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Technical specifications on harmonised epidemiological indicators for biological hazards to be covered by meat inspection of farmed game

Epidemiological indicators for meat inspection of farmed game

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SCIENTIFIC REPORT OF EFSA

Technical specifications on harmonised epidemiological indicators for biological hazards to be covered by meat inspection of farmed game[1]

European Food Safety Authority[2,3]

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ABSTRACT

In this report, harmonised epidemiological indicators are proposed for foodborne biological hazards to public health that are related to farmed game and meat thereof and that can be addressed within meat inspection. These hazards include *Salmonella, Toxoplasma*, *Trichinella* and *Mycobacterium* in farmed wild boar and deer. An epidemiological indicator is defined as the prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard that correlates to the human health risk caused by the hazard. The indicators can be used by the European Commission and 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 revised meat inspection system for farmed game meat outlined in the European Food Safety Authority scientific opinion, particularly to help categorise slaughter batches, animals and slaughterhouses according to the risk related to the hazards and process hygiene or to enable surveillance for the possible emergence of the hazard. Depending on the purpose and the epidemiological situation, risk managers should decide on the most appropriate indicator(s) to use, either alone or in combination, at national, regional, slaughterhouse or farm/herd level. Member States are invited to report data generated by the implementation of the indicators 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.

KEY WORDS

Meat inspection, epidemiological indicators, farmed game, *Salmonella*, *Toxoplasma, Trichinella*, *Mycobacterium*.

SUMMARY

The European Commission has requested that the European Food Safety Authority provide technical assistance on harmonised epidemiological criteria (harmonised epidemiological indicators, HEIs) for specific public health hazards in food and animals, to be used by risk managers when they consider that the current methods of 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 scientific opinion on the public health hazards to be covered by inspection of meat from farmed game (EFSA BIOHAZ Panel, 2013a) and this report under this mandate concern the meat inspection of farmed game and they were published in June 2013.

In this report, harmonised epidemiological indicators are proposed for foodborne biological hazards to public health that are related to farmed game and meat thereof and that can be addressed within meat inspection. These hazards include *Salmonella* and *Trichinella* in farmed wild boar as well as *Toxoplasma* and *Mycobacterium* in farmed wild boar and farmed deer. An epidemiological indicator is understood to mean the prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard (such as audits or evaluation of process hygiene) that correlates to a human health risk caused by the hazard. The epidemiological indicators can be used by the European Commission and Member States to consider when adaptations to meat inspection methods may be relevant and to enable Member States to carry out risk analysis to support any such decisions. It is foreseen that the epidemiological indicators will be used in the revised meat inspection system for farmed game outlined in the scientific opinion on the public health hazards to be covered by inspection of meat from farmed game, particularly to help to categorise slaughter batches, animals and slaughterhouses according to the risks related to particular hazards or level of process hygiene.

The risk managers should decide on the most appropriate use of the epidemiological indicators at 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 combination. Most of the epidemiological indicators are proposed for farmed wild boar and deer and their carcasses at the farm or slaughterhouse level. Auditing of farms for controlled husbandry conditions was not considered feasible as an indicator for hazards in farmed wild boar and deer production.

The proposed indicators for *Salmonella*, *Toxoplasma* and *Trichinella* may be applied to classify slaughter batches and animals according to the infection status or risks related to the hazard. An indicator for *Salmonella* may also be used to evaluate the measures taken in slaughterhouses to control the hazard or to assess process hygiene. In case of *Mycobacterium,* epidemiological indicators are suggested to enable surveillance for the possible emergence of the hazard.

Comparable data from European Union Member States were available for only one of the proposed epidemiological indicators, relating to *Trichinella.*

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 at which the sampling should take place, the sampling strategy, the type and details of the specimen to be taken, the diagnostic or analytical method to be used, and a case definition.

The implementation of the proposed epidemiological indicators will generate additional data that will provide a more precise picture of the epidemiological situation in the EU and these data may be used to update the indicators, when appropriate. It is recommended that the Member States report the data generated from implementation of these indicators in accordance with and using the framework prescribed in Directive 2003/99/EC. The proposed indicators should be reviewed regularly in the light of new information and the data generated by their implementation.

TABLE OF CONTENTS

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.

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[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, pp. 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, pp. 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.

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[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, pp. 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, pp. 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, pp. 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, pp. 55–205.

TECHNICAL SPECIFICATIONS

1. Introduction

There are a number of foodborne diseases affecting humans that can be related to the consumption of meat from farmed game. These hazards include parasites, bacteria and a virus (EFSA BIOHAZ Panel, 2013a). The relevant hazards related to farmed game meat 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 foodborne hazards, and in fact *Trichinella* is directly targeted through the current meat inspection procedures for wild boar and *Mycobacterium* through inspection procedures for wild boar and farmed deer (Regulation (EC) No 853/2004). However, most of the other biological hazards related to farmed game and meat thereof are not specifically addressed by the meat inspection system in place in the European Union (EU).

It is possible to use the data on the prevalence and incidence of biological hazards in animals, meat and humans as one aspect of the criteria when determining and ranking the importance to human health of the hazards to be covered by meat inspection. These epidemiological criteria or indicators may be used by risk managers when considering adaptations to current meat inspection methods for farmed game. In the case of *Trichinella*, *Mycobacterium*, *Toxoplasma* and *Salmonella*, relevant prevalence and foodborne outbreak 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. Data on the incidence of foodborne 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 from EFSA on the public health hazards to be covered by inspection of meat from farmed game (EFSA BIOHAZ Panel, 2013a) proposes some changes in the meat inspection of farmed game as regards 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 Scientific Opinion.

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[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, pp. 1–7.

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.

Biosecurity - Implementation of measures that reduce the risk of introduction and/or 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.

