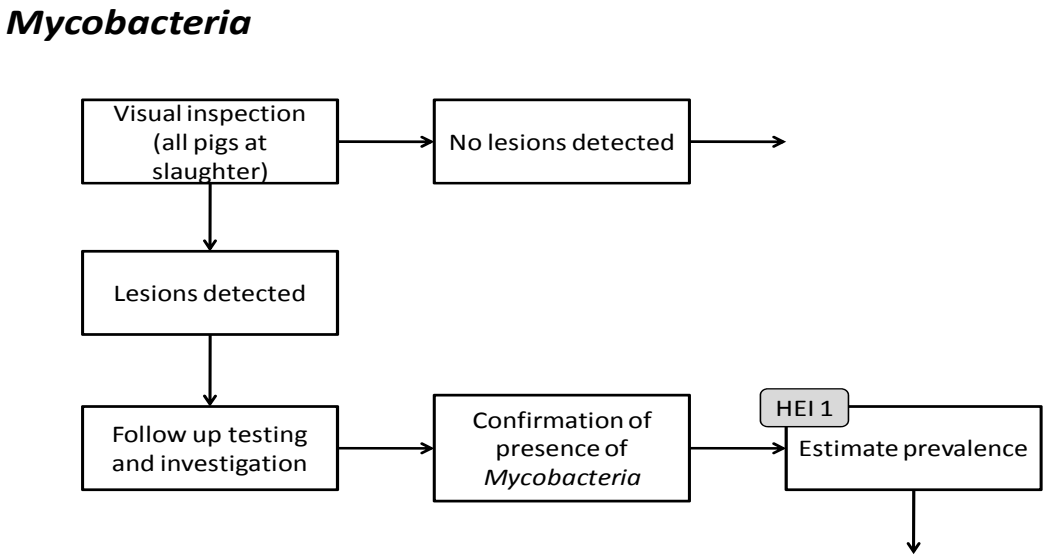


Figure 9: Schematic diagramme illustrating the harmonised epidemiological indicators for mycobacteria in pigs

4.6.6. Harmonised monitoring requirements

Animal population

- All pigs at slaughter.

Stage of the food chain

- At slaughterhouse.

Sampling

- HEI 1
    - Target population: All slaughter pigs.
    - Epidemiological unit: The farm.
    - Sampling strategy:
        - Initial visual inspection: Census – all pigs visually inspected,
        - Suspect lesions: All suspect lesions followed up with further investigation.
    - Survey interval:
        - Ongoing inspection as part of routine meat inspection,

- Periodic (e.g. annual) assessment of prevalence to be compared to threshold prevalence values determined by risk managers. If the prevalence exceeds the threshold, modifications to meat inspection may be introduced.

Type and details of samples

- All suspected lesions observed during the visual meat inspection are sampled and sent to a diagnostic laboratory for subsequent investigation.

Diagnostic / analytical methods

- Methods used are microscopy, Ziehl-Neelsen staining, culture and molecular characterization for epidemiological purposes, such as restriction fragment length polymorphism (RFLP), spoligotyping and/or mycobacterial interspersed repetitive unit - variable-number tandem repeat (MIRU-VNTR).

Case definition

- Finding of *Mycobacterium* species known to be a human pathogen in suspected lesion.

5. SAMPLING STRATEGIES TO BE USED WHEN ESTIMATING EPIDEMIOLOGICAL INDICATORS

The sampling strategy or plan describes the methodology used for selecting the sample from the population (EFSA, 2006c). The strategy should be aligned to the objectives of the surveillance (representative or risk-based), the population of interest, as well as the constraints of the environment in which sampling is to be undertaken. This section provides a number of examples of different sampling strategies that may be used in the collection of data for HEIs (Table 9).

Details on methods for calculating appropriate sample sizes are described in Annex 3.

Table 9: Examples of sampling strategies for different sampling locations and populations of interest

Sampling location	Possible units of interest	Example sampling strategies
Slaughterhouse	Slaughterhouse (e.g. *Salmonella*) Farm (e.g. *Toxoplasma*) Region / country (e.g. *Trichinella*)	• Plant input: systematic • Plant output: systematic • Systematic • Stratified, multi-stage random
On-farm	Farm (e.g. *Salmonella*)	• Grouped random • Systematic • Two-stage
Wildlife	Region / country (e.g. *Trichinella*)	• Hunter samples • Structured field sampling (shooting) • Mortality sampling • Sentinel holdings

5.1. Slaughterhouse sampling

The objective of sampling in slaughterhouses for HEIs is primarily to estimate prevalence, and therefore representative sampling of the slaughter population is required. The most common sampling strategy to be used in a slaughterhouse is systematic sampling, but stratified multi-stage sampling strategies can also be used.

Systematic sampling involves selection of individuals at regular intervals from an ordered population[12]. The population of a slaughterhouse is intrinsically ordered due to the sequence of animals in the processing chain. The details of implementation of systematic sampling depend on the population of interest:

Slaughterhouse

HEIs for *Salmonella* include indicators used to assess in-coming and out-going *Salmonella* prevalence in carcases, and measures taken to control *Salmonella* are therefore based on sampling before and after processing. The population of interest in this case is the slaughter pigs and carcase population in the slaughterhouse, and the sample should be representative of this population.

These HEIs may be assessed periodically, for instance, annually, but for each assessment, the period of measurement should be relatively short, although at the same time it needs to capture the range of

[12] *Random* systematic sampling is the same, except that the first animal is chosen at random. In an abattoir population that has no intrinsic cyclic structure, the distinction between random systematic and non-random systematic sampling is likely to be academic. If sampling is started with the first animal slaughtered on day 1 of the study, the sample is still very likely to be representative of the population.

variability in slaughterhouse processes that may affect hygiene - for instance, changes in personnel between shifts, and cleaning cycles. It is therefore preferable to collect a sample over an appropriate time period, for instance several days or a week.

To plan sampling, it is first necessary to know the sample size (*n*) and the population size (*N*).

The sample size may be calculated using the approach illustrated in Annex 3. The population size is the total number of pigs processed by the slaughterhouse during the period of the measurement of the HEI (e.g. all pigs for one week). The sampling interval is calculated as *N/n*. For example, if a sample size of 350 was required, and the population was 22,000, the sampling interval would be 22,000/350 or 62.8 (rounded down to 62 in order to achieve a sample size a little larger than planned).

To target the slaughterhouse input, faecal content would be collected from every 62nd animal slaughtered, and to cover the slaughterhouse output, swabs would be taken from every 62nd carcase after processing. In order to assess the impact of the slaughter process on *Salmonella* contamination, the same carcase may be sampled in both steps.

Practically, reliably identifying selected animals (e.g. every 62nd animal) over a period of several days may pose some challenges and suitable systems would need to be developed for different slaughterhouses. Options include:

- Simple systems, such as having a person responsible for counting the interval, and tagging each selected carcase in a way that makes it easy for workers further down the chain to identify and take samples;
- More complex systems requiring improved information technology infrastructure, which count and flag animals automatically.

Farm

When the population of interest is the individual farms submitting slaughter pigs (e.g. for HEIs for *Toxoplasma* that aim to characterise farm risk), systematic sampling at the slaughterhouses is appropriate, but becomes more complicated. Again, farm-level characterisation is likely to take place over a defined sampling period, but good food chain information is required to ensure that the farm of origin can be identified for all animals processed at the slaughterhouse. Sample sizes are calculated on a per-farm basis, and the population size is the total number of animals slaughtered from each farm during the defined study period.

The issues of practical implementation are even more complicated in this case, as running counts of the sampling interval need to be maintained for each farm. If mixed batches of pigs from multiple farms are processed, this can provide some logistical challenges and is best implemented using automated systems.

A further practical consideration is the difficulty in predicting the number of animals that may be submitted for slaughter by different farms during the study period. One practical solution is to continue sampling (potentially beyond the planned sampling period) until the desired sample size has been achieved. However, if the target sample size for a particular farm is reached before the end of the sampling period, there is a danger that the shorter period may not be representative of output from that farm. In this case it is preferable to continue sampling, and then to randomly subsample from the collected specimens to reduce to the desired sample size.

Region or country

When the population of interest is the live or slaughter population for a region or the entire country, multiple slaughterhouses participate in the study and a stratified design must be used. One such design for a baseline study is provided in Annex III of the EFSA Scientific Opinion on "Risk assessment and mitigation options of *Salmonella* in pig production" (EFSA, 2006b). This design involves:

- Stratification by slaughterhouse, with sample size proportional to the number of animals slaughtered,
- Stratification by month within a year, with an equal sample size per month,
- A first-stage random sample (with replacement) of a day within a month (with selection of one day for each sample required),
- A second-stage random sample within a day, with selection of one animal per selected day (noting that a day may be selected multiple times, resulting in multiple animals on that day).

This design involves continuous sampling for one year. However, for HEIs it is likely that indicators may be measured over a shorter period.

5.2. On-farm sampling

On-farm sampling can be used to estimate HEIs that aim to characterise farm status. In all cases, the population of interest is therefore the farm. As the whole farm population can be sampled (rather than just the slaughter animals), it may provide a better approach to measure the overall farm status rather than just one age class (fattening pigs). The objective of HEIs based on on-farm sampling is generally to measure the prevalence of hazards (in which case representative sampling must be used), but in some cases, it may be to classify the farm as either free or infected with a hazard (in which case either representative sampling or, more efficiently, risk-based sampling can be applied). Risk-based sampling involves first identifying and characterising subpopulations at higher risk of being infected, and then preferentially sampling from those high risk subpopulations. However, the selection of individual animals from within identified subpopulations should still follow representative sampling principles. This discussion will therefore focus on representative sampling strategies for on-farm sampling:

Systematic sampling

Systematic sampling, as described for use in slaughterhouses, can be applied on-farm. While the population is not strictly sequenced (as it is not in a processing chain), it may be conceptually sequenced by listing each house and pen, and counting pigs within each pen. In practice, sampling would involve determining the sampling interval, then walking through the entire farm counting pigs, and sampling every pig that matches the sampling interval. This approach will generate a representative sample and requires little planning (only identification of the population size, as well as calculation of the sample size and sampling interval), but involves individually counting every pig on the farm during sampling, which may be time consuming on a large farm.

Simple random sampling from a grouped population

This approach uses simple random sampling, and therefore assures a representative sample, but takes advantage of the grouped nature of the population on a pig farm. Instead of requiring a sampling frame which lists all pigs individually, a list of groups (pens) is compiled, with the number of pigs in each pen. Random numbers are then generated, and used to identify the pen and the pig number within the selected pen. The pen is uniquely identified, but the pig within the selected pen has, at this stage, only a nominal identifier (e.g. the 7^{th} pig). To identify the specific pig, all pigs in the pen are counted in arbitrary sequence by somebody who does not know the number of the selected pig. This process momentarily applies an identifier to each animal and allows the selected animal to be uniquely identified. This approach is described in more detail in Cameron (1999). It involves somewhat more preparation before sampling but can significantly reduce the time required to select the sample, compared to systematic sampling, as not all pens need to be visited.

Multi-stage sampling

The third common approach used for on-farm sampling is multi-stage random sampling. Two-stage sampling may involve generating a list of pens and selecting a number at random, and then selecting animals at random from the selected pens. This approach appears very similar to the previous

approach and the work-load is very similar. However, it is statistically less efficient, such that, for a given sample size, the estimate based on multi-stage sampling will always be less precise than with systematic or simple random sampling as described above. Multi-stage sampling is an important and useful approach when single-stage approaches are not logistically possible but, for on-farm sampling, the previous two strategies are generally preferable.

5.3. Wildlife sampling

Only one of the suggested HEIs (for *Trichinella*) involves sampling of wildlife. The objective of this HEI is to assess the probability that the wildlife population is free from infection. As described above, methodologies exist to deal with risk-based sampling approaches when demonstrating freedom. While these methods were designed to take advantage of surveillance targeting high risk populations, they are equally applicable for dealing with unavoidable sampling biases in surveillance, as long as the relative risk of the sampled population (relative to the rest of the population) can be estimated. In some cases, the sampled wildlife population may be at higher risk than the non-sampled population, for instance, when infection makes animals easier to capture or observe, resulting in a relative risk greater than one. In others, it may make them less likely to be included in the sample, for instance, when many diseased animals, such as migratory birds, die before they have a chance to be observed. In this case, the relative risk will be less than one.

Estimating relative risks in wildlife sampling will often depend on expert opinion. Such estimates invariably have some uncertainty and sometimes variability. Tools to deal explicitly with uncertainty and variability are available, including the use of stochastic simulation. The application of these techniques to the analysis of surveillance data are illustrated in Martin et al. (2007a, 2007b) and Martin (2008).

While analytical approaches for dealing with biases in wildlife sampling are available, field sampling techniques are also critically important. In particular, it is important to make a significant effort to achieve good coverage of the entire population. In some cases, this may mean targeting a number of susceptible species, as well as different habitats, age and sex classes. Morrison et al. (2008) provide some guidance in this difficult field.

A number of sampling strategies are commonly used for wildlife studies. Sampling strategies which may be appropriate for estimating *Trichinella* HEIs (in which case the target population includes wild carnivorous mammals, mostly wild boars and foxes), include hunter samples, structured field sampling (shooting), mortality sampling and sentinel holdings.

Hunter samples

Wild boars are an effective indicator of the presence of *Trichinella* in the wildlife population and, in some MSs, hunting wild boars is a popular activity resulting in a large number of carcases per year. In these countries, programmes involving the routine testing of hunted boar carcases are able to generate a large number of samples. The advantages of this approach include low cost for specimen collection, and a potentially large sample. The disadvantages, as with most wildlife sampling, relate to biases and coverage, problems which are always present and hard to quantify.

Spatial and temporal coverage of wild boars are both likely to be incomplete, as boar populations and hunting are more common in some areas than others. These areas are unlikely to correspond to the risk areas for transmission to commercial pig farms. Hunting is also restricted to a defined season, making it impossible to have representative year-round samples.

In addition, the class of animal sampled is likely to be biased towards larger and possibly slower animals. Despite these shortcomings, hunter samples provide a valuable surveillance resource in those countries with large wild boar populations and hunter communities. Efforts are required to ensure that the best possible coverage is obtained and that possible biases are understood and taken into account.

Structured field sampling

Another approach used in some MSs is the use of structured, active sampling involving shooting of foxes or wild boars. This allows the study to be designed in such a way as to ensure good spatial and temporal coverage, but it is much more expensive, and generally results in a much smaller sample size due to cost constraints. Biases are still likely due to the visibility and 'shootability' of different age classes of animals. When emphasis is placed on the status of wildlife populations and it can be afforded, this approach can provide relatively good quality data.

Mortality sampling

Mortality sampling involves the collection of specimens from wildlife found dead, and either reported by the public or collected in a structured programme. This forms part of the general wildlife surveillance system in a number of countries but could be used to supplement *Trichinella* surveillance as well. The most common samples come from animals hit by vehicles and found by the road side. These have the advantage of being able to be identified while they are still relatively fresh. This approach requires special infrastructure for the identification of dead animals (either actively or via public reporting) and the collection of specimens. There are likely to be significant temporal and spatial biases related to traffic density and forest locations, public reporting biases and distances to be travelled to collect samples. This type of strategy alone is unlikely to provide adequate data to support estimation of HEIs but may be combined with others.

Sentinel holdings

Sentinel animals are commonly used in surveillance for a range of diseases (e.g. avian influenza) but have received little attention for *Trichinella* surveillance. Despite this, all outdoor farms may be currently considered as sentinels for the infection.

A sentinel holding is a defined group of animals that are kept under conditions that provide the best possible opportunity for them to become infected if the disease is present in wildlife (i.e. intentionally very poor biosecurity conditions). The logic is that if the sentinel animals fail to become infected, then the wildlife populations that they are in contact with are very unlikely to be infected. Sentinel holdings can be established in strategic areas to provide good spatial coverage, including in high risk areas. Animals can be regularly sampled and tested, and replaced with known negative animals.

The advantages of sentinel holdings include the ability to define the distribution and coverage of surveillance, the potential for ongoing sampling, and low sample collection costs (compared, for example to structured shooting programmes). There may be some costs in the establishment of holdings (although they may be run as commercial enterprises). Their main value is for the demonstration of freedom, as once animals are infected, they can provide no new information about disease incidence.

6. COMPARABLE DATA ON THE HARMONISED EPIDEMIOLOGICAL INDICATORS

Comparable data on the proposed harmonised epidemiological indicators from the EU MSs are available for only a few of the proposed indicators. This is because the indicators are in many cases quite specific, closely defining the animal population targeted as well as the specimen to be taken and the analytical method to be used.

The comparable data available from the annual reporting of zoonotic agents in accordance with Directive 2003/99/EC and from the EU-wide baseline surveys on zoonotic agents in food and animals are presented in the following tables.

6.1. *Trichinella*

Comparable data is available from the annual reporting in accordance with Directive 2003/99/EC on *Trichinella* findings from wild boars and carnivorous mammal wildlife species (HEI 4; Table 10).