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

Controlled husbandry conditions - A type of animal husbandry in which farmed animals are kept at all times and for their whole life under conditions that effectively exclude all relevant risk factors or maintain a constant level of risk. Such conditions are controlled by the food business operator with regard to feeding, hygiene and the biosecurity of the holding.

Farmed deer - All species of deer that are farmed. The species include particularly red deer (*Cervus elaphus*) and fallow deer (*Dama dama*), but other species such as roe deer (*Capreolus capreolus*), sika deer (*Cervus nippon*) and wapiti deer (*Cervus canadensis*) may also be farmed.

Farmed game - According to Regulation (EC) No 853/2004 "Farmed game" is defined as "farmed ratites (e.g. ostrich) and farmed land mammals other than domestic bovine (including *Bubalus* and bison species), porcine, ovine and caprine animals and domestic solipeds (mammals with a single hoof on each foot e.g. horse)". In line with the Commission's mandate, in this report lagomorphs, i.e. farmed rabbits and hares, are considered to be farmed game despite the fact that Regulation 853/2004/EC addresses farmed game and farmed lagomorphs separately. Of these animal species, only animals that are bred, reared and slaughtered in captivity are considered farmed game.

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

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

Slaughterhouse – An 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, concentration or incidence of the hazard at a certain stage of the food chain that correlates to the human health risk caused by the hazard. Indirect indicators of the hazards, such as audits of farms or evaluation of process hygiene, are also covered.

The purpose of the harmonised epidemiological indicators proposed in this report is to enable the European Commission (EC) and MSs to consider whether adaptations to meat inspection methods may be made at the Member State 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. The hazards addressed in this report were those covered by the current meat inspection protocols and those identified in the complementary EFSA scientific opinion on the public health hazards to be covered by inspection of meat from farmed game (EFSA BIOHAZ Panel, 2013a) as being the most relevant in the context of meat inspection of farmed game. The epidemiological indicators provide information to be used in the integrated food safety assurance system outlined in the EFSA scientific opinion. This applies particularly in the process of classification of the farms/herds/flocks and slaughterhouses according to risk related to a particular hazard as well as the setting of related targets. The indicators, either alone or in combination, may be used by risk managers at the national, regional, slaughterhouse or farm/flock/herd level depending on the purpose.

The principles applied in the identification of the appropriate indicators in this report 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 is situated.

- The key epidemiological indicator for a given hazard will almost always be the prevalence (or concentration (counts)) of the hazard in the animal population or in the food.

- The identification of a range of risk factors is not, in itself, sufficient. The impact of these risk factors on public health must also be estimated when amendments to the current meat inspection methods are considered. The impact may be measured by estimating the prevalence (or concentration) 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 term of reference (ToR)):

- The hazard and, when appropriate, its life cycle is described. The current epidemiological situation within the EU, as regards both animals and humans, is evaluated, and the role of farmed game as the source of human infections is discussed for each hazard.

- For each hazard, the main farmed game food chain 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 receive the highest scores are selected.

Following the selection of the harmonised epidemiological indicators, the available data from the annual reporting in accordance with Directive 2003/99/EC were reviewed for comparable data from MSs. These comparable data are presented in chapter 7 (the second ToR).

In the cases in which no comparable data are 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 at which the sampling should take place, the type and details of the specimen to be taken, the 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.

3.2. The biological hazards addressed

The first ToR of the mandate for technical assistance from the Commission asks for the harmonised epidemiological indicators to be defined for specific hazards already covered by current meat inspection (such as trichinellosis, tuberculosis, cysticercosis, etc.). In the case of meat inspection of farmed game, *Trichinella* in wild boar and *Mycobacterium* (tuberculosis) in farmed deer and farmed wild boar are such hazards.

In addition, according to the first ToR, the epidemiological indicators for possible additional hazards identified in a scientific opinion on the hazards to be covered by inspection of meat from farmed game (EFSA BIOHAZ Panel, 2013a), which can be used to consider adaptations of meat inspection methodology, should be addressed as well. The EFSA scientific opinion identifies *Toxoplasma* in farmed deer and farmed wild boar and *Salmonella* in farmed wild boar as such hazards.

4. Farmed game production system

To support its work on the opinions and reports on the public health hazards to be covered by inspection of meat from farmed game EFSA organised a technical hearing with the relevant EU stakeholder organisations. The outcome of this technical hearing is published as an event report on the EFSA website (EFSA, 2012). Moreover, in 2012, the Biological Monitoring unit (BIOMO) of EFSA carried out a questionnaire survey (hereafter referred as the BIOMO questionnaire survey)[11] among the members of the Task Force on Zoonoses Data Collection, EFSA's network representing the reporting MSs and some other reporting European countries, to obtain information about the production of and farming systems for wild boar and farmed deer in place. A total of 11 countries replied to the questionnaire as regards wild boar and 17 countries as regards farmed deer.