Table 10: *Trichinella* testing and positive findings in wild boars and carnivorous wildlife[(a)] 2004 - 2009 in the EU

Member State	Wild boars												Carnivores											
	2004		2005		2006		2007		2008		2009		2004		2005		2006		2007		2008		2009	
	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P
Austria	31947	0	3.713	0					11555	0													10	0
Belgium	8167	1	11128	0	9284	0	13713	1	15177	0	10744	0	215	1	175	0	57	0	97	0	61	0	142	0
Bulgaria					2511	8	563	2	4307	34	6780	0					3	1	1		94	3	1	0
Cyprus															3	0							6	0
Czech Republic	11966	0	60442	0	27554	1	71525	0	78911	0	75000	0		0										
Denmark																					286	2	248	0
Estonia	6185	10	2668	3	2581	12	2717	10	4255	12	4380	40		0	31	10	30	9	56	13	63	11	72	23
Finland	4	0			2	0	21	1	12	1	19	0	693	162	665	163	656	165	674	122	974	275	730	220
France	26287	0	5782	0	28458	0	947	0	44708	0	23596	0	70	1	60	0			82	4	40	3	357	3
Germany	102726	1	390570	5	85719	3	134757	7	173642	15	164178	14	5653	0	4928	0	1543	1	3344	0	4222	2	4463	1
Greece	32	0									192	0												
Hungary	42110	0			30000	10			9	4	37455	1		1							1046	25	230	7
Ireland		0																			897	2	436	3
Italy	35006	0	20055	0	30929	0	19445	11	17360	29	42294	3	2	0			435	10	254	0	571	3	1060	4
Latvia	1022	12	982	17	1262	11	1546	15	2040	17	2214	31					1	0	2	1	102	76	29	20
Lithuania	9168	78	9011	46	9285	60			18150	62	24680	86	54	7	38	11	54	4			13	1	11	2
Luxembourg	1482	0	585	0	270	0	544	0	877	0	883	0			9	0	23	0	23	0	20	0	24	0
Malta																								
Poland	76698	240	91312	260	78650	321	86146	235	103612	524	50583	619					1	1					4	0
Portugal	213	0			10	0	450	0	2152	0	1852	0	208	10					6	0	3	0	22	2
Romania					4179	27	4371	31	7313	27	7911	54					73	7	63	8	164	22	115	21
Slovakia	15063	2	13199	16	10106	7	11978	5	12960	2	12605	4	715	90	385	44	733	100	627	123	18	0	220	13
Slovenia	5472	1	1421	0	475	1	1196	0	1496	1	847	1			37	0	56	0	1344	7	49	0	42	0
Spain	82563	121	128608	206	70566	172	51718	103	81248	182	64557	104	139	0					364	1				
Sweden	6191	1	6962	0	11226	0	17545	2	27131	1	47902	2	257	8	247	9	389	7	528	8	703	9	784	20
The Netherlands	811	34	366	1	311	0	1330	1	3585	0	2610	0											22	0
The United Kingdom							2023	0	31	0	159	0	1048	0	666	0	700	0	600	1	600	0	731	1
Norway									1	0			3	0	4	0	1	0	1	0			1	0
Switzerland			2655	0	1883	0	2475	0																

(a): Carnivorous wildlife include badgers, bears, foxes, hedgehogs, lynx, marten, minks, other mustelides, otter, polecats, raccoon dogs, wolverine, wolves.

6.2. *Salmonella*

In the case of *Salmonella*, the EU-wide baseline surveys provide comparable data from the MSs on *Salmonella* in holdings of breeding pigs (HEI 1; Tables 11 and 12), and on *Salmonella* in pig carcases at the end of the slaughter line (HEI 6; Table 13).

Table 11: Prevalence of *Salmonella*-positive pigs breeding holdings [(a)], *Salmonella* EU baseline survey, 2008[(b)] (EFSA, 2009b).

Member State	N[(c)]	*Salmonella*		*S.* Typhimurium		*S.* Derby		*Salmonella* other than *S.* Typhimurium and/or *S.* Derby[(d)]	
		% prev.	95 %CI[(e)]	% prev.	95 %CI	% prev.	95 %CI	% prev.	95 %CI
Austria	79	6.3	3.2-13.2	3.8	1.8-10.0	1.3	0.4-6.0	1.3	0.4-6.0
Belgium	16	18.8	7.3-45.1	12.5	4.2-37.8	6.3	1.4-29.7	6.3	1.4-29.7
Bulgaria	47	2.1	1.6-8.2	0	0.0-4.9	0	0.0-4.9	2.1	1.6-8.2
Cyprus	4	50.0	50.0-50.0	0	0.0-0.0	25.0	25.0-25.0	25.0	25.0-25.0
Czech Republic	106	10.4	7.2-15.9	3.8	2.1-7.7	0.9	0.5-4.1	5.7	3.6-10.3
Denmark	95	41.1	34.4-48.9	15.8	11.3-22.6	12.6	9.1-18.8	17.9	13.4-24.7
Estonia	6	0	0.0-14.3	0	0.0-14.3	0	0.0-14.3	0	0.0-14.3
Finland	50	0	0.0-6.1	0	0.0-6.1	0	0.0-6.1	0	0.0-6.1
France	157	50.3	44.2-57.1	7.0	4.5-11.4	25.5	20.5-31.7	26.8	21.8-33.2
Germany	46	28.3	18.4-42.6	8.7	3.9-20.3	10.9	5.3-22.9	6.5	2.6-17.4
Hungary	40	30.0	30.0-30.0	10.0	10.0-10.0	7.5	7.5-7.5	15.0	15.0-15.0
Ireland	40	52.5	51.2-53.7	17.5	17.1-19.5	20.0	19.5-22.0	17.5	17.1-19.5
Italy	43	51.2	39.2-65.1	7.0	2.7-17.7	16.3	9.1-29.0	16.3	9.1-29.0
Latvia	5	20.0	14.3-42.9	0	0.0-28.6	20.0	14.3-42.9	20.0	14.3-42.9
Lithuania	10	0	0.0-9.1	0	0.0-9.1	0	0.0-9.1	0	0.0-9.1
Luxembourg	3	33.3	33.3-33.3	0	0.0-0.0	0	0.0-0.0	33.3	33.3-33.3
Netherlands	109	57.8	50.0-66.2	13.8	9.3-20.9	18.3	12.9-26.1	38.5	31.3-47.2
Poland	144	6.9	3.9-12.3	2.8	1.1-6.9	1.4	0.4-4.9	3.5	1.6-7.9
Portugal	33	45.5	38.5-53.8	9.1	7.7-17.9	9.1	7.7-17.9	33.3	28.2-43.6
Slovakia	96	11.5	9.0-16.4	2.1	1.5-5.2	3.1	2.2-6.7	6.3	4.5-10.4
Slovenia	27	0	0.0-9.1	0	0.0-9.1	0	0.0-9.1	0	0.0-9.1
Spain	150	64.0	57.8-70.4	14.0	10.4-19.5	10.0	7.0-14.9	53.3	47.2-60.0
Sweden	57	1.8	1.3-6.3	1.8	1.3-6.3	0	0.0-3.8	0	0.0-3.8
United Kingdom	67	52.2	44.6-61.5	19.4	13.8-27.7	14.9	10.0-23.1	29.9	23.1-39.2
European Union	**1,377[(f)]**	**28.7**	**26.3-31.0**	**7.8**	**6.1-9.5**	**8.9**	**7.4-10.5**	**15.9**	**14.2-17.6**
Norway	108	0	0.0-2.2	0	0.0-2.2	0	0.0-2.2	0	0.0-2.2
Switzerland	71	15.5	12.6-20.7	4.2	3.4-8.0	1.4	1.1-4.6	8.5	6.6-13.8

(a): One holding can be positive for more than one serovar

(b): Greece, Malta and Romania did not conduct the survey and two non-MSs: Norway and Switzerland, participated.

(c): N is the total number of sampled holdings in each country.

(d): Untypeable *Salmonella* strains, as well as the partially typed *Salmonella* strains "4,5,12:i:-", "4,12:i:-", "4,5,12:-:-", were not included in the outcome "*Salmonella* other than *S.* Typhimurium and *S.* Derby". Instead, untypeable, partially typed, and non-typed *Salmonella* isolates were only included in the outcome variable "*Salmonella*".

(e): 95 % confidence interval (CI) based on a finite population approach.

(f): Total number of breeding holdings with at least 50 breeding pigs sampled in the EU.

Table 12: Prevalence of *Salmonella*-positive pigs production holdings[(a)], *Salmonella* EU baseline survey, 2008[(b)] (EFSA, 2009b).

Member State	N[(c)]	*Salmonella*		*S.* Typhimurium		*S.* Derby		*Salmonella* other than *S.* Typhimurium and/or *S.* Derby[(d)]	
		% prev.	95 %CI[(e)]	% prev.	95 %CI	% prev.	95 %CI	% prev.	95 %CI
Austria	173	5.8	3.2-10.3	0	0.0-2.1	0.6	0.1-3.2	5.2	2.8-9.6
Belgium	209	36.4	30.5-43.1	11.0	7.6-15.9	10.0	6.8-14.8	21.5	16.7-27.6
Bulgaria	25	0	0.0-13.5	0	0.0-13.5	0	0.0-13.5	0	0.0-13.5
Cyprus	60	18.3	13.8-26.4	0	0.0-4.6	8.3	5.7-14.9	8.3	5.7-14.9
Czech Republic	161	15.5	10.9-21.9	2.5	1.0-6.1	3.7	1.8-7.8	11.2	7.4-17.0
Denmark	198	41.4	35.2-48.4	12.6	8.9-17.9	14.6	10.6-20.2	18.7	14.1-24.7
Estonia	28	3.6	3.6-3.6	0	0.0-0.0	0	0.0-0.0	0	0.0-0.0
Finland	157	0	0.0-2.1	0	0.0-2.1	0	0.0-2.1	0	0.0-2.1
France	186	38.7	32.2-46.0	3.2	1.5-6.9	20.4	15.4-26.9	19.9	14.9-26.3
Germany	155	20.6	15.2-27.8	3.2	1.4-7.3	8.4	5.0-13.9	9.0	5.5-14.7
Hungary	141	27.7	22.1-34.6	1.4	0.6-4.5	12.8	8.9-18.6	14.2	10.1-20.2
Ireland	149	47.7	42.3-53.8	17.4	13.8-22.6	13.4	10.2-18.4	26.2	21.6-32.1
Italy	171	43.9	36.9-51.5	5.8	3.3-10.4	12.3	8.3-18.1	11.7	7.8-17.4
Latvia	28	28.6	20.5-41.0	0	0.0-7.7	3.6	2.6-12.8	25.0	17.9-38.5
Lithuania	72	8.3	7.1-12.9	0	0.0-2.4	0	0.0-2.4	8.3	7.1-12.9
Luxembourg	41	22.0	22.0-22.0	2.4	2.4-2.4	17.1	17.1-17.1	7.3	7.3-7.3
Netherlands	212	55.7	49.4-62.2	8.0	5.2-12.4	17.0	12.8-22.5	42.5	36.4-49.2
Poland	178	9.6	6.1-14.8	1.7	0.6-4.8	2.8	1.2-6.4	5.1	2.7-9.4
Portugal	134	43.3	35.6-52.0	13.4	8.8-20.3	5.2	2.6-10.4	29.9	23.0-38.2
Slovakia	96	18.8	12.6-27.7	3.1	1.2-8.7	4.2	1.8-10.1	13.5	8.3-21.8
Slovenia	87	10.3	5.7-18.7	0	0.0-4.1	1.1	0.3-6.2	10.3	5.7-18.7
Spain	209	53.1	46.6-60.0	12.4	8.7-17.7	6.7	4.1-10.9	42.6	36.3-49.5
Sweden	150	0	0.0-2.4	0	0.0-2.4	0	0.0-2.4	0	0.0-2.4
United Kingdom	191	44.0	37.8-50.9	9.9	6.7-14.8	11.0	7.5-16.0	31.9	26.3-38.7
European Union	**3,050[(f)]**	**33.3**	**30.9-35.7**	**6.6**	**5.3-7.9**	**9.0**	**7.6-10.5**	**21.6**	**19.5-23.6**
Norway	143	0	0.0-2.5	0	0.0-2.5	0	0.0-2.5	0	0.0-2.5
Switzerland	154	11.7	7.9-17.3	1.9	0.7-5.2	1.9	0.7-5.2	7.8	4.9-12.8

(a): One holding can be positive for more than one serovar.

(b): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

(c): N is the total number of sampled holdings in each country.

(d): Untypeable *Salmonella* strains, as well as the partially typed *Salmonella* strains "4,5,12:i:-", "4,12:i:-", "4,5,12:-:-", were not included in the outcome "*Salmonella* other than *S.* Typhimurium and *S.* Derby". Instead, untypeable, partially typed, and non-typed *Salmonella* isolates were only included in the outcome variable "*Salmonella*".

(e): 95 % confidence interval (CI) based on a finite population approach.

(f): Total number of breeding holdings with at least 50 breeding pigs sampled in the EU.

Table 13: Observed prevalence of pig carcases contaminated with *Salmonella*, with 95 % confidence intervals, in 13 MSs, *Salmonella* EU baseline survey, 2006-2007 (EFSA, 2008)

		***Salmonella* spp.**		***S.* Typhimurium**		***S.* Derby**		**Serovars other than *S.* Typhimurium and *S.* Derby**	
Member State	**N**	**% prev.**	**CI**	**% prev.**	**CI**	**% prev.**	**CI**	**% prev.**	**CI**
Austria	617	1.2	0.4 - 3.7	0.4	0.1 - 1.4	0.7	0.1 - 4.6	0.2	0.0 – 1.4
Belgium	381	18.8	14.1 - 24.6	10.9	6.9 - 16.8	3.8	2.1 - 6.7	3.1	1.9 – 4.9
Cyprus	359	3.3	3.2 - 3.4	0.5	0.5 - 0.5	0		2.8	2.6 – 3.0
Czech Republic	417	3.7	2.2 - 6.3	1.3	0.5 - 3.5	0.9	0.3 - 2.6	1.3	0.5 – 3.2
Denmark	344	3.3	1.3 - 8.5	1.6	0.6 - 4.2	0.5	0.2 - 1.5	1.3	0.4 – 4.8
France	413	17.6	11.8 - 25.4	7.0	3.9 - 12.1	5.9	3.3 - 10.5	4.8	2.6 – 8.7
Ireland	422	20.0	10.8 - 34	11.7	6.4 - 20.5	3.5	1.4 - 8.8	4.6	2.4 – 8.7
Latvia	391	3.3	1.2 - 8.9	0		0.5	0.1 - 3.2	2.9	0.9 – 9.1
Lithuania	461	1.6	0.6 - 4	0.6	0.2 - 2.3	0.5	0.1 - 1.4	0.7	0.3 – 1.6
Poland	447	1.3	0.5 - 3.2	0.5	0.1 - 1.7	0.6	0.2 - 2.5	0.1	0.0 – 0.8
Slovenia	441	0		0		0		0	
Sweden	402	0		0		0		0	
The United Kingdom	641	13.5	9.9 - 18.1	7.2	5.3 - 9.7	3.1	1.8 - 5.2	3.8	2.2 – 6.6
13 MS-group	5,736	8.3	6.3 – 11.0	3.9	2.8 - 5.5	2.6	1.7 - 3.9	2.3	1.6 – 3.5

The observed prevalence accounts for the aspects of clustering and of weighting.

N = number of tested carcases (surface swabbing); % prev. = observed prevalence estimate; CI = 95 % confidence interval.

The '*S.* Typhimurium', '*S.* Derby' and '*Salmonella* serovars other than *S.* Typhimurium and *S.* Derby' prevalence estimates do not add up to the '*Salmonella* spp.' prevalence estimates due to some rounding errors in the estimation process.

7. CONCLUSIONS AND RECOMMENDATIONS

ToR 1: Define harmonised epidemiological criteria for specific hazards already covered by current meat inspection (trichinellosis, tuberculosis, cysticercosis, …) and for possible additional hazards identified in the Scientific Opinion on the hazards to be covered by inspection of meat (see Annex 1 of the mandate), which can be used to consider adaptations of meat inspection methodology (e.g. prevalence, status of infection.

Conclusions

- In this report harmonised epidemiological indicators (HEIs) are proposed for food-borne biological hazards related to pigs and pork in the context of the Scientific Opinion on meat inspection of swine (EFSA, 2011). These hazards include *Trichinella*, mycobacteria and *Cysticercus* (*Taenia solium*) that are already covered by meat inspection of pigs as well as *Salmonella, Yersinia enterocolitica* and *Toxoplasma gondii* that were identified by the Scientific Opinion itself. An epidemiological indicator is understood to mean the prevalence or incidence of the hazard at a certain stage of the food chain or an indirect measure of the hazards, such as audits of farms, that correlates to a human health risk caused by the hazard.

- The epidemiological indicators proposed in this report will provide relevant information to the risk managers (i.e. the European Commission and the Member States), in order to consider whether adaptations in meat inspection methods may be relevant and to enable the Member States to carry out a risk analysis to support such decisions. It is also foreseen that the epidemiological indicators will be used in the pork safety assurance framework proposed by the Scientific Opinion, particularly to help categorise the farms/herds and slaughterhouses according to risk related to a particular hazard, as well as setting appropriate targets for final chilled carcases. Thus, the indicators can facilitate the implementation of risk-based meat inspection.

- In cases of rare biological hazards in EU pig production, epidemiological indicators are suggested to enable surveillance for possible emergence of such hazards. This is the case for mycobacteria and *Cysticercus*.