4.1. Farmed deer

In Europe, 280 000 deer are farmed yearly, predominantly red deer and fallow deer, but the numbers of farms and size of production varies widely between countries (EFSA, 2012). In this report only animals that are bred, reared and slaughtered in captivity are considered farmed deer. The BIOMO questionnaire survey revealed that the number of holdings in the responding countries varied from approximately 1 800 to around 15, with two countries reporting no farmed deer holdings and the majority reporting 200–800 holdings. In most countries the holdings held around 15–30 animals, although a few countries had averages around 150–200. Generally, the picture was either many small herds or a few larger herds in a country. Results from the questionnaires concur fairly well with those of the technical hearing held by EFSA, which mentions that the country with the most deer holdings has 4 600 and the average size of an EU holding is 27 animals (EFSA, 2012). In all responding countries farmed deer are reared extensively outdoors on grass with access to outdoor shelters depending on the local weather conditions. Farmed deer are produced in a similar way to extensively reared beef cattle, but require higher fences, special handling facilities and trained keepers to accommodate the temperament of the deer (Teagasc, online). Farmed deer are mainly raised on grass (pastures), but the feed is usually supplemented with vitamins, minerals, hay, straw and concentrates, especially in the winter. In northern countries, only young stock (calves) are housed during their first winter to protect them against the elements (Scottish Venison, online). Drinking water is often surface water, but in some responding countries water with drinking water quality was provided. Some responding countries mentioned that rodent control was practised, when the deer were kept inside, but access by cats was common all year around. The majority of farmed deer are slaughtered at around 15–17 months of age. The maximum age for slaughter is 27 months, after which the meat becomes progressively tougher. Older culled deer are predominantly used in processed products. Most deer are killed on-farm in order to avoid the stress of transport, and for them an ante-mortem inspection is carried out on-farm. Bleeding is done immediately after killing and, if facilities are available, evisceration can also be done on-farm. All farmed deer are then transported to slaughter houses for further processing and the number of deer slaughter per day is usually very low (EFSA, 2012). Live transported live deer must have their antlers removed on-farm. Breeding is seasonal and individual premises tend to deliver animals for slaughter at most once a year (BDFPA, online).

4.2. Farmed wild boar

In general, very little information is available on farmed wild boar populations in the EU (EFSA, 2012; EFSA BIOHAZ Panel, 2013a). The BIOMO questionnaire survey revealed that the number of holdings of farmed wild boar in countries is small and each holding usually has fewer than 30 animals per holding. Some countries report having a few larger holdings with around 150 animals, but there was some confusion in the replies at to whether these were farmed wild boar or wild boar reared for hunting. Farmed wild boar are reared in external systems or as backyard pigs. Husbandry conditions often attempt to mimic their natural habitat, allowing access to woodland and surface water. However,

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[11] EFSA (European Food Safety Authority), 2013. Internal report on results of questionnaires on farmed game production systems and husbandry practices in EU in 2012. 2013:IN-254.

many are reared on pastureland in large paddocks with arks or other free range shelters, similar to the production of free range pigs (Booth, 1995). Feed, including compound feed, grass, vegetables, silage, hay, fruits and grain, is always provided. Most responding countries reported that drinking water was derived from wells, public water sources and sometimes natural water sources. Rodent controls were applied on some farms and cats mostly had a free access to the premises. Breeding is seasonal and one sow usually produces one litter a year. The pigs are slaughtered at around the age of 9–12 months (CALU, 2007). The animals are usually killed and bled at the farm after a required ante-mortem inspection and transported to slaughter houses for processing.

4.3. Controlled husbandry conditions

As farmed deer and farmed wild boar are mainly reared outdoors, and in the light of the information received on their husbandry conditions, it is considered problematic to adequately control access of free-roaming animals, such as cats or wildlife, to the farm premises. This control of access would effectively reduce the risk of introducing biological hazards that are commonly found in these animals to the farm. Therefore, controlled husbandry conditions were not considered relevant for farmed game species. Furthermore, owing to the lack of options for controlling the environment and risk factors on-farm (risk of introduction), it is unlikely that the biological hazard status of one slaughter batch will be a predictor for the status of the following slaughter batches from the same farm.

Farming practices may change in the future and, if production becomes more intensive and moves towards indoor rearing, it is proposed that the controlled housing conditions for pigs would be appropriate to farmed wild boar and the controlled husbandry conditions for cattle or small ruminants would be appropriate for deer.

The BIOMO questionnaire survey revealed some confusion about the definition of farmed game in the EU. Many farmed game holdings reported that they were rearing animals to be released for hunting. These animals would be considered in this document as hunted game and they would be slaughtered according to a different set of meat inspection rules, without ante-mortem inspection, independently of how they were originally reared. Thus, it appears that the real number of farmed gamed holdings in the EU is not precisely known.

5. Epidemiological indicators for the biological hazards

5.1. *Salmonella* in wild boar

5.1.1. Introduction

Salmonella has long been recognised as an important zoonotic pathogen 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 subspecies and most zoonotic *Salmonella* belong to the subspecies *S. enterica* subsp. *enterica*. In the following text, the organisms are identified by genus followed by serovar (e.g. *S. enterica* subsp. *enterica* serovar Typhimurium or *S.* Typhimurium). More than 2 600 serovars of *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, following an incubation period of 12–36 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. When *Salmonella* causes systemic infections, such as septicaemia, effective antimicrobials are essential for treatment. Salmonellosis has also been associated with long-term and sometimes chronic sequelae, e.g. reactive arthritis. Mortality is usually low, and less than 1 % of reported *Salmonella* cases in humans have been fatal.

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 into food processing areas and are allowed to multiply in food (e.g. owing 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.

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 (EFSA and ECDC, 2013).

5.1.2. Current situation and trends in the EU

In the framework of the annual data collection in accordance with Directive 2003/99/EC some data on the occurrence of *Salmonella* in wild boar (including farmed, wild and of unspecified origin) are submitted by EU MSs. During the years 2004–2011 a total of 2 070 wild boar were reported to be tested for *Salmonella* and 326 (15.7 %) of these samples were found positive (EFSA, 2005, 2006a, 2007a, 2009c, 2010; EFSA and ECDC, 2011, 2012, 2013). Regarding meat from wild boar, 1 075 single meat samples were tested in the same time period, and in 15 (1.4 %) of them *Salmonella* was detected. In meat at batch level 2 (3.4 %) out of 58 batches tested were contaminated with *Salmonella.* Data reported to originate from clinical cases are not included in the above description.

Wild boar have also been shown to be a reservoir of *Salmonella* in a number of scientific publications. Data on the occurrence of *Salmonella* sero-converted wild boar are presented in Table 1, and data on the occurrence of cultured *Salmonella* in wild boar are presented in Table 2. The data in Table 1 indicate that a high proportion of wild boar are harbouring antibodies against *Salmonella* O antigens. The presence of antibodies indicates that the animal has been challenged with *Salmonella* at a certain point but does not necessarily reflect the infection status of the animal at the time of testing.

As can be seen in Table 2, *Salmonella* can also frequently be isolated from wild boar. The diversity of serovars in wild boar is considerable, as shown in Table 3.

Table 1: Occurrence of antibodies against selected O antigens of *Salmonella* spp. in hunted wild boar

Country	No of samples	Detected serogroups	Positive (%)	Reference
Greece	140	NI[a]	9 (15 %)	Billinis, 2013
Italy	383	B, C1, D	255 (67 %)	Zottola et al., 2013
Italy	342	B, C1, D	66 (19 %)	Montagnaro et al., 2010
Slovenia	178	B, C1, D	85 (47 %)	Vengust et al., 2006

(a): No information.

Table 2: Occurrence of *Salmonella* in hunted wild boar by culturing

Country	Sample type	No of samples	Positive (%)	Reference
Australia	Faeces	543	223 (41 %)	Cowled et al., 2012
Italy	Faeces	2 365	441 (19 %)	Magnino et al., 2011
Italy	Carcasses	65	0	Avagnina et al., 2012
Italy	Faeces	499	54 (11 %)	Zottola et al., 2013
Portugal	Faeces	77	17 (22 %)	Vieira-Pinto et al., 2011
Spain	Faeces	148	70 (47 %)	Mentaberre et al., 2013
Sweden	Faeces	66	0	Wahlstrom et al., 2003
Switzerland	Tonsils	158	8 (5 %)	Wacheck et al., 2010
Switzerland	Faeces	73	0	Wacheck et al., 2010
United States	Faeces	161	8 (5 %)	Thakur et al., 2011

Table 3: *Salmonella* serovars found in strains isolated from hunted wild boar

Country	Serovar[a]	Reference
Italy	Coeln (81), Typhimurium (74), Ball (43), Thompson (37), Veneziana (37), Enteritidis (18), Infantis (5)[b]	Magnino et al., 2011
Italy	Fischerhuette (4), Veneziana 83), Napoli (3), Kottbus (3), Thompson (3), Toulon (2), Burgas (1), Cholerasuis (1), Ferruch (1), Paratyphi (1), Stanleyville (1), Tennelhone (1), Typhimurium (1)[c]	Zottola et al., 2013
Portugal	Typhimurium (11), Rissen (6)	Vieira-Pinto et al., 2011
Spain	Meleagridis (20), Anatum (9), Muenster (9), Enteritidis (4), Newport (4), Mbandaka (2), Otmarschen (2), Spartel (2), Infantis (1), Kottbus (1), Mikawasima (1), Offa (1), Sangera (1), Stanley (1), Stoneferry (1), Tomegbe (1)[d]	Mentaberre et al., 2013
Switzerland	Enteritidis (6), Stourbridge (1), Veneziana (1)	Wacheck et al., 2010
United States	Bareilly, Berta, Braenderup, Inverness[e]	Thakur et al., 2011

(a): Number of strains in brackets.
(b): Subspecies *S*. diarizonae (IIIb) was also isolated.
(c): Subspecies *S*. salamae (II), arizonae (IIIa), diarizonae (IIIb) and houtenae (IV) were also isolated.
(d): Subspecies *S*. arizonae (IIIa) and some other *Salmonella* sp. serovars were also isolated.
(e): Subspecies III and IV were also isolated.

5.1.3. Farmed wild boar as a source of *Salmonella* infection for humans

In the framework of data collection on foodborne outbreaks in accordance with Directive 2003/99/EC, no *Salmonella* outbreaks caused by farmed wild boar meat were reported in 2007–2011 (EFSA, 2009c, 2010; EFSA and ECDC, 2011, 2012, 2013). However, Nogareda et al. (2011) reported an outbreak of *S*. Enteritidis in France from the year 2011, which was associated with consumption of meat from hunted wild boar. The importance of farmed wild boar as a source of human salmonellosis is not clear. Wild boar harbours different *Salmonella* serovars, and several of these serovars have also been

identified in human cases. The annual consumption of meat from game (farmed and wild game) is estimated to be low in Europe, and *Salmonella* has only sporadically been detected from the carcase or meat cuts of wild boar (Paulsen et al., 2012).

5.1.4. Risk and protective factors

There is not much knowledge about the risk and protective factors for *Salmonella* in farmed wild boar. It has not been possible to find any systematic studies on risk factors, neither in the scientific literature nor by consulting the different MSs. The controlled housing conditions that are used for production of slaughter pigs are not used for the production of farmed wild boar. Farmed wild boar are fenced, but they still have the opportunity to interact with animals from the wild fauna, including rodents and birds. The potential contact between farmed wild boar and free-living wild animals may result in transmission of *Salmonella* between the animals. Farmed wild boar may further be exposed to *Salmonella* if the provided feedstuffs are contaminated with *Salmonella*. The omnivorous nature of wild boar and the fact that farmed wild boar often drink surface water (as recorded in the BIOMO questionnaire survey) are factors that increase the risk for transmission of *Salmonella* between animals. In fact, farming wild boar may provide a higher risk for enteric pathogens such as *Salmonella* owing to crowding of animals and their closer contact with humans and other farm animals than free-living wild boar (Paulsen et al., 2012). The impact of the general occurrence of *Salmonella* in livestock, pet and wildlife animals in different geographical settings is not known, but a high density of *Salmonella*-infected animals could well be a risk factor for the presence of *Salmonella* in wild farmed boar living in the same area.