- The risk managers should decide on the most appropriate use of the epidemiological indicators. Depending on the purpose and the epidemiological situation of the country, the indicators may be applied at national, regional, slaughterhouse or farm/herd level and they can be used alone or in different combinations. The epidemiological indicators may be used in the classification of the countries, regions or farms according to the infection status related to the hazards. In addition, some indicators may be used to evaluate the measures taken in the slaughterhouses to control a specific hazard.

- Most epidemiological indicators are suggested for a subpopulation of pigs at the farm or for pig carcases on the slaughter line using visual, serological or bacteriological testing methods. Some epidemiological indicators that are assessed by auditing apply for example to controlled housing conditions on farms or to transport and slaughterhouse conditions.

- The proposed harmonised epidemiological indicators are listed in Table 14.

Recommendations

- It is recommended that the Commission and the Member States define the harmonised requirements for the controlled housing conditions at farms related to the specific hazards as well as for the conditions of transport and lairage of slaughter pigs referred to in the epidemiological indicators.

- For some biological hazards addressed, there is a need for more research to clarify the factors placing pigs at risk of infection and the role of pork as a source of human infections. This seems to be particularly the case for *Toxoplasma gondii* and *Yersinia enterocolitica*. Therefore, the Member States are invited to support such studies at the national level.

- The proposed epidemiological indicators will generate data that will provide information on the epidemiological situation in the EU and this data can be used to update the epidemiological indicators, when appropriate. It is recommended that the Member States report the data generated from implementation and monitoring of the indicators within the framework of annual reporting in accordance with Directive 2003/99/EC.

- The harmonised epidemiological indicators proposed by this report should be reviewed regularly in light of new information and the data generated from monitoring of them.

ToR 2: Provide a summary of comparable data from Member States based on the above defined harmonised epidemiological criteria, if existing (e.g. from ongoing monitoring in humans, food or animals).

Conclusions

- Comparable data from the EU Member States was only available for a few of the proposed epidemiological indicators. This was the case for some indicators for *Trichinella* and *Salmonella* where such data were provided by annual reporting on zoonotic agents under Directive 2003/99/EC or from the EU-wide baseline surveys. These data are summarised in chapter 6 of this report.

ToR 3: Recommend methodologies and minimum monitoring/inspection requirements to provide comparable data on such harmonised epidemiological criteria, in particular if comparable data are missing. These criteria should also be achievable in small Member States.

Conclusions

- For each epidemiological indicator the key elements of minimum monitoring or inspection requirements are defined. This includes the animal population to be targeted, the stage of the food chain where the sampling should take place, type and details of the specimen to be taken, diagnostic or analytical method to be used, and a case definition.

- A general description is provided on how to choose the sampling strategy for the different types of indicators and also specifically for each indicator. Guidance on sample size determination and sampling are given to guide the Member States in the implementation and monitoring of the indicators.

Recommendations

- It is recommended that the Commission and the Member States organise training to ensure harmonised implementation of the monitoring and inspection requirements.The proposed harmonised epidemiological indicators are summarised in the following table (Table 14):

Table 14: Proposed harmonised epidemiological indicators for pigs

Indicators (animal/ food category/other)	Food chain stage	Analytical /diagnostic method	Specimen
Salmonella			
HEI 1 *Salmonella* in breeding pigs	Farm	Microbiology (detection and serotyping)	Pooled faeces sample
HEI 2 *Salmonella* in fattening pigs prior to slaughter	Farm	Microbiology (detection and serotyping)	Pooled faeces sample
HEI 3 Controlled housing conditions on the farm (both for breeding pigs and fattening pigs)	Farm	Auditing	Not applicable
HEI 4 Transport and lairage conditions (both for breeding pigs and fattening pigs)	Transport and slaughterhouse	Auditing	Not applicable
HEI 5 *Salmonella* in fattening pigs – in-coming to slaughter process (evisceration stage)	Slaughterhouse	Microbiology (detection and serotyping)	Ileal contents
HEI 6 *Salmonella* in fattening pigs – carcases after slaughter process before chilling	Slaughterhouse	Microbiology (detection and serotyping)	Carcase swabs
HEI 7 *Salmonella* in fattening pigs – carcases after slaughter process and after chilling	Slaughterhouse	Microbiology (detection and serotyping)	Carcase swabs
Yersinia enterocolitica			
HEI 1 *Yersinia enterocolitica* in fattening pigs - in-coming to slaughter process (evisceration stage)	Slaughterhouse	Microbiology (detection and biotyping)	Tonsils or rectal content
HEI 2 Slaughter method: separation of head	Slaughterhouse	Auditing	Not applicable
HEI 3 *Yersinia enterocolitica* in fattening pigs – carcases after slaughter process before chilling	Slaughterhouse	Microbiology (detection and biotyping)	Carcase swabs
HEI 4 *Yersinia enterocolitica* in fattening pigs – carcases after slaughter process and after chilling	Slaughterhouse	Microbiology (detection and biotyping)	Carcase swabs

Toxoplasma			
HEI 1 Farms with officially recognised controlled housing conditions (including control of cats and boots)	Farm	Auditing	Not applicable
HEI 2 *Toxoplasma* in breeding pigs from officially recognised controlled housing conditions	Slaughterhouse	Serology	Blood
HEI 3 *Toxoplasma* in all pigs from non-officially recognised controlled housing conditions	Slaughterhouse	Serology	Blood
Trichinella			
HEI 1 *Trichinella* in free-range and backyard pigs (both fattening and breeding pigs)	Slaughterhouse	Digestion	Meat
HEI 2 *Trichinella* in pigs from non-officially recognised controlled housing conditions (both fattening and breeding pigs)	Slaughterhouse	Digestion	Meat
HEI 3 Farms with officially recognised controlled housing conditions and *Trichinella* free status	Farm	Auditing	Not applicable
HEI 4 *Trichinella* in wildlife (e.g. wild boar, bear, raccoon dog, fox, jackal, wolf, lynx, wild cats, genet, mustelids)	Environment	Digestion	Meat
***Cysticercus* (*Taenia solium*)**			
HEI 1 *Cysticercus* cysts in pigs (both fattening and breeding pigs).	Slaughterhouse	Visual meat inspection + PCR for confirmation	Meat
Mycobacteria			
HEI 1 Human pathogenic mycobacteria in pigs at slaughter	Slaughterhouse	Visual meat inspection + Microbiology	Suspected lesions

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9. APPENDICES

Annex 1. Proposed requirements for controlled housing conditions on farms

Trichinella

According to Annex IV to the Commission Regulation (EC) No 2075/2005:

A. The following requirements must be met by food business operators to obtain official recognition of holdings as free from *Trichinella*:

(a) the operator must have taken all practical precautions with regard to building construction and maintenance in order to prevent rodents, any other kind of mammals and large carnivorous birds from having access to buildings where animals are kept;

(b) the operator must apply a pest-control programme, in particular for rodents, effectively to prevent infestation of pigs. The operator must keep records of the programme to the satisfaction of the competent authority;

(c) the operator must ensure that all feed has been obtained from a facility that produces feed in accordance with the principles described in Regulation (EC) No 183/2005 of the European Parliament of 12 January 2005 and of the Council laying down requirements for feed hygiene[13];

(d) the operator must store feed intended for *Trichinella* susceptible species in closed silos or other containers that are impenetrable to rodents. All other feed supplies must be heat-treated or produced and stored to the satisfaction of the competent authority;

(e) the operator must ensure that dead animals are collected for disposal by sanitary means within 24 hours of death. However, dead piglets may be collected and stored on the holding in a properly closed container pending disposal;

(f) if a rubbish dump is located in the neighbourhood of the holding, the operator must inform the competent authority. Subsequently, the competent authority must assess the risks involved and decide whether the holding is to be recognised as free from *Trichinella*;

(g) the operator must ensure that piglets coming onto the holding from outside and pigs purchased are born and bred under controlled housing conditions in integrated production systems;

(h) the operator must ensure that pigs are identified so each animal can be traced back to the holding;

(i) the operator may introduce new animals, onto the holding only if they:
- come from in holdings officially recognised as free from *Trichinella*; or
- are accompanied by a certificate authenticated by the competent authority in the exporting country stating that the animal comes from a holding recognised as free from *Trichinella*; or
- are kept in isolation until the results of a serological test approved by the Community reference laboratory prove to be negative. Serological sampling must commence only after the animals have been on the holding for four weeks;

(j) the operator shall ensure that no pigs intended for slaughter have had outdoor access during the entire production period;

(k) outdoor access during the first few weeks of life before weaning shall be permitted if all the following conditions are met:
- no *Trichinella* infestations have been diagnosed in domestic animals in the country in the past 10 years;
- an annual surveillance programme exists for wildlife susceptible to *Trichinella*. The programme shall be risk-based and shall be conducted in an area epidemiologically related to

[13] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1–22.

the geographical location of the *Trichinella*-free farms. The programme shall test the relevant indicator species on the basis of previous findings. The results shall show a prevalence of *Trichinella* in indicator animals below 0.5 %;
- when outdoors, the animals shall be in properly fenced areas;
- the monitoring program referred to in Article 11 shall be in place and monitoring shall be more frequent on the holdings involved;
- all sows and boars kept for breeding purposes on the holding shall be systematically sampled at slaughter for examination using the reference method of detection described in Chapter I of Annex I or one of the equivalent methods described in Chapter II of Annex I, and
- steps shall be taken to prevent access by large carnivorous and omnivorous birds (e.g. crows, birds of prey) (EFSA, 2005c).

B. Food business operators of holdings recognised as free from *Trichinella* shall inform the competent authority where any of the requirements is no longer fulfilled or where any other change has occurred that might affect the *Trichinella*-free status of the holding.

Toxoplasma

The requirements for controlled housing conditions are to a large extent the same as for *Trichinella* in pigs, complemented by:

- Use of specific boots and their cleaning and disinfection in the building where pigs are kept;
- The control of cats and prevention of their access to barns, feed storage, water sources and bedding.

Salmonella

The following factors are essential for biosecurity to prevent *Salmonella* infections of pigs at the farm level:

- Frequency of sows' faeces removal in the farrowing room;
- Emptying the pit below the slatted floor between two successive batches of sows in the farrowing room;
- *Salmonella* contamination of the finishing room prior to loading of a new batch of pigs;
- Duration of the down period in the post-weaning room;
- Cleaning and disinfection of building;
- Type of feeding during the fattening phase;
- All in/ all out production systems;
- Quarantine;
- Prevention of the access of wildlife and pets;
- Ventilation of buildings;
- Prevention of the access of rodents and wild birds;
- Insect control;
- Overall protection of the buildings: hurdles, sanitary/changing room, specific clothes for the workers, for visitors, monitoring of visitors;
- Quality of water used.

Annex 2. Food chain, risk and risk-reducing factors, possible human health epidemiological indicators and their evaluation

Salmonella

1. Identification of potential epidemiological indicators

Table 15: Potential epidemiological indicators for *Salmonella* in pigs

	Availability of prevalence data	Data availability to divide population into groups between which the risk varies	Suggested epidemiological indicator (HEI)
Farm (including contribution from wildlife)			
Risk factor 1 Replacement pigs (buy-in replacement stock from *Salmonella* negative / positive herds)	Data on *Salmonella* in breeding animals readily available.	Data readily available for breeding herds (only supply replacement stock to integrated herds) and integrated breeding herds (buy in breeding stock or rear their own).	*Salmonella* status of supply breeding herd.
Risk factor 2 Feed (possibly *Salmonella* positive)	Some data available from the industry and official controls of feed.	Possible to gather.	*Salmonella* prevalence in feed or occurrence in feed mill.
Risk factor 3 On farm conditions (biosecurity)	Data on *Salmonella* in fattening animals readily available.	Data readily available from audits of farms.	*Salmonella* status of fattening pigs at the farm. Microbiology. Auditing of on-farm structures and procedures for biosecurity.
Transport to slaughterhouse			
Risk factor 1 Loading and transport – cross-contamination	Data available from research and studies on impact of transport on *Salmonella* prevalence in pigs.	It is possible to obtain such data.	Microbiology on transport vehicles. Measurement of time of transport.

Slaughterhouse			
Risk factor 1 Lairage: Cross-contamination Stress during lairage Incoming animals infected with *Salmonella*	Data available from research and studies on impact of lairage on *Salmonella* prevalence in pigs.	It is possible to obtain such data.	Microbiology: Samples taken from the caeca or ileum would provide an overall indication of bacterial load due to • on-farm conditions, • the time taken o from animal loading o transport o lairage to sticking, • animal mixing and, • associated stress due to these steps in the delivery chain. Auditing of the transport, handling, unloading and lairage procedures. Recording the overall time from loading to slaughter and the time spent in the various constituent parts of this process.
Risk factor 2 Dressing techniques	Data available on the carcases.	Surveys on surface sampling of carcases easily carried out. Limited data available to show differences between dressing techniques in slaughterhouses.	Carcase swabs prior to chilling.

Processing of meat and products thereof			
Risk factor 1 Boning hall – mincing Further processing Cross-contamination due to operatives, poor procedures and dirty equipment	Data available on the prevalence of *Salmonella* in processed pork products.	Data from carcase swabs prior to boning could be considered as a baseline indicator.	Microbiological testing. End product testing.
Retail			
Risk factor 1 Temperature abuses Risk factor 2 Cross-contaminations		A temperature above 12 °C is considered high risk for *Salmonella* growth.	
Consumer			
Risk factor 1 Undercooking or eating raw meat			
Risk factor 2 Temperature abuses		Temperature of the refrigerator.	

2. Evaluation of suggested indicators

Table 16: Suggested epidemiological indicators for *Salmonella* in pigs

Weighting factor				30 %	40 %	15 %	15 %	
Indicators (animal/ food category)	**Food chain stage**	**Analytical/ diagnostic method**	**Specimen**	**Quality of indicator (0,1,2)***	**Appropriateness of indicator (0,1,2)***	**Data availability (0,1,2)***	**Feasibility (0,1,2)***	**Total points**
Breeding pigs	Farm	Microbiology	Faeces	2	2	2	1	1.85
Breeding pigs	Farm	Serology	Blood/ meat juice	1	1	1	1	1
Fattening pigs	Farm	Microbiology	Faeces	2	2	2	1	1.85
Fattening pigs	Farm	Serology	Blood/ meat juice	1	1	1	1	1
Feed	Farm/ Feed mill	Microbiology	Feed sample	2	1	0	1	1.15
Bio-security and controlled housing conditions	Farm	Auditing	Not applicable	1	2	1	1	1.4
Loading and transport vehicles	Transport/ Slaughterhouse	Microbiology	Swabs on transport vehicles	1	1	0	1	0.85
Loading, transport and lairage	Transport/ Slaughterhouse	Auditing	Not applicable	1	2	1	1	1.4
Fattening pigs – incoming to slaughter process (evisceration stage)	Slaughterhouse	Microbiology	Lymph nodes	2	1	1	1	1.3
Fattening pigs – incoming to slaughter process (evisceration stage)	Slaughterhouse	Microbiology	Ileal contents	2	2	2	1	1.85

Fattening pigs at end of slaughter line before chilling	Slaughterhouse	Microbiology	Carcase swab	2	2	2	1	1.85
Fattening pigs at end of slaughter line after chilling	Slaughterhouse	Microbiology	Carcase swab	2	2	2	1	1.85
Pork products	Processing plant	Microbiology	Swab	2	1	1	1	1.3

* The justification for the score may be given in footnotes.
0= bad, 1 = moderate, 2 = good

Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
Appropriateness of indicator = how well the indicator correlates to human health risk caused by the hazard and to the possibility/need to amend the meat inspection method.
Data availability = is there data already available or is it easy to get the data needed.
Feasibility = how laborious is the sampling and testing procedure.

Yersinia enterocolitica

1. Identification of potential epidemiological indicators

Table 17: Potential epidemiological indicators for *Yersinia enterocolitica* in pigs

	Availability of prevalence data	**Data availability to divide population into groups between which the risk varies**	**Suggested epidemiological indicator (HEI)**
Farm (including contribution from wildlife)			
Risk factor 1 Age of the pigs	Prevalence data on slaughtered pigs is very scarce in EU data. Some prevalence data available in the literature.	It is possible to obtain such data. Sows are less colonized than slaughter pigs.	Prevalence of serological responses (in serum or meat juice) to *Y. enterocolitica* in slaughter pigs.
Risk factor 2 Production system	Some prevalence data available in the literature.	It is possible to obtain such data. Prevalence in slaughter pigs from fattening farms higher than from farrow-to-finish farms. Prevalence in organic pigs lower than in conventional reared ones.	Prevalence of serological responses (in serum or meat juice) to *Y. enterocolitica* in slaughter pigs.
Transport to slaughterhouse			
Risk factor 1 Contamination during transport and lairage	One paper published (Fukushima et al., 1990)		
Slaughterhouse			
Risk factor 1 Faecal contamination	Data on carcase contamination is scarce in EU. Limited data available in the literature.	It is possible to obtain such data. Sows – slaughter pigs.	Enumeration of *Y. enterocolitica* in faeces from slaughter pigs. Detection and enumeration of carcase contamination with *Y. enterocolitica* using carcase samples from slaughter pigs.