If farmed wild boar are killed and eviscerated on the farm before being transported to the slaughterhouse for further processing, this practice could also lead to an increased risk of contamination of the carcases with enteric bacterial pathogens if the evisceration is done under poor hygiene conditions.

Wild boar meat is in most cases treated in the same way as pork by consumers. The meat is eaten as cuts or minced meat, and some of the meat is used for the production of ready-to-eat meat products, such as salami and cured ham. If wild boar meat is used in the production of meat products it is particularly important that the preservation process (fermentation, salt content, pH control, heat treatment, etc.) ensures that the products are safe with regard to *Salmonella*.

5.1.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected for *Salmonella* in farmed wild boar (Table 4 and Figure 1).

Table 4: Harmonised epidemiological indicators (HEIs) for Salmonella for farmed wild boar

Indicators (animal/food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
HEI 1: *Salmonella* in farmed wild boar before slaughter	Farm	Microbiology (detection, isolation and serotyping)	Pooled faeces sample
HEI 2: *Salmonella* in/on farmed wild boar carcasses after slaughter but before chilling	Slaughterhouse	Microbiology (detection, isolation and serotyping)	Carcass 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 Appendix A.

Microbiological testing of either pooled faeces samples or carcase swabs are the analytical methods proposed for the HEIs. The microbiological testing will provide isolates that can be serotyped, and investigated further with adequate typing techniques such as antimicrobial resistance testing. The

typing results will be useful in assessing the human pathogenic potential of the serovars that prevail in farmed wild boar.

HEI 1 focuses on the provision of information on the occurrence of the *Salmonella* and the serovars present on the farm producing wild boar. The harmonised epidemiological indicator (HEI) gives information on the *Salmonella* infection status of the incoming slaughter batches to the slaughterhouse, thus facilitating the classification of the slaughter batches into high and low risk. Regular sampling of wild boar from the same farm will enable the *Salmonella* status of the farm to be trended over time and provide historical information about the farm.

HEI 2 focuses on providing an indicator of the process hygiene on a slaughter line by measuring the presence of *Salmonella* on wild boar carcases before chilling. The HEI also provides information on the *Salmonella* contamination of the wild boar carcases and meat that is placed on the market. Sampling by swabbing is performed prior to chilling rather than after it, as it is easier to recover and cultivate *Salmonella* bacteria before the chilling of the carcase. The historical data from the implementation of HEI 2 gives information on the performance of the slaughterhouse with regard to process hygiene and *Salmonella* control.

The scientific opinion on public health hazards to be covered by inspection of meat (swine) (EFSA Panel on Biological Hazards (BIOHAZ), 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, 2011). This is because pathogens occur in animals/on carcases relatively rarely, are also affected 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 and so is more related to the setting of targets for slaughterhouses.

Serological testing for *Salmonella* has not been included as a HEI. The reason for this is that it seems that there is a high prevalence of seroconverted wild boar and the serological testing indicates past exposure to *Salmonella* but does not determine whether the animals are infected with *Salmonella* at the time of sampling. Furthermore, serological testing provides only limited information about the occurrence of the different *Salmonella* serovars. However, in geographical areas with a low general occurrence of *Salmonella*, serological surveillance might be a useful tool to monitor the *Salmonella* status of farms producing wild boar.

Salmonella
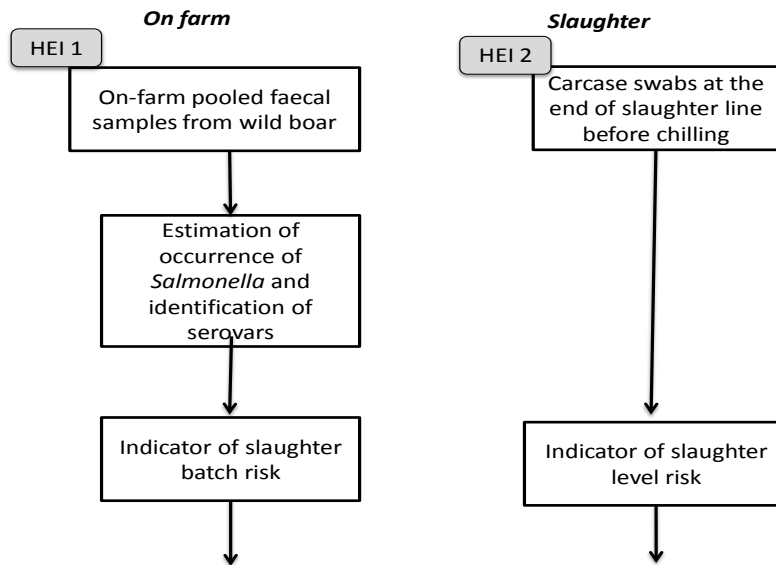
Figure 1: Schematic diagram illustrating the harmonised epidemiological indicators for *Salmonella* in farmed wild boar

5.1.6.   Harmonised monitoring requirements

Animal population

- At farm: farmed wild boar.

- At slaughterhouse: carcases of slaughtered wild boar.

Stage of the food chain

- The farm for wild boar.

- The slaughterhouse for carcases.

Sampling

- HEI 1

  o   Target population: farmed wild boar destined for slaughter.

  o   Epidemiological unit: the group of animals that is ready for slaughter within a period of one month.

  o   Sampling strategy: representative sampling using a standardised methodology, e.g. by subdividing enclosures into smaller areas and using systematic or random strategies to select samples (see Annex 3 to the scientific report on technical specifications on harmonised epidemiological indicators for public health hazards to be covered by meat inspection of swine (EFSA, 2011)).

  o   Sample size: sufficient sample size to detect at least one positive sample if the group is *Salmonella* positive. Sample size will depend on the size of the epidemiological group to be tested and the sensitivity of the pooled sample and can be calculated according to principles described in Annex 3 to the scientific report on technical specifications on harmonised epidemiological indicators for public health hazards to be covered by meat inspection of swine (EFSA, 2011).