Risk factor 2 Removal of the pluck set and tonsils	Data on carcase contamination is scarce in EU data. Limited data available in the literature.	Sows – slaughter pigs.	Enumeration of *Y. enterocolitica* in tonsils from slaughter pigs. Detection and enumeration of carcase contamination with *Y. enterocolitica* using carcase samples from slaughter pigs.
Risk factor 3 Splitting of the carcase	Data on carcase contamination is scarce in EU data. Limited data available in the literature.	Sows – slaughter pigs	Enumeration of *Y. enterocolitica* in tonsils from slaughter pigs. Auditing of slaughter process (separation of the head).
Processing of meat and products thereof			
Risk factor 1 Cross-contamination during handling of carcases and pork meat	Data on contamination is scarce both in EU data and the literature.		
Risk factor 2 Possible outgrowth of *Y. enterocolitica* during chilled storage of fresh pork	Data on growth in pork is scarce in the literature.		
Retail			
Risk factor 1 Cross-contamination during handling of carcases and pork meat	Data on contamination is scarce both in EU data and literature.		
Risk factor 2 Possible outgrowth of *Y. enterocolitica* during chilled storage of fresh pork	Data on growth in pork is scarce in the literature.		
Consumer			
Risk factor 1 Age	Reliable human data only available from a limited number of EU countries.	Prevalence highest in young children	Reliable data from humans. Data on the age of infected humans.
Risk factor 2 Eating raw or undercooked pork	Data available in the literature.		

2. Evaluation of suggested indicators

Table 18: Suggested epidemiological indicators for *Yersinia enterocolitica* in pigs

Weighting factor				30 %	40 %	15 %	15 %	
Indicators (animal/ food category)	**Food chain stage**	**Analytical/ diagnostic method**	**Specimen**	**Quality of Indicator (0,1,2)***	**Appropriateness of Indicator (0,1,2)***	**Data availability (0,1,2)***	**Feasibility (0,1,2)***	**Total points**
Fattening pigs	Farm/ Slaughterhouse	Serology	Blood/meat juice	1	0	1	1	0.6
Fattening pigs	Slaughterhouse	Microbiology – (detection)	Rectal content	2	1	1	2	1.45
Fattening pigs	Slaughterhouse	Microbiology – (detection)	Tonsils	2	1	1	2	1.45
Fattening pigs at end of slaughter line before chilling	Slaughterhouse	Microbiology (detection)	Carcase swabs	2	1	1	2	1.45
Fattening pigs at end of slaughter line after chilling	Slaughterhouse	Microbiology (detection)	Carcase swabs	2	1	1	2	1.45
Type slaughter process-separation of head	Slaughterhouse	Auditing	Not applicable	2	1	2	2	1.6
Incidence of human yersiniosis cases	Consumers	Microbiology (detection)	Faeces	1	1	1	1	1

* The justification for the score may be given in footnotes.
0= bad, 1 = moderate, 2 = good

Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
Appropriateness of indicator = how well the indicator correlates to human health risk caused by the hazard and to the possibility/need to amend the meat inspection method.
Data availability = is there already data available or is it easy to get the data needed.
Feasibility = how laborious is the sampling and testing procedure.

Toxoplasma

1. **Identification of potential epidemiological indicators**

Table 19: Potential epidemiological indicators for *Toxoplasma* in pigs

	Availability of prevalence data	**Data availability to divide population into groups between which the risk varies**	**Suggested epidemiological indicator (HEI)**
Farm (including contribution from wildlife)			
Risk factor 1 Age of the pig and length of exposure – higher prevalence in breeding pigs (sows and boars) Risk factor 2 Presence of cats Risk factor 3 Presence of outdoor facilities Risk factor 4 Size of the farm Risk factor 5 Level of cleaning and disinfection and use of rodenticides Risk factor 6 Use of water from private sources (wells)	Data on *Toxoplasma* in animals available from annual monitoring in the EU.	Very little data available for different types of production systems or ages of animals but it is possible to obtain this information.	Detection of antibodies to *T. gondii* by a blood test on: - outdoor pigs - indoor pigs under controlled conditions - breeding pigs Submission of dead piglets to diagnostic laboratories for necropsy and histological evaluation. Auditing of the housing conditions and biosecurity on farms.
Transport to slaughterhouse			
Risk factor 1	-	-	-

Slaughterhouse			
Risk factor 1	-	-	-
Processing of meat and products thereof			
Risk factor 1	-	-	-
Retail			
Risk factor 1	-	-	-
Consumer			
Risk factor 1 Pregnant women Risk factor 2 Eating raw or undercooked meat	Data on human cases available from annual reporting.	Some countries have data available because of positive screening of pregnant women.	Positive screening of pregnant women (but it will not tell us if the infection is from pork or other sources).

2. Evaluation of suggested indicators

Table 20: Suggested epidemiological indicators for *Toxoplasma* in pigs

Weighting factor				30 %	40 %	15 %	15 %	
Indicators (animal/ food category/ other)	**Food chain stage**	**Analytical/ diagnostic method**	**Specimen**	**Quality of indicator (0,1,2)***	**Appropriateness of indicator (0,1,2)***	**Data availability (0,1,2)***	**Feasibility (0,1,2)***	**Total points**
Detection of antibodies to *T. gondii* by a blood test on all pigs not from officially recognised controlled housing conditions	Slaughterhouse	Serology[(a)]	Blood	1	2	1	1[(a), (b)]	1.4
Detection of antibodies to *T. gondii* by a blood test on indoor pigs under controlled conditions	Slaughterhouse	Serology[(a)]	Blood	1	1	1	0[(a), (b)]	0.85
Detection of antibodies to *T. gondii* by a blood test on breeding pigs from officially recognised controlled housing conditions	Slaughterhouse	Serology[(a)]	Blood	1	2	1	1[(a), (b)]	1.4
Submission of dead piglets to diagnostic laboratories for necropsy and histological evaluation	Farm	Histology	Tissue	1	1	0	0[(c)]	0.7
Auditing of farms with controlled housing conditions**	Farm	Auditing	Not applicable	1	2	1	1	1.4

* The justification for the score may be given in footnotes.
**To address cats and boots and similar requirements than for *Trichinella*.
0= bad, 1 = moderate, 2 = good
(a): For serology: lack of standard methods commercially available. There are different tests and antigens that have not been validated. Need to make standards serum samples available. A positive result does not indicate that all muscle samples contain viable parasites.
(b): A serological test is not cheap.
(c): Submission of pigs from the farm to a laboratory will increase the cost.

Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
Appropriateness of indicator = how well the indicator correlates to human health risk caused by the hazard and to possibility/need to amend meat inspection method.
Data availability = is there already data available or is it easy to get the data needed.
Feasibility = how laborious is the sampling and testing procedure.

Trichinella

1. **Identification of potential epidemiological indicators**

Table 21: Potential epidemiological indicators for *Trichinella* in pigs

	Availability of prevalence data	Data availability to divide population into groups between which the risk varies	Suggested epidemiological indicator (HEI)
Farm (including contribution from wildlife)			
Risk factor 1 Ingestion of flesh from wildlife	High – due to the routine controls at the slaughterhouses: meat inspection shows a high sensitivity.	Possible to collect data on indoor high containment level pigs/ free-ranging and backyard pigs at the slaughterhouse.	Prevalence of *Trichinella* spp. infection in: - indoor pigs from controlled housing conditions, - indoor pigs from non-controlled housing conditions, - free-ranging and backyard pigs. Standard of the controlled housing conditions of the farm. Prevalence of *Trichinella* in wildlife.
Risk factor 2 Ingestion of pork scraps	High – due to the routine controls at the slaughterhouses: meat inspection shows a high sensitivity.	Possible to collect data on indoor high containment level pigs/ free-ranging and backyard pigs at the slaughterhouse.	Prevalence of *Trichinella* spp. infection in: - indoor pigs from controlled housing conditions, indoor pigs from non-controlled housing conditions, - free-ranging and backyard pigs.
Transport to slaughterhouse			
Risk factor 1	-	-	-
Slaughterhouse			

Risk factor 1	-	-	-
Processing of meat and products thereof			
Risk factor 1	-	-	-
Retail			
Risk factor 1	-	-	-
Consumer			
Risk factor 1 Consumption of raw or undercooked pork	In most of the EU countries incidence data on human trichinellosis is available	High risk – consumers who ingest raw pork and raw pork products	

2. Evaluation of suggested indicators

Table 22: Suggested epidemiological indicators for *Trichinella*

Weighting factor				30 %	40 %	15 %	15 %	
Indicators (animal/ food category/ other)	**Food chain stage**	**Analytical/ diagnostic method**	**Specimen**	**Quality of Indicator (0,1,2)***	**Appropriateness of Indicator (0,1,2)***	**Data availability (0,1,2)***	**Feasibility (0,1,2)***	**Total points**
Free-ranging pigs	Farm	Serology[(a)]	Blood	0[(b)]	0	2	0	0.3
Free-ranging fattening pigs	Slaughterhouse	Digestion	Meat	2	2	2	2	2.0
Free-ranging sows and boars	Slaughterhouse	Digestion	Meat	2	2	2	2	2.0
Backyard pigs	Farm	Serology[(a)]	Blood	0[(b)]	0	2	0	0.3
Backyard pigs	Slaughterhouse	Digestion	Meat	2	2	2	1	1.85
Backyard sows and boars	Slaughterhouse	Digestion	Meat	2	2	2	1	1.85
Fattening pigs from controlled housing conditions	Farm	Serology[(a)]	Blood	1	1	1	0	0.85
Fattening pigs from controlled housing conditions	Slaughterhouse	Serology[(a)]	Blood	1	1	2	0[(c)]	1.0
Fattening pigs from controlled housing conditions	Slaughterhouse	Digestion	Meat	2	1	2	0[(c)]	1.3
Sows and boars from controlled housing conditions	Farm	Serology[(a)]	Blood	1	1	1	0	0.85
Sows and boars from controlled housing conditions	Slaughterhouse	Serology[(a)]	Blood	1	1	2	0	1.0

Sows and boars from controlled housing conditions	Slaughterhouse	Digestion	Meat	2	1	2	0	1.3
Indoor pigs from non-controlled housing conditions (both fattening and breeding pigs)	Farm	Serology[(a)]	Blood	1	1	1	1	1.0
Indoor pigs from non-controlled housing conditions (both fattening and breeding pigs)	Slaughterhouse	Serology[(a)]	Blood	1	1	2	1	1.15
Indoor pigs from non-controlled housing conditions (both fattening and breeding pigs)	Slaughterhouse	Digestion	Meat	2	2	2	2	2.0
Wildlife: potentially infected mammal species (e.g. wild boars, raccoon dogs, foxes, bears)	Slaughterhouse	Digestion	Meat	2	2	2	2	2.0
Auditing of farms with controlled housing **	Farm	Auditing	Not applicable	2	2	2	1	1.85

* The justification for the score may be given in footnotes.
**Make reference to legislation.
0= bad, 1 = moderate, 2 = good
(a): ELISA antibody detection only for monitoring purposes, not for diagnosis.
(b): Possible cross-reaction with other pathogens.
(c): The cost is not justified by the risk, since no infection has been detected in the last 20 years.

Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
Appropriateness of indicator = how well the indicator correlates to human health risk caused by the hazard and to the possibility/need to amend the meat inspection method.
Data availability = is there already data available or is it easy to get the data needed.
Feasibility = how laborious is the sampling and testing procedure.

Cysticercus

1. **Identification of potential epidemiological indicators**

Table 23: Potential epidemiological indicators for *Cysticercus* (*Taenia solium*) in pigs

	Availability of prevalence data	Data availability to divide population into groups between which the risk varies	Suggested epidemiological indicator (HEI)
Farm (including contribution from wildlife)			
Risk factor 1 Access to outdoors	Poor - prevalence data on slaughtered pigs: meat inspection has low sensitivity; confusion cysticercosis due to *T. solium* and *T. hydatigena*.	Possible to collect data on indoor/ outdoor pigs at slaughterhouses.	Confirmation of *T. solium* porcine cysticercosis by meat inspection and confirmation of species by molecular method. Prevalence of cysticercosis in outdoor pigs.
Transport to slaughterhouse			
Risk factor 1	-	-	-
Slaughterhouse			
Risk factor 1	-	-	-
Processing of meat and products thereof			
Risk factor 1	-	-	-
Retail			
Risk factor 1	-	-	-
Consumer			
Risk factor 1 Eating raw or undercooked meat	NA		

2. Evaluation of suggested indicators

Table 24: Suggested epidemiological indicators for *Cysticercus* (*Taenia solium*) in pigs

Weighting factor				30 %	40 %	15 %	15 %	
Indicators (animal/ food category/ other)	**Food chain stage**	**Analytical/ diagnostic method**	**Specimen**	**Quality of Indicator (0,1,2)***	**Appropriateness of Indicator (0,1,2)***	**Data availability (0,1,2)***	**Feasibility (0,1,2)***	**Total points**
All pigs (both fattening and breeding pigs), particularly free-range pigs	Slaughterhouse	PCR[(a)]	Meat (suspected lesion)	2[(b)]	2[(c)]	1[(d)]	1[(e)]	1.7
Free-ranging pigs	Farm/ Slaughterhouse[(f)]	Serology[(g)]	Blood	0[(h)]	1	0[(d)]	1	0.55
Free-ranging pigs	Slaughterhouse	PCR[(a)]	Meat (suspected lesion	2	2	1[(d)]	1[(e)]	1.7

* The justification for the score may be given in footnotes.
0= bad, 1 = moderate, 2 = good
(a): Confirmation of parasitological diagnosis.
(b): Molecular test will give unequivocal answer on whether or not the lesion is due to *Taenia solium.* Sensitivity of PCR is high, in case of calcified lesions it is possible that no DNA can be extracted.
(c): This indicator will inform on whether active transmission is still occurring.
(d): No data available but could be collected.
(e): Sampling is easy, for testing a molecular lab needed. Price estimation for DNA extraction and PCR is high but considering the small number of samples expected this is feasible.
(f): Serology can be performed both on farm and at slaughterhouse.
(g): ELISA antigen detection.
(h): Possible cross-reaction with *Taenia hydatigena.*

Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
Appropriateness of indicator = how well the indicator correlates to human health risk caused by the hazard and to the possibility/need to amend the meat inspection method.
Data availability = is there already data available or is it easy to get the data needed.
Feasibility = how laborious is the sampling and testing procedure.

Mycobacteria

1. Identification of potential epidemiological indicators

Table 25: Potential epidemiological indicators for mycobacteria in pigs

	Availability of prevalence data	Data availability to divide population into groups between which the risk varies	Suggested epidemiological indicator (HEI)
Farm (including contribution from wildlife)			
Risk factor 1 Production system –access to outdoors and contact with wildlife	Some prevalence data available in the literature. Some prevalence data available in the literature of wildlife (e.g. Boadella et al., 2011).	It is possible to obtain such data. Because of contacts with the wildlife, the prevalence in outdoor pig herds, free-ranging pig herds and domesticated wild boar holdings is higher than in conventional pigs.	Prevalence of serological responses (in serum or meat juice) to *Mycobacterium* spp. in slaughter pigs in contact with the wildlife. Detection of *Mycobacterium* spp. in suspected lesions and/or pharyngeal and sub-maxillary lymph nodes in all pigs or pigs from populations at risk. Confirmation by skin testing is possible in serial. Prevalence of serological responses (in serum or meat juice) to *Mycobacterium bovis* in wildlife to substantiate freedom of disease as well as maintenance of the status.
Transport to slaughterhouse			
Risk factor 1	-	-	-
Slaughterhouse			
Risk factor 1	-	-	-
Processing of meat and products thereof			
Risk factor 1	-	-	-

Retail			
Risk factor 1	-	-	-
Consumer			
Risk factor 1	-	-	-

2. Evaluation of suggested indicators

Table 26: Suggested epidemiological indicators for mycobacteria in pigs

Weighting factor				30 %	40 %	15 %	15 %	
Indicators (animal/ food category)	**Food chain stage**	**Analytical/ diagnostic method**	**Specimen**	**Quality of Indicator (0,1,2)***	**Appropriateness of Indicator (0,1,2)***	**Data availability (0,1,2)***	**Feasibility (0,1,2)***	**Total points**
Slaughter pigs	Farm/ Slaughterhouse	Serology	Blood/ meat juice	1	0	1	1	0.6
Slaughter pigs	Farm	Allergy (skin test)	Animal	2	1	0	0	1.0
All pigs	Slaughterhouse	Detection	Suspected lesions, e.g. tonsils and spleen	2	2	1	2	1.85
Wild boars	Slaughterhouse	Serology	Blood/meat juice	1	0	0	0	0.3

* The justification for the score may be given in footnotes.
0= bad, 1 = moderate, 2 = good

Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
Appropriateness of indicator = how well the indicator correlates to human health risk caused by the hazard and to the possibility/need to amend the meat inspection method.
Data availability = is there already data available or is it easy to get the data needed.
Feasibility = how laborious is the sampling and testing procedure.

Annex 3. General guidelines for sample size determination and sampling

1. Introduction

Harmonised epidemiological indicators (HEIs) for meat inspection take two distinct forms: measures of the prevalence of a hazard (to be compared to a defined threshold), and data to support claims of freedom from the hazard (in which case the probability of freedom is compared to a defined threshold). While in both cases sampling and data collection procedures may be similar, the underlying theory and approach to calculating sample size are very different. This section provides guidance for planning and implementing data collection based on these two different types of HEI.