- o Survey interval: all groups of wild boar destined for slaughter sampled a maximum of one month before dispatch.

- HEI 2

  - o Target population: all carcasses of wild boar after the slaughter process prior to chilling.

  - o Epidemiological unit: the carcase at the slaughterhouse.

  - o Sampling strategy: representative sampling by random or systematic selection of carcasses for testing.

  - o Sample size: adequate number of carcasses to assess the *Salmonella* status of the slaughterhouse throughput after processing before chilling.

  - o Survey interval: initial survey of the slaughterhouses in order to determine the occurrence of *Salmonella* on carcasses. Repeated at a frequency to be determined by risk managers adequate to characterise the slaughterhouse risk.

Type and details of sample

- Pooled faecal samples either from animals or from groups of animals ready for slaughter, as foreseen in the EU baseline survey on *Salmonella* in breeding pigs (EFSA, 2009a).

- Carcase surface samples of farmed wild boar carcasses at the slaughterhouse as foreseen in the EU baseline survey on *Salmonella* in slaughter pigs (EFSA, 2008a).

Diagnostic/analytical methods

- ISO 6579 Annex D (ISO, 2007): DETECTION of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage (to be used for faecal samples) (ISO, 2007).

- ISO 6579:2002: microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp. (to be used for carcass swab samples) (ISO, 2002).

Alternative methods certified and validated against the ISO methods may also be used, if isolates are obtained from the positive samples.

Serotyping is to be performed in accordance with the current edition of the Kaufmann White Scheme (Grimont and Weill, 2007).

Case definition

Finding *Salmonella* in a sample.

5.2. *Toxoplasma* in deer and wild boar

5.2.1. Introduction

Toxoplasma gondii is a zoonotic parasite of significant public health concern (Richomme et al., 2010). This parasite is ubiquitous and infections (toxoplasmosis) are common in humans and warm-blooded animals (mammals and birds), which are intermediate hosts. Wild and domestic cats are the most important definite host and other felids (such as lynxes in Europe) can also act as definite hosts. Several epidemiological studies have shown a worldwide distribution of *T. gondii* antibodies in domestic and wild animals in Europe and in other continents (EFSA, 2007b). The prevalence varies from 0 to 100 % according to animal species, type of animal farming and handling, geographical region and age (EFSA, 2007b). In several studies, it has been shown that the prevalence increases with the age of the animals (Berger-Schoch et al., 2011; Garcia-Bocanegra et al., 2012a; Lopes et al., 2011). The prevalence in the intermediate hosts depends on the presence of cats or other felids in their environment. The highest prevalence in wildlife is found in humid and tropical countries.

Humans become horizontally (1, 2) and vertically (3) infected with *T. gondii* through different routes: (1) ingestion of sporulated oocysts from the environment; (2) consumption of tissue cysts in infected animal tissue; and (3) congenital (pre-natal) infection (Figure 2) (Cenci-Goga et al., 2011; Robert-Gangneux and Darde, 2012). The variation in human seroprevalence can be due to dietary habits such as method of cooking, quality of water, hand washing, vegetable cleaning, etc. *T. gondii* infections are usually subclinical or asymptomatic in immunocompetent persons. When illness occurs, it is usually mild with flu-like symptoms that last for several weeks. If infection is acquired during pregnancy, it can cause transplacental transmission of tachyzoites followed by abortion or congenital malformation affecting the brain, eyes and organs of the fetus. Infants often show no symptoms at birth but develop them later in life with potential loss of vision and mental disability. Encysted bradyzoites of *T. gondii* remain in the body and can be reactivated if the person becomes immunosuppressed.

The detection of *Toxoplasma* infection in animals relies primarily on serological assays (Robert-Gangneux and Darde, 2012). The sensitivity and specificity depends on the animal species and cut-off values used. Polymerase chain reaction (PCR) methods have been developed to detect parasite DNA in blood, fluid and tissues. The specificity of this test is almost 100 % but the difficulty of extracting DNA and concentrating large sample quantities results in limited sensitivity (Cenci-Goga et al., 2011).
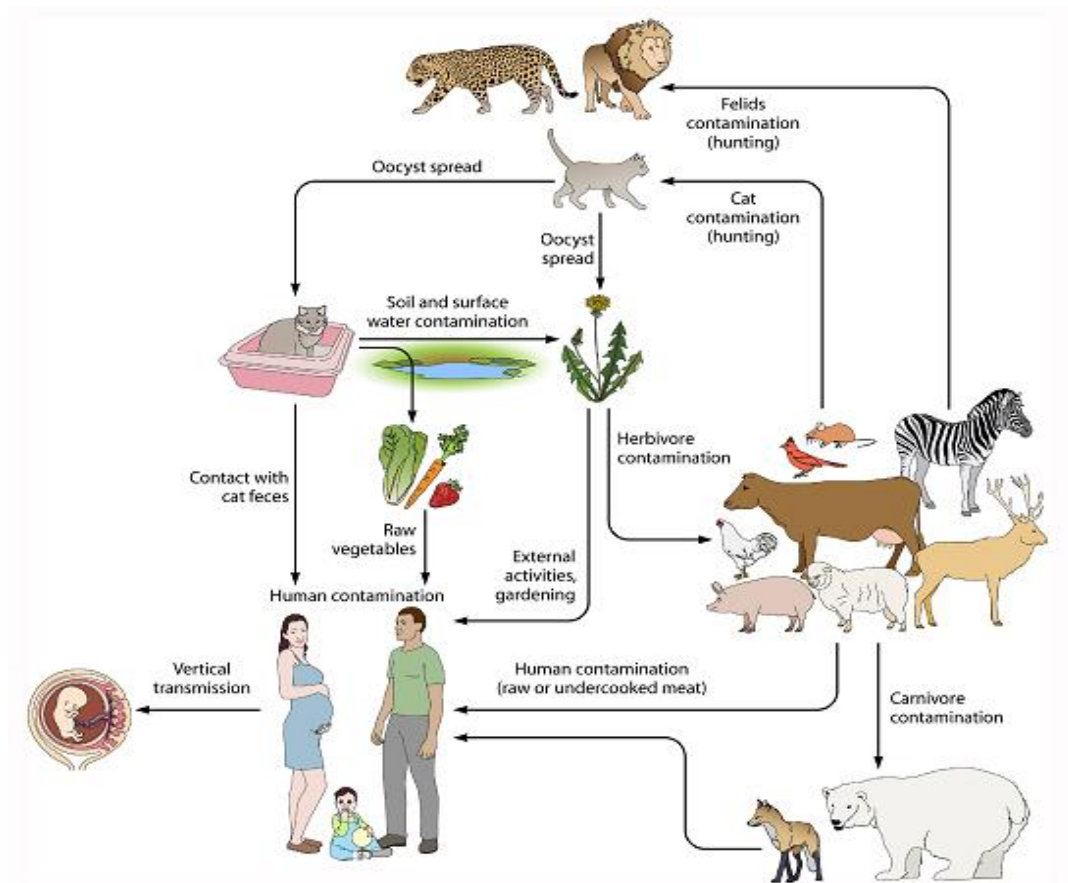
Source: © Robert-Gangneux F and Darde ML, 2012. Epidemiology of and Diagnostic Strategies for Toxoplasmosis. Clinical Microbiology Reviews, 25, 264-296. Copyright license obtained from the American Society for Microbiology.