General guidance for the design of surveys has previously been provided by EFSA (EFSA, 2006c), covering the major elements of such a protocol and discussing various aspects to consider for each element.

2. General principles

A number of principles are common to the two types of HEI.

Definition of the target population

It is important to have a clear understanding of the target population[14] for the HEI. This is the population to which the indicator refers, and may vary according to different HEIs. For instance, demonstration that the wildlife population is free from infection with a particular hazard may be used as an HEI for some hazards (such as *Trichinella*). In this case, the target population consists of all wildlife species susceptible to *Trichinella* within a defined geographical area (for example, the entire territory of a MS, or a subdivision of that country). On the other hand, some indicators may operate at the farm level. In this case, the target population may be either all animals on a specified farm, or all slaughter animals leaving the farm.

A clear definition of the target population is essential in order to address issues of representativeness and sample size calculations.

Sampling frame

The sampling frame is a list of each member of the population, from which the sample is drawn by random sampling. The requirements for an ideal sampling frame are that:

- There are no missing members,
- There is no duplication,
- Each member is uniquely and reliably identified (so that it can be sampled if selected).

Many sampling frames do not completely meet these requirements, but the degree to which these requirements are not met influences the validity of the results.

Quality assurance

When analysing data to estimate an HEI, one is normally presented with a list of numbers (often test results). Analysis of this data is often straight forward; however the validity of the results of this analysis depends not just on the analytical techniques used, but on the way in which the data was

[14] Target population means the immediate population to which the study results will be extrapolated. The subjects (items, animals, batches) included in the study would be derived from the target population (Dohoo et al., 2003 in Report of Task Force on Zoonoses Data Collection on Guidance Document on Good Practices for Design of Field Surveys (EFSA, 2006c)).

collected. Valid and reliable indicators can only be estimated if the data collection systems are undertaken in an appropriate way. It is often not possible for an analyst to tell the difference between data that was collected appropriately and that which was collected incorrectly.

Quality assurance systems for data collection allow the analyst and other users of that data to have confidence that the process meets certain basic requirements. It is important that any surveillance to gather data for HEIs should have some sort of quality assurance system in place. This would normally consist of:

- Documentation of data collection procedures[15],
- Training of staff involved in data collection,
- Systems which are able to verify that the required procedures are actually followed. These may include:
    - supervision,
    - record keeping (for instance, if formal random sampling is used, a record of the random numbers selected and the corresponding animal identification numbers),
    - auditing (periodic follow-up surveys to re-test animals to confirm that the results obtained through the routine work match the audit results).

Quality control systems should also include explicit mechanisms to detect when the data collection system is not functioning correctly, and measures that must be taken to correct them.

Data produced from a quality controlled system should be accompanied by meta-data, which defines how the data was collected and the quality standards to which it conforms.

3. Estimation of prevalence

Key points

- The sample must be representative of the population of interest. This may be achieved by the application of proper survey sampling procedures, including:
    - Census: test all animals in the population of interest,
    - Formal random sampling (simple random sampling or a more complex multistage and / or stratified design using random sampling at each stage),
    - Systematic sampling.

  In practice, a combination of these techniques is often used.
- Sample size is determined by:
    - Population variance (expressed in terms of the expected prevalence),
    - Desired precision (calculated based on the difference between the expected prevalence and defined threshold for decision making),
    - Size of the population of interest (only significant in relatively small populations),
    - Sampling method used (e.g. stratified sampling increases precision; multi-stage sampling decreases precision but increases convenience, etc.).
- Measurement errors must be avoided by understanding the measurement system and correcting for biases (including correcting for imperfect sensitivity and/or specificity of tests).

[15] This may be achieved by adhering to the guidelines published in Report of Task Force on Zoonoses Data Collection on Guidance Document on Good Practices for Design of Field Surveys (EFSA, 2006c).

- The method used to analyse the data to calculate prevalence should be correctly matched to the sampling scheme to avoid analysis bias.

Quality of measures for prevalence estimates

When estimating prevalence, the quality of estimates may be measured in terms of systematic and random error. These concepts are probabilistic and are based on the idea that a particular process to estimate prevalence may be repeated many times. Each time the process is repeated, a different result may be obtained.

Systematic error is when the mean result of an estimate is different from the true value, which is otherwise known as bias. There are a number of different possible sources of bias.

Random error describes the spread of the different results, and is due to the random effects inherent in sampling and testing. If there is no bias, repeated results will still vary, but the mean will be equal to the true value. If the level of variation in the results is small, there is little random error and the estimate is relatively precise. If there is a high level of variation, due to high random error, the estimate is imprecise. Systematic and random errors arise from different sources and may operate independently.

Avoiding bias (systematic error)

The main sources of bias are sampling bias, measurement bias and analysis bias.

Sampling bias

Sampling bias occurs when the process used to select animals or specimens in a survey results in a sample which is not representative of the population of interest. This means that the characteristic being measured (for instance, the prevalence of a hazard) is different in the sample compared to the prevalence in the population of interest.

Sampling bias is caused by the use of an inappropriate selection method. Inappropriate methods include convenience sampling (the closest, easiest, or fastest animals to sample), purposive sampling (selecting animals based on some preconceived criterion), risk-based sampling (selecting animals with a specific risk factor related to the hazard), and haphazard sampling (pseudo-random sampling where animals are chosen by a person who attempts to simulate randomness). All these sampling methods result in bias. Sampling bias can also occur when there is a strong discrepancy between the population and the available sampling frame. A biased sample (for instance due to risk-based sampling) may be desirable when the objective of the surveillance is to detect disease, but yields incorrect estimates when the objective is to measure prevalence.

Unlike other sources of bias, it is impossible to detect or correct sampling bias after sampling has been completed. Poor sampling strategies will therefore often lead to undetected bias, leading to incorrect conclusions from the data. For this reason, it is very important to take steps to ensure that sampling bias is avoided.

Representative (hence unbiased) samples can only be reliably achieved using one of the following two general approaches:

- Probability sampling, using formal random sampling. In this approach, individuals are selected from the sampling frame using a truly random process (e.g. tossing dice, or random number tables), such that each member of the population has a known, non-zero probability of selection. Simple random sampling (a specific case of random sampling, in which each member has the same probably of selection), is the most common implementation;

- Systematic sampling, in which the population is arranged (at least notionally) in a sequence, and individuals are selected from that sequence at regular intervals (e.g. every 10th animal). Systematic sampling is not a probability sampling approach but will generate a representative sample under most conditions. Systematic sampling can be easily transformed into a probability sampling technique ('random systematic sampling') by choosing the first animal in the sequence using a formal random process. Using the above example of a sampling interval of 10, the first animal would be selected using a random number between 1 and 10.

There are a range of more complex sampling designs, including clustered, multi-stage and stratified sampling; however these only influence survey precision and efficiency. In order to avoid bias, they must be combined with either random or systematic sampling.

Practical implementation of this basic sampling theory in the field is often complex, and it is rarely possible to achieve a perfectly representative sample, given real-world constraints. Guidance in practical field implementation of representative sampling strategies is available in a number of texts including Cameron (1999). It is sometimes suggested that convenience sampling is acceptable when practical constraints make formal probability sampling impossible. However, in virtually all situations, systematic sampling methods can be adapted to even the most challenging situations. For instance, systematic sampling from a fast moving chain in an slaughterhouse (where resources for counting carcases are not available) can be achieved by sampling at regular time intervals (e.g. by using an alarm that sounds, say, every two minutes). The next carcase to pass after the alarm has sounded is the one to be sampled.

Measurement bias

Measurement bias is the result of a systematic error at the point of measurement. Classically, this is illustrated with the concept of a survey which aims to estimate the average weight of animals in a population, but uses scales that are incorrectly calibrated so that there is a systematic error in the measurement of every animal. For HEIs, a more pertinent example is the use of screening or diagnostic tests with imperfect sensitivity or specificity, resulting in systematic misclassification.
Unlike selection bias, measurement bias can be corrected after data collection, if the nature of the bias is known. In the example of poorly calibrated scales, if the scales read 30 grams too heavy for each animal, the correct mean can be calculated by simply subtracting 30 grams from the estimated mean. For imperfect sensitivity (Se) and specificity (Sp), true prevalence (TP) can be calculated from apparent prevalence (AP) using the following formula:

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1}$$

This formula (or more advanced versions of it) is implemented in a number of epidemiological software suites, including EpiTools (referenced below under 'Software tools for calculations').

Analysis bias

Analysis bias refers to a situation where the approach used to analyse the data is inappropriate for the way in which the data was collected, and results in an estimate which is systematically different from the true value.

A simple example of analysis bias is given in the following Section 6 'Examples calculations'. In this example, the survey design samples an equal number of animals from populations of different sizes, meaning that animals have unequal sampling probabilities. Weighting is therefore required to reflect the size of the source populations.

Analysis bias can be avoided by clearly understanding the study design and using appropriate analytical methods to analyse the results.

Achieving the required precision (controlling random error)

The precision of an estimate of prevalence is usually expressed in terms of a confidence interval (absolute precision). Formally speaking, a 95 % confidence interval indicates the range in which the estimated prevalence would fall 95 % of the time if the study was repeated using the same design many times. A narrow confidence interval indicates that the study was able to measure the prevalence more precisely and there is less uncertainty about the true value.

Precision may also be expressed in relative terms, especially when the prevalence is very low. The width of the confidence interval is described as a proportion of the estimated prevalence. A relative precision of 50 % with a prevalence of 1 % would be equivalent to an absolute precision of 0.5 % to 1.5 %.

The factors that influence the precision of a prevalence estimate are:

- Confidence level: by convention, this is set at 95 %. Different values should not be used as it complicates interpretation of the results.
- Population size: this has an effect only when the size of the population is small relative to the sample size. With large populations, there is no significant impact of population size on precision.
- Variance: if the degree of variance in the population is high, precision will be lower, and vice versa. In prevalence studies, variance is often expressed in terms of the prevalence of the hazard in the population. Variance is low when the prevalence is either very low or very high (because most members of the population are similar, either mostly infected or mostly uninfected). Variance is highest when the prevalence is 50 %.
- Sample size: increasing the sample size increases the precision of the prevalence estimate.
- Survey design: one-stage surveys using simple random sampling achieve the highest precision for a given sample size. As the design becomes more complex (e.g. two-stage surveys), the precision decreases for a given sample size.

Based on these relationships it is possible to determine the appropriate sample size that is required to achieve a specified level of precision. Examples of software tools for these calculations are listed below. In order to calculate the appropriate sample size, the following inputs are required:

- Survey design: in order to choose the software tool implementing the appropriate formula.
- Expected prevalence (as a measure of variance): this is an unfortunate statistical paradox – it is necessary to know the result of the study (prevalence in the population), in order to correctly design and size the study. Often estimates are available, but if uncertain, it is good practice to err towards 50 %.
- Population size.
- Desired precision. Selecting this value is explained in the section ‘Examples calculations’.

Software tools for calculations

A wide variety of statistical software is available to plan prevalence surveys and calculate sample sizes. Stand-alone free Windows software packages for calculation of sample size with simple one-stage survey designs include EpiCalc[16] and WinEpiScope[17]. The EpiTools[18] web-based suite of epidemiological calculators provides tools for the design and analysis of both simple one-stage and more complex two-stage surveys using a variety of sampling designs. This site also has tools for a range of other surveillance tasks, including demonstration of freedom as discussed below.

[16] http://www.brixtonhealth.com/epicalc.html
[17] http://www.clive.ed.ac.uk/cliveCatalogueItem.asp?id=B6BC9009-C10F-4393-A22D-48F436516AC4
[18] http://epitools.ausvet.com.au

4. Demonstration of freedom

When measuring prevalence, the value being estimated is a number. In contrast, when testing whether a population is free from a hazard or not, the result is 'yes' or 'no', qualified by a measure of confidence (probability of freedom). This difference means that different approaches need to be used when estimating HEIs related to freedom from infection compared to measuring the prevalence of infection.

Key points

- Representative or risk-based sampling approaches may be used.
- Risk-based approaches may be more efficient, but the effect of selected risk factors must be well understood and quantified.
- Sampling must provide some coverage of all important parts of the population (for example, with respect to location, time, species / breed, age and sex). Different sub-populations may have different levels of coverage (with risk-based sampling), but none should be excluded unless they are not at risk from the disease.
- To claim freedom, all final results must be negative. Any positive test result that is assumed to be a false positive must be conclusively demonstrated to be truly negative. If this is not the case, the population cannot be considered free from the hazard.
- The following factors should be taken into consideration:
    - The results of historical disease surveillance,
    - The risk of introduction of the hazard into the population,
    - The performance of tests used (sensitivity and specificity),
    - The use of one or more confirmatory tests to exclude false positives in the screening test, and the combined sensitivity and specificity of the test system, and
    - Agreed standard design prevalence, indicating the hypothetical level of disease in the population against which the probability of detection is calculated.

Concepts

It is rarely possible to prove beyond doubt that a population is free from infection. Instead, probabilistic approaches are used. The quality of surveillance to demonstrate freedom is measured in terms of the **sensitivity of the surveillance**, or the probability that, if disease were present in the population, that the surveillance would detect at least one positive animal. If disease were present at a high level, it would be easy to find and the sensitivity of the surveillance would be high, but low levels of disease are difficult to detect. To provide a standard for the evaluation of the sensitivity of surveillance, it is therefore necessary to define a standard hypothetical level of disease (the ***design prevalence*** or P*) against which sensitivity is calculated.

While survey sensitivity provides a measure of the quality of the surveillance, it does not address the prime question – is the population free from infection or not. This is expressed using the **probability of freedom**. The probability of freedom is analogous to the negative predictive value of a diagnostic test, and can be calculated using Bayes' Theorem, using the survey sensitivity and an estimate of the prior probability that the population is free from infection. The concept of the prior probability allows us to incorporate historical surveillance data, adjusted to take into account the risk of introduction of disease, into our current estimates of the probability of freedom.

Sampling strategies

Studies to demonstrate freedom from infection can use a range of sampling strategies. Representative techniques (as described in the section on prevalence estimation) can be used. However risk-based approaches are generally more efficient. The intuitive explanation for this is that, for a given sample size, there is a greater chance of finding diseased animals (and therefore greater survey sensitivity) if

one looks in sub-populations that have a higher risk of being infected, compared to representative surveys.

Risk-based surveillance requires good information about the risk-structure of the population. In order to quantify the benefits of a risk-based approach on the surveillance sensitivity, it is necessary to know or estimate:

- The relative risk of the targeted sub-population compared to the rest of the population. This indicates whether the risk factor chosen is a more or less efficient way to identify infected animals if infection were present.
- The degree of targeting of the high risk population. This is evaluated by comparing the proportion of the population in the high risk group, to the proportion of the surveillance sample in the high risk group. If these two proportions are the same, the surveillance is representative. If the surveillance proportion is higher than the population proportion, the surveillance is targeting the high risk population.

It is possible to use multiple risk factors when designing surveillance, and these factors may operate at the group level (e.g. farm management), or at the individual level (e.g. sex or age). However, once all risk groups have been identified, sampling of individual animals within the same risk group should, where possible, be carried out using representative techniques (random or systematic) to avoid introducing unknown selection biases.

It is also possible to increase the sensitivity of surveillance by targeting not only those sub-populations with a higher risk of being infected, but also those with a higher probability of being detected. Factors influencing the performance of the screening test may therefore also be included in the surveillance design.

Risk-based approaches are most efficient when only very high risk sub-populations are targeted. However, completely ignoring other parts of the population is not recommended, as this decreased coverage lowers confidence in the surveillance. Survey design therefore involves a balance between targeting high risk populations to maximise sensitivity, while maintaining a reasonable level of coverage of all other parts of the population (spatially as well as by age, sex and other relevant factors).

Data analysis and sample size calculation

Data analysis to estimate HEIs related to freedom from infection is normally undertaken in two parts. The first is to estimate the sensitivity of a study or surveillance exercise (surveillance in a defined period of time). Parameters required for this calculation include:

- The number of animals tested,
- The risk groups from which the animals were drawn,
- The relative risk for each risk factor considered in the design,
- The population proportion for each risk group,
- The size of the population (only important when the sample size is large relative to the size of the population),
- The sensitivity of any individual animal tests used in the surveillance, and
- The design prevalence, which is usually based on existing standards, where available.

The second step is to combine this estimate with historical data to estimate the probability of freedom. Here, the parameters required are:

- The sensitivity of surveillance in each previous time period (calculated using the same parameter as listed above),
- The risk of introduction of infection into the population, for each time period considered. This can be calculated using risk analysis techniques,

- A starting prior probability of freedom for time period zero, which, by convention, is set to 0.5.

The formulae for calculating sensitivity and probability of freedom are complex and modelling techniques are often used. Fortunately, there are software tools to simplify these calculations, facilitating both data analysis and allowing sample size calculations to be made for a range of designs.

Software tools

Several different software packages provide tools for the analysis of representative surveillance to demonstrate freedom from disease. However the EpiTools suite of epidemiological calculators, referenced previously, is one of the only systems to provide a range of tools for analysis of risk-based surveillance, including sample size calculation.