Figure 2: Life cycle of *Toxoplasma*

5.2.2. Current situation and trends in the EU

In the framework of the annual data collection in accordance with Directive 2003/99/EC some data on the occurrence of *Toxoplasma* in game animals (farmed, wild and of unspecified origin) have been submitted by MSs. This information is summarised in Table 5 for deer and wild boar. *Toxoplasma* antibodies were commonly reported from both animal species (EFSA, 2006b, 2007a, 2009b, 2010; EFSA and ECDC, 2011, 2012, 2013).

Table 5: Occurrence of *Toxoplasma* in deer and wild boar.[a] Data reported to EFSA (2003/99/EC) by MSs and some other reporting countries during 2005–2011

Animal	Period of reporting	No of animals	No of positives	%
Deer	2008–2011	779	219	28.1
Wild boar[b]	2007–2011	2871	399	13.9

(a): All data reported to EFSA are presented, including data for non-farmed deer and wild boar.
(b): In Switzerland, *Toxoplasma* was detected in 1 of 150 (0.7 %) carcasses.

As results from several field studies show, *Toxoplasma* antibodies have frequently been detected in deer and wild boar in Europe (Table 6). The presence of *T. gondii* antibodies in wild deer and boar was not studied in several European countries during 2005 and 2010 (Table 6). The presence of *Toxoplasma* antibodies varied between 7 % and 60 % in wild deer and between 7 % and 40 % in wild boar. These antibodies were most frequently detected in wild deer (60 %) and wild boar (40 %) in France. The presence of *Toxoplasma* antibodies in wild boar was correlated with high density of animals in southern Spain (Gauss et al., 2005). Wild roe deer, which is one of the main deer species in

Europe, have most often been studied (Table 6). In one study conducted in Sweden, viable cysts of *T. gondii* were found in the muscle from several deer species (Malmsten et al., 2011). Halova (Halova et al., 2013) detected *T. gondii* antibodies in 7 % of the deer and, using PCR, *T. gondii* was detected in 4 % (3/71) of diaphragm samples.

Table 6: Presence of *Toxoplasma* antibodies in hunted deer and wild boar in Europe reported between 2005 and 2012

Country	Animal species	No of animals	No of positives	%	Reference
Belgium	Roe deer	73	38	52	De Craeye et al., 2011
Czech Republic	Red deer	377	169	45	Bartova et al., 2007
	Roe deer	79	19	24	
	Fallow deer	143	24	17	
	Wild boar	565	148	26	Bartova et al., 2006
Finland	White-tailed deer	135	36	27	Jokelainen et al., 2010
	Roe deer	17	3	20	
	Wild boar[a]	197	95	33	Jokelainen et al., 2012
France	Roe deer	60	36	60	Aubert et al., 2010
	Wild boar	1399	566	40	Richomme et al., 2010
Ireland	Deer	315	22	7	Halova et al., 2013
Netherlands	Wild boar	973	262	27	Opsteegh et al., 2011
Slovak Republic	Wild boar	320	65	8	Antolova et al., 2007
Spain	Red deer	441	69	16	Gauss et al., 2006
	Fallow deer	79	18	23	
	Roe deer	33	7	21	
	Roe deer	278	109	39	Gamarra et al., 2008
	Ibex	531	146	27	Garcia-Bocanegra et al., 2012a
	Wild boar	507	185	36	Gauss et al., 2005
Sweden	Roe deer	199	68	34	Malmsten et al., 2011
Switzerland	Wild boar	150	10	7	Berger-Schoch et al., 2011

(a): In this study only the wild boar were farmed.