5. Small populations

Small populations may pose special challenges when measuring harmonised epidemiological indicators. The definition of a small population is relative to other parameters in the survey design and the nature of the population. For a national level survey, a small population may be represented by a small MS with only a small number of farms or slaughterhouse. At the farm level, small populations are commonly present in every MS, and are represented by farms with small herd sizes.

The main problem with small populations is when sample size calculations either provide a required sample size that is greater than the population size, or when sampling every unit in the population fails to meet the survey objectives.

Estimation of prevalence

For surveys to estimate prevalence, small populations generally do not pose a problem. Sample size calculations should take the size of the population into account. As the size of the sample approaches that of the population, the sample size required to achieve a given precision decreases (relative to the sample size required for a large population). At a certain point, the sample size required is equal to the population size, in which case no sampling is required, and every animal is tested instead (i.e. a census). When all animals in the population are sampled, there is no possibility of random error, and the prevalence is directly measured rather than estimated.

Demonstration of freedom

In contrast, small populations can pose significant problems when aiming to demonstrate freedom from disease or infection. However, there is still some doubt about the status of a population, because of imperfect test performance, even when every member of that population has been tested. For small populations and tests with relatively poor sensitivity, it may not be possible to achieve the target survey sensitivity even by testing all animals.

In practice, there are only two solutions to this problem, both of which are somewhat inadequate. The first is to use a test with higher sensitivity, if one is available. The second is to artificially combine the small population (e.g. a small herd) with other small populations to create a single larger population. This is based on the assumption that all the sub-populations that are combined share approximately the same risk factors.

A third alternative is to acknowledge that demonstrating freedom in small populations is difficult, and that it may not be possible to achieve target survey sensitivities in all cases. Other risk mitigation strategies may be required to account for this lack of certainty.

6. Examples calculations

Example of analysis bias

A survey is undertaken for a population consisting of 4 farms, to estimate the overall prevalence of a hazard. The results of the survey are shown below (Table 27):

Table 27: Population, sample size and test results of an example survey involving four farms

Farm ID	Population	Specimens	Positive
1	100	10	2
2	100	10	2
3	700	10	5
4	100	10	1
Total	**1000**	**40**	**10**

A naive analysis to estimate prevalence would use the normal formula:

$$P = \frac{Number\ sampled\ positive}{Total\ number\ sampled}$$

$$= \frac{10}{40} = 25\%$$

The correct analysis would take into account the different population sizes for each farm and the different prevalence on each farm (Table 28):

Table 28: Illustration of correct analysis of data from table 27, weighted by population size

Farm ID	Population	Specimens	Positive	Prevalence	Estimated positive
1	100	10	2	20 %	20
2	100	10	2	20 %	20
3	700	10	5	50 %	350
4	100	10	1	10 %	10
Total	**1000**	**40**	**10**		**400**

Using this approach, the estimate of the overall prevalence is:

$$P = \frac{Estimated\ total\ positive\ animals}{Total\ population}$$

$$= \frac{400}{1000} = 40\%$$

The true population prevalence is 40 %, but incorrect analysis (failing to weight for farm size) resulted in a biased estimate of 25 %.

Determining the required precision

Figure 10 below shows a typical confidence interval around an estimate of an HEI. In this example, the estimated prevalence is 10 % and the 95 % confidence interval extends from 8 % to 12 % as indicated by the red lines marking the 2.5th and 97.5th percentiles (i.e. containing 95 % of the area under the curve). Based in this confidence interval, it is not known what the true value is but it is most likely to lie between 8 % and 12 %.

The dashed green line shows the threshold against which the HEI will be measured, as determined by the risk manager. If the HEI is above the threshold, one action will be taken, and if it is below the threshold, a different action will be taken.

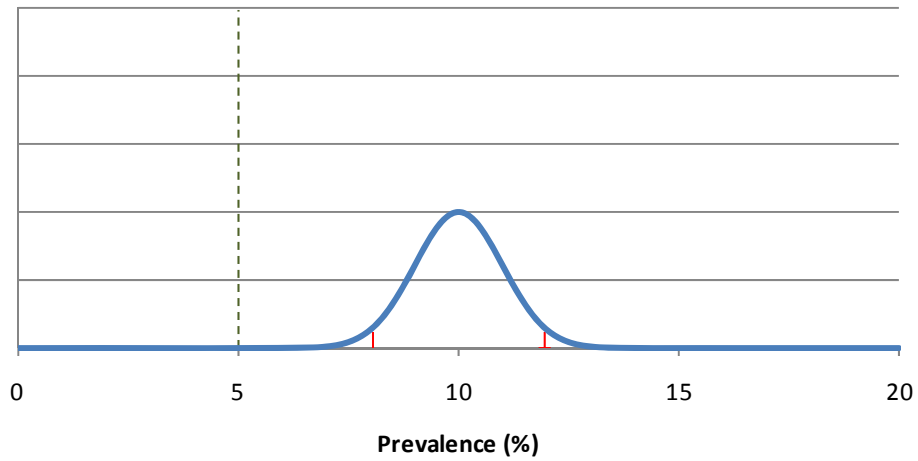

Figure 10: 95 % confidence interval around an estimate of prevalence of 10 % used as an HEI

In this example, it is clear that the estimated prevalence (10 %) is greater than the threshold (5 %). As the true prevalence is uncertain, even the most pessimistic value (the lower limit of the 95 % confidence interval, or 8 %) is still greater than the threshold. This means that the results of our study allow us to take the decision with confidence.

In a second example illustrated in Figure 11 below, the estimated prevalence is now 6.7 %. While this is still greater than the threshold of 5 %, the lower limit of the confidence interval is now 4.7 %. This means that while the best guess at the true prevalence is 6.7 %, there is a reasonable chance that it could actually be less than the threshold of 5 %. However, it is no longer sure if the HEI is greater than or less than the threshold because the confidence interval overlaps the threshold. Therefore the decision can no longer be made with confidence.

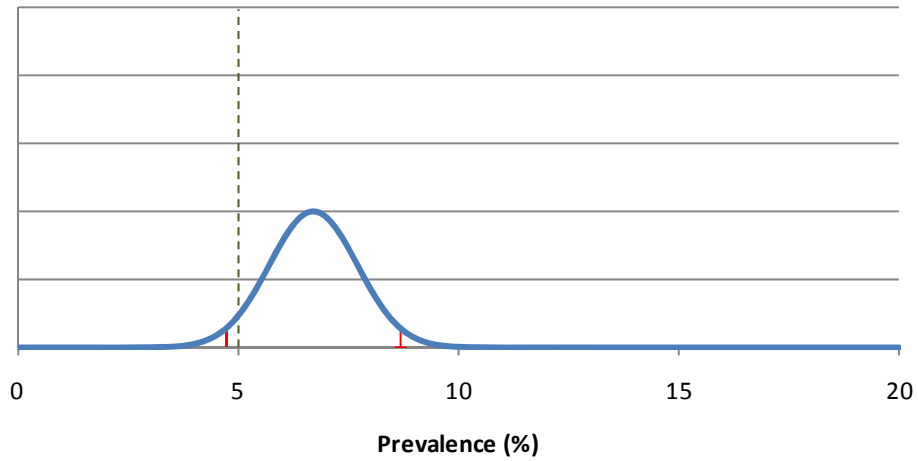

Figure 11: 95 % confidence interval around an estimate of prevalence of 6.7 % used as an HEI

If the estimated prevalence is 6.7 %, it is necessary to achieve a greater precision to be able to distinguish confidently between the estimated prevalence and the threshold. By increasing the sample size, it is possible to make the confidence interval narrower. Figure 12 below illustrates the results of the analysis of a study with a larger sample size in the same population. The estimated prevalence is still 6.7 %, but the lower 95 % confidence limit (5.7 %) is now clearly greater than the threshold of 5 %.

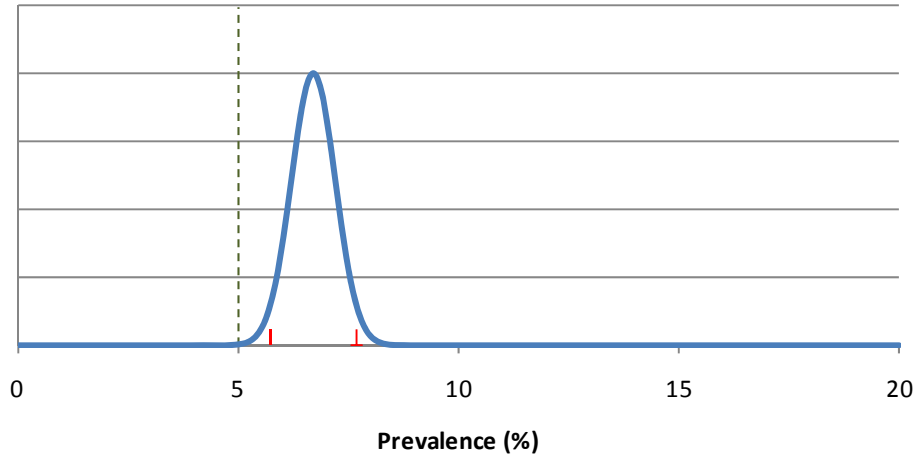

Figure 12: 95 % confidence interval around an estimate of prevalence of 6.7 % based on a study with a larger sample size

Figure 13 shows a final example in which the minimum sample size has been calculated to provide a precision which is adequate to determine confidently if the HEI is greater than the threshold or not (with 95 % confidence).

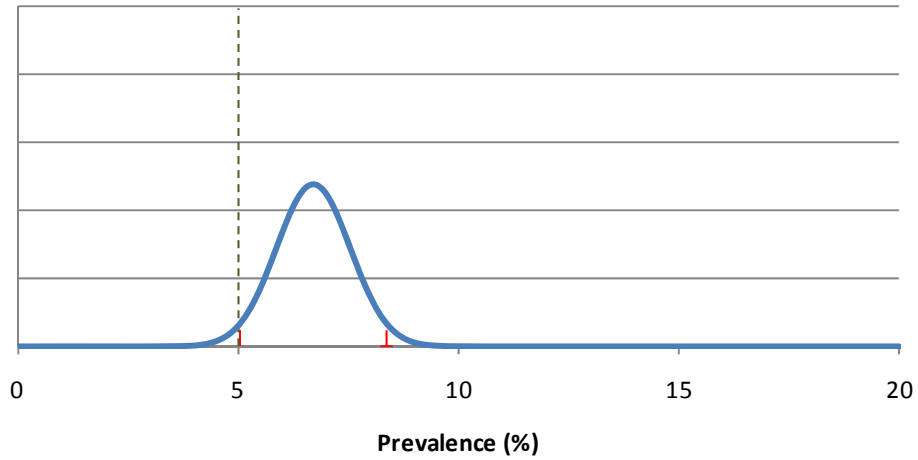

Figure 13: 95 % confidence interval around an estimate of prevalence of 6.7 % using the minimum sample size required to distinguish the prevalence from a threshold prevalence of 5 %

These examples illustrate an approach for determining the required precision when planning the sample size of study to estimate an HEI. If *a* is half the size of the 95 % confidence interval, T is the threshold prevalence set by the risk manager, and $\hat{p}$ is the estimated population prevalence, the required precision, expressed in terms of *a* can be calculated as:

$$a = \hat{P} - T$$

Using the example above:

$$a = \hat{P} - T$$
$$= 6.7 - 5$$
$$= 1.7$$

This means that a precision of ±1.7 % should be used when calculating the sample size if the expected prevalence is 6.7 %. In contrast, if the expected prevalence is 20 %, a precision of ±15 % would be required (resulting in a much smaller sample size).

Example of using EpiTools for estimation of prevalence

Consider an example of an HEI based on estimated prevalence at the herd level. The threshold for decision making set by the risk manager is 1 % prevalence. If the prevalence is higher than 1 %, specific risk mitigation actions need to be taken, but if it is lower than 1 %, meat inspection procedures for animals from that farm may be simplified.

Sample size calculation

The estimated prevalence on Farm A is 5 % and the population size is 1,000 animals. What sample size is required?

These calculations will be illustrated using EpiTools from http://epitools.ausvet.com.au. First, the required precision is calculated based on the formula above, giving a value of ±4 %.

From the main screen, select **Sample size calculations → Estimate a single proportion.**

There are four input values required. Prevalence and probability values are entered as proportions (between 0 and 1) and not percentages.

- Estimated true proportion: Enter 0.05.
- Confidence level: This should never be changed. Leave it at 0.95.
- Desired precision (±): Enter 0.04.
- Population size: Enter 1000.

Click the **Submit** button, then scroll down to see the results. Two sample size figures are listed, one for infinite populations (ignoring the sample size value entered) and one for a population with the specified sample size. In this case we want the second value, Population = 1,000. The result is 245 animals.

This means that if 245 animals are sampled, and the result is a prevalence of 5 % as expected, the precision will be ±4 % which will allow us to conclude with 95 % confidence that the true prevalence is greater than 1 %. However, we may find that the true prevalence is greater or lower than expected. Therefore, the results need to be analysed in order to determine whether valid conclusions can be drawn.

Data analysis

Consider now that the survey has been conducted using a sample size of 245 animals. Samples were tested with a screening test that had a sensitivity of 92 % and a specificity of 99 %. After testing, 11 positive results were found. The estimated prevalence needs to be calculated taking into account the

measurement bias caused by the imperfect sensitivity and specificity of the test, as well as estimating the width of the 95 % confidence interval.

Using EpiTools again, select **Estimating true prevalence → Estimated true prevalence with an imperfect test**.

The input values required are:

- Sample size: Enter 245.
- Number positive: Enter 11.
- Test sensitivity: Enter 0.92.
- Test specificity: Enter 0.99.
- Sample size for sensitivity estimation: Leave blank. This and the next value are used to account for uncertainty in the sensitivity and specificity values but, for this example, they will be assumed to be known fixed values.
- Sample size for specificity estimation: Leave blank.
- Confidence level: Leave as 0.95.

Click the **Submit** button, then scroll down to see the results. A range of different (but usually very similar) results are presented, based on different published algorithms. The text of the page indicates the most commonly preferred approach, which in this case is Blaker's Exact.

On the third line, the appropriate result is shown. The results indicate that:

- Estimation of the prevalence, correcting for sensitivity and specificity of the test, would have given a value of 4.5 % with a 95 % confidence interval of 2.5 % to 7.9 % (first row of the results).
- After correcting for the test performance, the estimated true prevalence is 3.8 % with a confidence interval of 1.4 % to 7.4 % (third row of results).

This illustrates the potential importance of correcting for test performance. Based on these results, it could be confidently concluded that the prevalence was greater than the 1 % threshold.

Example of using EpiTools for demonstration of freedom

While more complex two-stage designs can be handled, this example will illustrate a simple one-stage survey design. The objective is to undertake surveillance to demonstrate freedom from disease in a herd. If disease were present, older animals are more likely to be seropositive than younger animals, with a relative risk of 3. The proportion of older animals on the farm is 20 % but this group is targeted and makes up 70 % of the sample. The sensitivity of the ELISA used is 98 %, and the aim is to achieve a surveillance sensitivity of 95 % based on a 5 % design prevalence.

Sample size calculation

Using EpiTools, select **Risk-based surveillance → Calculate sample size for simple risk-based surveillance**.

The following parameters are required:

- Relative risk: Enter 3.
- Population proportion in high risk group: Enter 0.2.

- Surveillance proportion in high risk group: Enter 0.7.
- Design prevalence: Enter 0.05.
- Test sensitivity: Enter 0.98.
- Target surveillance sensitivity: Enter 0.95.

Click **Submit** and scroll down to the results. The table compares the required sample size for risk-based versus representative sampling. For risk-based, a sample size of 25 animals from the high risk group, and 10 from the low risk group (giving a total sample size of 35) would be required to achieve a surveillance sensitivity of 95 %. In contrast, representative sampling would need a sample size of 62 to achieve the same result. Consequently, using the risk-based approach results in a saving of 43 % in terms of sample size.

Analysing results

In order to claim freedom from infection, all final results from the surveillance must be negative. Tests with imperfect specificity will produce some false positive results, however normal procedures involve retesting these positive samples with more specific tests to confirm whether they are true or false positives. If, after one or more confirmatory tests in a test system, the result is negative, it is assumed that it was a false positive, and the result can be treated as a true negative. If however, the result remains positive, then it is assumed that it is a true positive, and the task of demonstrating freedom is no longer relevant.

To analyse the surveillance results using EpiTools, select **Risk-based surveillance → Calculate sensitivity of simple risk-based surveillance.** Enter the following values:

- Relative risk: Enter 3.
- Population proportion in high risk group: Enter 0.2.
- Surveillance proportion in high risk group: Enter 0.7.
- Design prevalence: Enter 0.05.
- Test sensitivity: Enter 0.98.
- Number tested: Enter 35.
- Prior confidence of freedom: Enter 0.8. This allows the calculation of the posterior probability of freedom. It is assumed that previous surveillance has been conducted and analysed, and the adjusted prior probability of freedom has been calculated as 80 %.

Click **Submit** and scroll down to see the results. Again, a comparison is made between risk-based and representative approaches. Using the risk-based approach, the survey sensitivity achieved is 98.8 % and the posterior probability of freedom is 99.7 %.

If the same sample size had been used but with representative sampling, the surveillance sensitivity would be only 91.9 % and the posterior probability of freedom would be 98 %.

Calculating the adjusted prior probability of freedom

For a series of time periods, the posterior probability of freedom for one time period is first adjusted for the risk of introduction of infection, and then used as the prior probability of freedom for the subsequent time period. Adjusting for the risk of introduction is undertaken using the following formula.

$$PriorPr(inf)_k = ((PriorPr(Inf)_{k-1}) + Pr(Intro)_k - [(PriorPr(Inf)_{k-1}) \times Pr(Intro)_k])$$

Where:

PriorPr(free)k = 1-PriorPr(Inf)k and

Pr(Inf)k is the probability of the introduction of infection in the kth year.

For example, if the posterior probability of freedom last year was 98 %, and the risk of introduction of infection was 1 % per annum:

$$PriorPr(inf)_k = 2\% + 1\% - (2\% * 1\%)$$
$$= 2.98\%$$

This gives a prior probability of freedom for the current year of 1-2.98 %, or 97.02 %.

Annex 4. Case study on application of harmonised epidemiological indicators for *Trichinella*

The objectives of this section are to:

- explain the approach used and purpose for HEIs for *Trichinella*,
- illustrate their use to support decisions for modification of meat inspection procedures, and
- examine the availability and suitability of existing data that may be used for the estimation of HEIs.

Current meat inspection procedures for *Trichinella*

The level of *Trichinella* infection (number of cysts per gram) and distribution of cysts varies significantly, and this influences the sensitivity of the digestion test, as does the skill of the operator. Under current testing standards using one gram of diaphragm, the sensitivity has been estimated to range between 40 % for low level infections up to nearly 100 % (Forbes and Gajadhar, 1999). Despite possibly poor sensitivity for low-level infections, public health surveillance indicates that virtually no cases have been contracted from meat that has undergone official inspection. This suggests that the digestion test is effective at identifying meat that is infected at a level adequate to cause human infection.

Modified meat inspection procedures for *Trichinella*

As there is no method for the reliable detection of *Trichinella* in pigs other than specific post-mortem tests (such as the digestion test), ongoing testing will be required to identify infected animals at slaughter. Harmonised epidemiological criteria should be able to provide the information that risk managers need to decide if modifications to meat inspection procedures can be introduced.

Assumptions

The most appropriate modifications available are based on the identification of very low risk populations which do not need to be tested. The following assumptions about risk are used to identify low risk populations:

- If there is adequate evidence that all the wildlife in a defined geographic area are free from *Trichinella* infection, and the domestic pig population has tested negative in the past, there is no risk of new infection in the domestic pig population. This is based on the assumption that the source of infection is wildlife. When evaluating evidence of the free status of wildlife it is important that possible external sources of infection (for instance, through movement of wildlife from infected areas) be taken into account.
- Pigs raised under controlled housing conditions, as defined by the EU legislation (Commission Regulation (EC) No 2075/2005) are not at risk of becoming infected, as they have no contact with possible sources of infection. Pigs raised in conditions that do not meet the definition of controlled housing, in areas where infection may be present in wildlife are at risk of being infected. This includes pigs raised outdoors, pigs with access to outdoor areas, and housed pigs without adequate controls. The infection status of these pigs needs to be determined before any decision can be made on modification of meat inspection procedures.
- The specificity of the digestion test is assumed to be virtually 100 %. This is demonstrated by the absence of positive results from millions of tests from animals from controlled housing, as described on the section on data analysis.

The HEIs proposed for *Trichinella* are presented in Section 4.4 of this report.

Application of HEIs

An example of the application of these indicators is shown in the flowchart below (Figure 14).

Wildlife

Firstly, if wildlife surveillance data is available, or if the risk managers decide that conducting adequate wildlife surveillance is feasible and cost-effective, and all results are negative, this can be analysed to estimate the probability that the population is free from *Trichinella* infection. The calculations are illustrated in the following section on data analysis which also provides a basis to determine the surveillance frequency and sample size required. If the probability of freedom is greater than a threshold defined by the risk managers (and there is prior evidence that the domestic population is also free from infection), it may be deemed that the wildlife population in the defined area is free from infection and that modified meat inspection procedures may be applied to all slaughter pigs from that area (for instance, complete cessation of *Trichinella* testing). Note that this decision is made at the level of a defined geographical areas (such as the entire country, or a zone within that country).

Controlled housing

If the above conditions are not met (either because of the absence of wildlife surveillance data, the presence of positives test results from wildlife, or inadequate surveillance to meet the defined threshold for probability of freedom), further risk factors can be considered. The key risk factor recommended is whether pigs are raised in controlled housing or not. If pigs are raised in controlled housing, based on the previously stated assumptions and definition, they are not at risk from *Trichinella* and modified meat inspection procedures may be adopted (i.e. cessation of testing for those pigs). However, in order to provide adequate assurance that an establishment meets the requirements of controlled housing, an audit is required (based on the EC regulation definition). This audit should be repeated at regular intervals (as discussed in the section on harmonised procedures for gathering HEIs). Establishments that fail to pass the audit are deemed not to have controlled housing. Note that decisions based on the use of controlled housing are made at the establishment level.

Other farms

Farms that are not exempt from *Trichinella* testing on the basis of freedom of infection in wildlife, or the use of (audited) controlled housing are at risk from *Trichinella* infection. In order to adopt modified meat inspection procedures for these establishments it is required to provide evidence that they are at adequately low risk of being infected (or that the prevalence of infection is below the threshold determined by risk managers).

If it is desired to implement modified meat inspection procedures, testing can be performed on a sample of pigs from these establishments in order to estimate the prevalence. If the prevalence based on this sample is below the defined threshold prevalence, meat inspection procedures may be modified. Otherwise, all pigs must continue to be tested, or other risk mitigation strategies should be implemented (such as the cooking of all meat from these establishments).

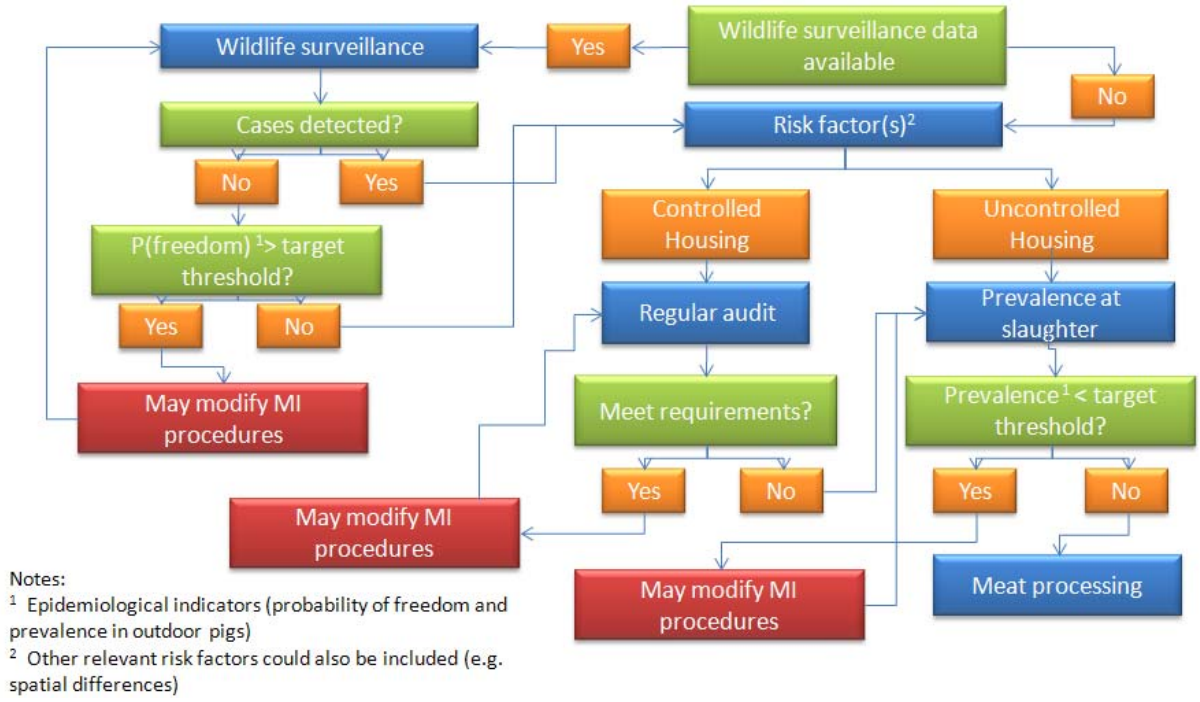

Figure 14: Example of the possible application of HEIs for *Trichinella* using a decision-tree approach

Estimation of HEIs

Wildlife surveillance

The HEI for wildlife is the prevalence of *Trichinella* in wildlife and is used to decide whether adequate evidence exists to assume that the wildlife population is free from infection, and therefore poses no risk for transfer of infection to the domestic population. The analysis is based on the methods described by Martin et al. (2007a, 2007b) and Martin (2008). Current surveillance data is combined with historical evidence of freedom to estimate the current probability that a population is free from infection, taking into account the risk of re-introduction of infection from outside. This analysis is carried out for a population in a specified geographical area, usually the entire territory of a MS or a zone within it.

Data required

Three types of data are required for this analysis:

- Current monitoring data: monitoring data from the current time period (year) is analysed to estimate its sensitivity. To be useful the requirements for current surveillance are:
    - It covers all the geographical area of interest. Risk-based approaches may be used to focus on areas of higher risk, but some monitoring should also be carried out in the lower risk areas. If there are areas without susceptible species, these may be omitted.
    - It covers all the susceptible species. Again, risk-based approaches may be used to focus on high risk species.
    - All final results must be negative. With imperfect specificity, it is possible and even likely that some screening results will be positive. However, these must be followed up with high-specificity confirmatory tests and must ultimately test negative. If the final result of any test is positive, then it must be concluded that the population is not free from infection.

- Historical monitoring data. If data from previous years exists, this can be used to determine the prior probability of freedom from infection. It is analysed in the same way as current surveillance data to determine the sensitivity of surveillance, and then combined using a Bayesian approach to estimate the historical probability of freedom from infection.
- An estimate of the probability of introduction of disease. After surveillance has been conducted, the probability of freedom from infection can be estimated. However, as there is a constant risk of introduction of infected wildlife into a previously free area, the probability of freedom decays over time. More surveillance is required to provide assurance that the disease has not been introduced. An estimate of the probability of introduction of disease into the MS's territory or the disease-free zone is required. This can be estimated using traditional risk analysis tools.

In addition to these monitoring data, two standards for the analysis and interpretation of the surveillance results are required:

- Design prevalence. The design prevalence specifies the hypothetical prevalence of disease that the surveillance would be able to detect if disease were present. This is sometimes called the 'minimum detectable prevalence' or the 'maximum acceptable prevalence'. This should be based on existing standards in EU legislation (Commission Regulation (EC) No 2075/2005). For *Trichinella*, the design prevalence for wildlife used in this example is 0.1 %.
- Target probability of freedom. This is the threshold above which a MS or zone may be considered free from disease. If evidence from surveillance fails to reach this threshold, the wildlife population is not considered free (even if no positives have been detected). This value is set by national risk mangers. Common values, depending on the level of risk averseness, are 95 % or 99 %.

Analytical approach

For the purposes of this illustration, it is assumed that risk-based sampling is not used, and that all regions and species carry the same risk of being infected with *Trichinella*. Furthermore, it is assumed that the wildlife population is freely mixing and that there are no independent subgroups. If risk-based approaches are used, or independent subgroups are present, analysis is more complicated, and is undertaken using scenario-trees as described in the previously cited publications. Detailed description of these advanced methods is beyond the scope of this document.

Estimation of surveillance sensitivity

For a given year *k*, the sensitivity of surveillance (SSe, or probability that at least one infected animal would be detected by the surveillance system if the population were infected at the design prevalence) can be calculated as:

$$SSe_k = 1 - (1 - P^* \times Se_k)^{n_k}$$

Where:

SSe_k is the surveillance sensitivity in year *k*,

P* is the design prevalence (0.1 %),

Se_k is the individual (digestion) test sensitivity (varying between 40 % and 100 %) in year *k*,

n_k is the number of animals included in the surveillance in year *k*.

The sensitivity of historical surveillance is calculated for each year in the same way. If no surveillance was conducted in a particular year, the sensitivity is 0.

Estimation of probability of freedom

The probability that the population is free from infection, given that the surveillance has not detected any infected animals (*PostPr(free)*, is analogous to the negative predictive value of a test and is calculated using Bayes' Theorem:

$$\text{PostPr}(free) = \frac{PriorPr(free)}{PriorPr(free) + \left[\left(1 - PriorPr(free)\right) \times (1 - SSe)\right]}$$

The prior probability of freedom, *PriorPr(free)* is based on the calculation of the posterior probability of freedom for the previous year, adjusted to take into account the likelihood of introduction of infection into the population during that year:

$$PriorPr(inf)_k = ((PriorPr(Inf)_{k-1}) + Pr(Intro)_k - [(PriorPr(Inf)_{k-1}) \times Pr(Intro)_k])$$

Where:

$\text{PriorPr(free)}_k = 1\text{-PriorPr(Inf)}_k$ and

Pr(Inf)_k is the probability of the introduction of infection in the *k*th year.

By convention, the prior probability of freedom for the year in which surveillance starts (before which no historical data is available) is set to 0.5.

Using the above formulae, the surveillance sensitivity is first calculated for each year, and then iteratively from the earliest surveillance, the probability of freedom for each year up to the current year is calculated.

Uncertainty and variability

The above calculations and examples imply that a point estimate for each value is known. Unfortunately, some values in the calculations are subject to either uncertainty or variability. While the number of animals included in the surveillance should be known, and the design prevalence and starting probability of freedom are fixed as standards, the individual test sensitivity and the probability of introduction are both estimates with some uncertainty and variability. To explicitly capture these, calculations may be performed stochastically, using defined distributions for test sensitivity and the probability of introduction, resulting not in a point estimate of the probability of freedom, but a distribution. In this case, it is common to use a lower percentile (e.g. 20th percentile) of the distribution to assess against the required threshold for demonstrating freedom. For example, the calculated distribution for the probability of freedom may range from 92 % to 99.99 %, and the threshold for accepting freedom has been set at 95 %. If the 20th percentile of the distribution is above 95 %, the population is accepted as free, but if it is below 95 %, not enough evidence has been gathered to demonstrate freedom. This can be addressed by either increasing the surveillance sample size (*n*) or by decreasing the level of uncertainty in the estimates for test sensitivity of probability of introduction of disease.

Practical implementation

Iterative calculation of the annual probability of freedom from *Trichinella* may appear complex and tedious. These calculations are most practically implemented using a spreadsheet template with all the formulae previously entered. In this way, all that is required is to enter the total number of animals tested each year and the probability of freedom is automatically updated.

Surveillance design

If the target for probability of freedom is defined, it is possible to design wildlife surveillance to meet the needs of the HEI. This section illustrates the principles of survey design.

For the first year of surveillance, the prior probability of freedom is 50 % (by convention). In order to generate enough evidence to demonstrate freedom immediately, the following approach can be used. If it is assumed that the target probability of freedom is 99 %, these values can be inserted into the version of Bayes' Theorem described above to calculate the required sensitivity of the surveillance to meet the target (in MS Excel, the solver tool makes this easy). The result is a target surveillance sensitivity of 98.989 %.

The sample size required to achieve this sensitivity can be calculated by solving the formula for surveillance sensitivity (previously shown) for *n*:

$$SSe_k = 1 - (1 - P^* \times Se)^{n_k}$$

$$n_k = \frac{\ln(1 - SSe_k)}{\ln(1 - \mathrm{P}^* \times Se)}$$

$$= \frac{\ln(1 - 99.989\%)}{\ln(1 - 0.1\% \times 40\%)}$$

$$= \frac{-4.59512}{-0.0004}$$

$$= 11{,}486$$

This indicates that a sample size of 11,486 animals would be required to achieve an adequate probability freedom immediately. This sample size would be halved if the assumed sensitivity of the digestion test were doubled (from 40 % to 80 %).

In subsequent years, prior evidence already exists that the population is free, so surveillance only needs to balance the risk of introduction of infection. If this is assumed to be 5 % per year, the subsequent sample size can be calculated as follows.

The adjusted prior probability of freedom in year 2 after surveillance in year 1 is given by the formula shown above:

$$PriorPr(inf)_k = ((PriorPr(Inf)_{k-1}) + Pr(Intro)_k - [(PriorPr(Inf)_{k-1}) \times Pr(Intro)_k])$$

$$= 99\% + 5\% - (99\% \times 5\%)$$
$$= 94.05\%$$

The required surveillance sensitivity to achieve the target probability of freedom (99 %) is again calculated with Bayes' Theorem, as described above, using the adjusted prior probability of 94.05 % just calculated. This results in a target surveillance sensitivity of 84.03 %. The sample size for the second year can then be calculated as:

$$SSe_k = 1 - (1 - P^* \times Se)^{n_k}$$

$$n_k = \frac{\ln(1 - SSe_k)}{\ln(1 - \mathrm{P}^* \times Se)}$$

$$= \frac{\ln(1 - 84.03\%)}{\ln(1 - 0.1\% \times 40\%)}$$

$$= \frac{-1.8346}{-0.0004}$$

$$= 4586$$

This is less than half the sample size required in the first year, but this sample size would need to be continued each year to balance the high risk of introduction of disease in wildlife.

This example has resulted in large sample sizes. In contrast, if the calculations were repeated using more optimistic parameters, the sample sizes would be smaller. For example, using:

- Target probability of freedom: 95 %.
- Digestion test sensitivity: 90 %.
- Probability of introduction of infection: 0.1 % (e.g. an island).

The first year sample size would be 3,679 animals, but, due to the very low risk of introduction, a sample of only 25 animals per year would then be adequate to maintain a probability of freedom of 95 %.

If high levels of wildlife sampling were not possible, but using the same optimistic assumptions, it would be possible to use a smaller sample size of 500 animals per year, and gradually accumulate evidence. Using this approach, it would be possible to achieve 95 % probability of freedom after 9 years of surveillance.

Prevalence at slaughter

If wildlife surveillance meeting the requirements described above is not feasible or desired, the second epidemiological indicator that may be used to justify modifications to meat inspection procedures is the prevalence of infection in individual herds without controlled housing (outdoors, free-range or housed herds not meeting the controlled housing definition). In contrast to the objective of wildlife surveillance (demonstrating that wildlife in a geographical area are free from *Trichinella* infection), the objective for this HEI is to measure the prevalence of infection in individual herds. If the infection is below a defined prevalence threshold, modified meat inspection procedures may be implemented.

Determining the threshold

A threshold of 7 infected animals detected per million is used as an example. Note that the actual figure for each MS will vary based on the approach used by the risk managers (as well as the assumed sensitivity of the digestion test).

Calculating the threshold for non-controlled housing

The calculated threshold represents a global prevalence. Pigs from controlled housing are not at risk of being infected with *Trichinella* and therefore act to dilute the prevalence of infected pigs from non-controlled housing.

$$P_{global} = \frac{P_{outdoor} \times N_{outdoor}}{N_{outdoor} + N_{indoor}}$$

$$P_{outdoor} = \frac{P_{global}(N_{outdoor} + N_{indoor})}{N_{outdoor}}$$

Where:

P is the prevalence

N is the total number o f pigs

indoor relates to the controlled housing and

outdoor relates to non-controlled housing

To illustrate, let us assume that:

N_{indoor} = 10 million
$N_{outdoor}$ = 1 million

$$P_{outdoor} = \frac{P_{global}(N_{outdoor} + N_{indoor})}{N_{outdoor}}$$

$$= \frac{0.000007 \times (10000000 + 1000000)}{1000000}$$

$$= 0.000077 \text{ or } 77 \text{ per million}$$

This means that the apparent prevalence from farms with non-controlled housing could be up to 77 per million and still not exceed the target global prevalence of 7 per million, due to the dilution effect from those farms with controlled housing.

At the farm level, this means that, in order to avoid testing all animals for *Trichinella* the farm would need to demonstrate that the prevalence is less than 0.0077 %. Traditional survey sample size calculations (for example using *HerdAcc* (Jordan and McEwen, 1998) or *FreeCalc* (Cameron and Baldock, 1998) indicate that a sample size of almost 40,000 animals would be required to achieve this. This is clearly impractical in most circumstances.

The conclusion (based on the values used in this example) would be that farms with non-controlled housing should continue to test all pigs. It may be possible after a number of years for large farms to have accumulated enough evidence to show that the prevalence is lower than the target threshold.

Examples of the results of analysis of existing data

Probability of freedom in wildlife

Data from the annual CSRs and EUSR on zoonoses and food-borne outbreaks reported by the EU MSs were used to illustrate the process of estimating the probability that each reporting MS was free from *Trichinella* in wildlife, using the analytical approach illustrated above.

The sensitivity of the digestion test was assumed to be 40 % and the probability of introduction of infection was assumed to be 5 % per year. A design prevalence of 0.1 % was used.

The analysis assumed that surveillance was representative and that all wildlife species shared the same risk of being infected.

For the analysis, wildlife were defined in terms of the data available in two columns, species_level_1 (the species of the animal) and species_level_2 (a categorisation of species groups). In

species_level_2, only those animals classified as 'wild' were included. The species listed in Table 29 were included. Species excluded were birds and herbivore species.

Table 29: Species included in the definition of 'wildlife' for *Trichinella* surveillance

Species	Total tests	Proportion
Wild boars	5546172	97.82 %
Foxes	77086	1.36 %
Other wildlife	18233	0.32 %
Wild animals	17962	0.32 %
Bears	2643	0.05 %
Lynx	2112	0.04 %
Raccoon dogs	1959	0.03 %
Badgers	1313	0.02 %
Rats	990	0.02 %
Rodents	535	0.01 %
Wolves	432	0.01 %
Minks	173	0.00 %
Marten	156	0.00 %
Raccoons	153	0.00 %
Polecats	110	0.00 %
Wolverine	6	0.00 %
Mice	5	0.00 %
Beech Marten	4	0.00 %
Weasel	2	0.00 %

The annual surveillance sensitivity (expressed as a proportion) for MSs was calculated and is shown in the Table 30. A sensitivity of zero indicates that no surveillance was performed in that year.

Table 30: Annual sensitivity of surveillance for *Trichinella* in wildlife in MSs

MS	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
1	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
2	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6342	0.2020	0.8281	0.9337
4	0.2007	0.3324	0.3647	0.6126	0.6945	0.3783	0.1106	0.2030	0.3015	0.3043
5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0854	0.0000	0.0000	0.0000	0.0103
6	0.9995	0.0000	0.0000	0.0000	1.0000	0.9034	1.0000	0.3743	1.0000	0.9999
7	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	1.0000	0.0000	0.3443	1.0000
8	0.6621	0.4660	0.6441	0.5756	0.2771	0.1362	0.1170	0.4126	0.7919	0.6511
9	0.0000	0.0000	0.0000	0.0000	0.8880	0.4420	0.1914	0.6380	0.4610	0.2993
10	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
11	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	1.0000	0.9997	0.9974	1.0000
12	0.0000	0.0000	0.0000	0.0000	0.0012	0.0016	0.0004	0.0004	0.0004	0.0004
13	0.0000	0.0000	0.0000	0.0000	0.9982	0.9956	0.9869	0.9936	0.9944	0.9941
14	0.5942	0.0773	0.1226	0.1258	0.2434	0.2354	0.2315	0.2440	0.3260	0.2589
15	0.0000	0.0000	0.0000	0.0000	0.9929	0.6603	0.6482	0.6703	0.8223	0.8316
16	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.8175	0.8303	0.9498	0.9597
17	0.0000	0.0000	0.0000	0.0000	0.9750	0.9732	0.9762	0.0000	0.9993	0.9999
18	0.4539	0.4517	0.7909	0.0000	0.1550	0.0000	0.0040	0.0036	0.4753	0.3988
19	0.0111	0.0000	0.1604	0.0135	0.0127	0.0000	0.0036	0.0000	0.0000	0.0739
20	0.0000	0.9962	0.0000	0.9991	0.9663	0.9891	0.9762	0.9960	0.9977	0.9872
21	0.2065	0.2184	0.9463	0.5767	0.9242	0.9441	0.9904	0.9993	1.0000	1.0000
22	0.9999	0.9955	1.0000	0.9608	1.0000	0.7736	0.0000	0.0000	0.9902	0.0040
23	0.0000	0.0000	0.0000	0.0000	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000
24	0.2093	0.0000	0.0000	0.0438	0.3425	0.2339	0.2443	0.6499	0.2231	0.3004
25	0.4521	0.7389	0.6616	0.6409	0.3665	0.4626	0.0000	0.0000	0.1272	0.1067
26	0.0000	0.0000	0.0000	0.0000	0.3356	0.3249	0.3972	0.4617	0.5759	0.5925
27	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

The annual probability of freedom from *Trichinella* in wildlife was calculated. When positive results were found, the probability of freedom was set to zero. For the subsequent year (if no further positives were found) the prior probability of freedom was set to 0.5. The cumulative results for these calculations for 2009 (the latest year for which data was available), are shown in Table 31. MSs with probability of freedom exceeding an example threshold of 99 % are highlighted in green.

Table 31: Probability of wildlife freedom from *Trichinella* in MSs in 2009, and evaluated status (using 99 % as the threshold for free status)

Country	P(free) 2009	Status
1	0.000 %	Infected
2	0.000 %	Infected
3	93.779 %	Infected
4	87.484 %	Infected
5	33.484 %	Infected
6	0.000 %	Infected
7	0.000 %	Infected
8	74.135 %	Infected
9	0.000 %	Infected
10	0.000 %	Infected
11	0.000 %	Infected
12	31.599 %	Infected
13	0.000 %	Infected
14	0.000 %	Infected
15	0.000 %	Infected
16	0.000 %	Infected
17	0.000 %	Infected
18	0.000 %	Infected
19	37.075 %	Infected
20	99.929 %	Free
21	0.000 %	Infected
22	94.852 %	Infected
23	100.000 %	Free
24	0.000 %	Infected
25	52.818 %	Infected
26	0.000 %	Infected
27	31.512 %	Infected

The progressive probability of freedom in three example countries by year is shown in Figure 15. Country A has a high level of surveillance and achieves high surveillance sensitivity each year (except 2000). While it detected the presence of *Trichinella* in 2004 and 2007 (dropping the probability of freedom) the high surveillance sensitivity meant that it was able to immediately regain a high probability of freedom.

Country B has a lower level of surveillance. Initially, this was adequate to increase the probability of freedom steadily, but from around 2005, the level of surveillance dropped and the probability of freedom decreased due to the risk of introduction of infection. In 2008, *Trichinella* was detected, and 2009 surveillance was inadequate to increase significantly the probability of freedom.

Country C has irregular surveillance, and, when it is carried out, the sensitivity is poor due to a small sample size. Apart from small fluctuations, the probability of freedom progressively decreases from the starting default value of 0.5, due to the ongoing risk of introduction of infection.

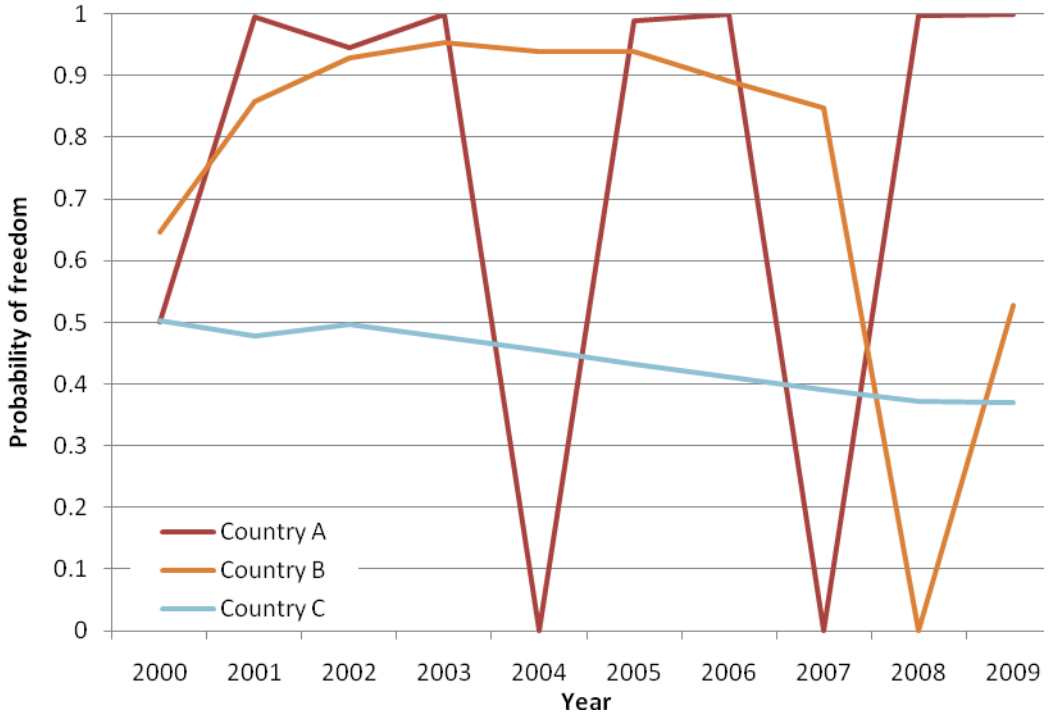

Figure 15: Annual probability of freedom from *Trichinella* in wildlife for three example countries.

a. Prevalence of *Trichinella* in establishments without controlled housing

Data from the CSRs and EUSRs and baseline surveys were used to illustrate the process to estimate the average prevalence of *Trichinella* in pigs from non-controlled housing and overall (all slaughter pigs including both controlled and non-controlled housing).

The key data was the number of pigs tested positive by MS and year. The denominator provided in the CSRs and EUSR (the number of tests performed) was judged to be unreliable, as different countries have different testing strategies including partial testing in some cases and dual testing (digestion and trichinoscopy) in others. Instead, the total number of slaughter pigs, based on baseline surveys, was used as the denominator.

The numerator was based on the CSRs and EUSR data and included all animals classified as 'pigs' in the species_level_1 field. It was assumed that all positive test results were from pigs in non-controlled housing.

Baseline surveys provided data on the number of farms classified as controlled housing and non-controlled housing for each MS. The denominator for estimation of the prevalence amongst pigs from non-controlled housing is the number of pigs in non-controlled housing. This figure was not directly available, but was estimated by assuming that the proportion of pigs from non-controlled housing was the same as the proportion of farms with non-controlled housing. This assumption is unlikely to be perfectly correct, as it is probable that farms with controlled housing will tend to be larger than those without, however, inadequate data were available to calculate the difference in average farm size.

The baseline surveys indicated that there were no farms with non-controlled housing in a number of countries. For these countries, only the overall prevalence could be calculated. The estimated average prevalence of *Trichinella* infected slaughter pigs from non-controlled housing farms and from all farms, broken down by country, is shown below. This average is calculated across all farms and all years for which data is available. Countries with an average prevalence greater than the example threshold prevalence (0.000668 %) are highlighted in red (Table 32).

Table 32: Estimated average *Trichinella* prevalence over all farms and years in pigs from non-controlled housing and all farmed pigs

Country	Non-controlled housing	All farms
1	0 %	0 %
2	0.00021 %	0.00000 %
3		0.00525 %
4		0.00257 %
5		0 %
6		0.00001 %
7	0.01009 %	0.00011 %
8		0 %
9		0.00005 %
10		0 %
11		0 %
12		0 %
13		0.01236 %
14	0.64055 %	0.00013 %
15		0 %
16	0 %	0 %
17		0 %
18	0.09320 %	0.00019 %
19		0 %
20		0 %
21	0.00208 %	0.00010 %
22	0 %	0 %
22		0 %
23	0 %	0 %
24	0.00862 %	0.00000 %
25	0 %	0 %
26	0.00121 %	0.00001 %
27		0 %

Conclusions on the use of existing data from the CSRs and EUSR

The analysis of wildlife and pigs testing data presented here indicates that it is possible to use existing data to illustrate the calculation of the HEIs to inform decisions on modifications to meat inspection procedures. However, some limitations were encountered.

Wildlife

The species_level_2 field in the EFSA's zoonoses database differentiates wildlife from other groups. The only species (species_level_1) for which this data has sometimes not been completed is 'pigs' so it is considered that this classification is a reliable means of identifying records relating to wildlife in the data. The species_level_1 field, providing the specific species of animals tested, has no missing data. There are a tiny number of animals for which the classification is unclear ('other animals') but the proportion is so small as to have no real impact.

In principle, the data from EFSA's zoonoses database are therefore appropriate for the analysis of region or country freedom from *Trichinella* infection. However, the most important constraint relates to information on the way the data was collected, its coverage and representativeness.

Wildlife surveillance is particularly challenging. The value of the EFSA's zoonoses database would be increased if they included information on the way in which surveillance was conducted, allowing analysts to assess the population coverage and degree of representativeness of the surveillance.

Non-controlled housing

The EFSA's zoonoses database contains no field allowing clear distinction between pigs from farms with controlled housing and those from farms without controlled housing. The baseline surveys are the only currently available source of data on the proportion of farms with and without controlled housing, but they only provide data at the farm rather than the animal level.

Conceptually, farms without controlled housing should be classified on a farm-by-farm basis. Nevertheless, if a global analysis, such as that performed here, indicates that the overall prevalence of *Trichinella* in a MS in both controlled and non-controlled housing combined is below the target threshold, it may provide risk managers with adequate information to decide to modify meat inspection procedures.

Annex 5. Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
CSR	Community Summary Report
CVO	Chief Veterinary Officer
DNA	Deoxyribonucleic acid
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ELISA	Enzyme linked immunosorbent assay
EU	European Union
EURL	European Union Reference Laboratory
EUSR	European Union Summary Report
FERG	Food-borne Disease Burden Epidemiology Reference Group
HEI	Harmonised Epidemiological Indicator
MS	Member State
MAA	*Mycobacterium avium* subsp. *avium*
MAC	*Mycobacterium avium* complex
MIRU-VNTR	Mycobacterial interspersed repetitive unit- variable number tandem repeat
MTC	*Mycobacterium tuberculosis* complex
NCC	Neurocysticercosis
NTM	Non-tuberculous mycobacteria
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
ToR	Terms of Reference
VNTR	Variable number tandem repeat
WHO	World Health Organisation