5.2.3. Farmed game as a source of infection for humans

Despite serological evidence demonstrating widespread exposure to *T. gondii* in wild and farmed game, the role of these animals for human infection remains unclear. However, some evidence is present and several studies have reported an association between eating raw/undercooked game and acute toxoplamosis. Cervids and wild boar were considered to be one source of *T. gondii* infections in humans (Gauss et al., 2005; Gauss et al., 2006). A European multicentre case–control study of acute *T. gondii* infection in pregnant women concluded that eating raw/undercooked "other meats" was a significant risk factor (Cook et al., 2000). The variable "other meats" excluded products such as beef, lamb, pork and cured meats, but included game meat. The population attributable factor of "other meats" varied between countries from 1 % to 16 %, probably associated with consumption habits. Clinical toxoplasmosis cases in humans who consumed undercooked venison were also reported by Dubey et al. (2009). Acute and ocular toxoplasmosis has been reported in hunters who consumed undercooked or raw meat from cervids (Ross et al., 2001) and wild boar (Choi et al., 1997).

5.2.4. Risk and risk-reducing factors

Outdoor production increases the exposure of animals to a contaminated environment (EFSA, 2007b; Cenci-Goga et al., 2011; Robert-Gangneux and Darde, 2012). Farmed deer and wild boar are usually kept on pasture and therefore they have an increased risk of infection owing to contamination of the environment with sporulated oocysts. Felines play an important role in the transmission of infection because they excrete oocysts in their faeces, thus contaminating the environment.

The seroprevalence has been shown to be higher in wild boar than in domestic pigs, which may be the result of higher exposure to *T. gondii* oocysts excreted in the faeces of infected rural cats or ingestion

of infected rodents, birds or carrion in the wild. The seropositivity of wild boar was related to the density of straying cats and climatic conditions. High temperatures and especially dryness decrease the survival of oocysts. The seroprevalence of about 30 % in Dutch wild boar was shown to be stable over a period of five years (Opsteegh et al., 2011).

Deer are herbivores and can only become infected with *T. gondii* through ingestion of sporulated oocysts in soil, vegetation or water or by congenital transmission. The contamination of the environment is linked to the shedding of oocysts by domestic and stray cats and wild felid species (Robert-Gangneux and Darde, 2012). In deer, a high prevalence can be partly explained by their particular susceptibility especially when living in environments that are highly contaminated with oocysts (Cenci-Goga et al., 2011).

The exclusion of cats from areas where wild boar and deer are farmed would prevent contamination of the environment with oocysts. Management measures should also include control of rodents on the farm (Garcia-Bocanegra et al., 2010).

Eating undercooked deer and wild boar meat may pose a risk of infection with *Toxoplasma*. The risk associated with the meat varies among different countries according to local eating habits and the prevalence in game meat. Cooking practices have changed, with an increase in barbecue cooking, whereby the meat is not fully cooked (Richomme et al., 2010). Results of a study on food preparation demonstrated that women who washed kitchen knives infrequently after cutting meat had an increased risk of *Toxoplasma* infection (Kapperud et al., 1996), suggesting cross-contamination as a mechanism of transmission (Kijlstra and Jongert, 2008).

The curing and fermentation of meat does not affect the *Toxoplasma* parasite immediately and the survival time of tissue cysts varies with the concentration of the salt solution and the storage temperature. Adequate measures, such as freezing of game meat before processing (Dubey et al., 2009; Cenci-Goga et al., 2011) or better cooking would prevent human toxoplasmosis.

5.2.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected for *Toxoplasma* in farmed deer and wild boar and meat thereof (Table 7 and Figure 3).

Table 7: Harmonised epidemiological indicators (HEIs) for *Toxoplasma* in farmed deer and farmed wild boar

Indicators (animal/food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
HEI 1: Detection of *Toxoplasma* antibodies in all farmed deer and wild boar	Slaughterhouse	Serology	Meat juice
HEI 2: Detection of *Toxoplasma* antibodies in the older animals (over one year) of farmed deer and wild boar	Slaughterhouse	Serology	Meat juice

The scheme describing the food chain and related risk and risk-reducing factors, as well as the evaluation of possible epidemiological indicators, are presented in Appendix A.

The outdoor rearing practices currently used excludes the possibility of effectively controlling risk factors and exposure to *T. gondii* or maintaining stable risk status on a farm. This applies particularly to preventing the access of cats to the premises where the animals are reared. Controlled husbandry conditions are therefore not relevant for farmed deer or farmed wild boar currently as regards *Toxoplasma*. Therefore, audits of farms were considered not useful as a HEI. However, should farming practices for these species change to more intensive and indoor systems, definitions for

controlled housing for pigs could be used for farmed wild boar and controlled husbandry conditions for ruminants might apply to farmed deer.

HEI 1 is focused on the serological testing of meat juice samples from all farmed deer and wild boar at slaughter. This information enables the classification of the animals or slaughter batches as low or high risk as regards *Toxoplasma*. It will also provide surveillance information on the prevalence of *Toxoplasma* in the relevant animal population in the country or region. Serological testing is not, however, a predictor of the infection status of animals or the herd because of the delay in antibody response after infection. The use of historical data from *Toxoplasma* testing for the risk categorisation of farms was not regarded as useful because, on the one hand, the *Toxoplasma* status of the animals can change rapidly (i.e. due to cats) and, on the other hand, only one or a couple of batches of farmed deer and wild boar from a farm might be slaughtered in a year, so the interval between slaughter batches is likely to be long.

HEI 2 is focused on serological testing of meat juice samples from older animals which have a higher exposure to *Toxoplasma* because of their longer life span. In deer, young animals might be up to two years old when slaughtered, whereas wild boar may be less than nine months old at slaughter. Furthermore, age categories "young" and "old" may vary between countries. Thus, old animals, which are at higher risk for *Toxoplasma* infection, are considered to have an age of more than one year. Information on the age of animals can be easily gathered from the food chain information system. HEI 2 can be applied as an alternative to HEI 1, particularly when *Toxoplasma* prevalence is expected to be low.
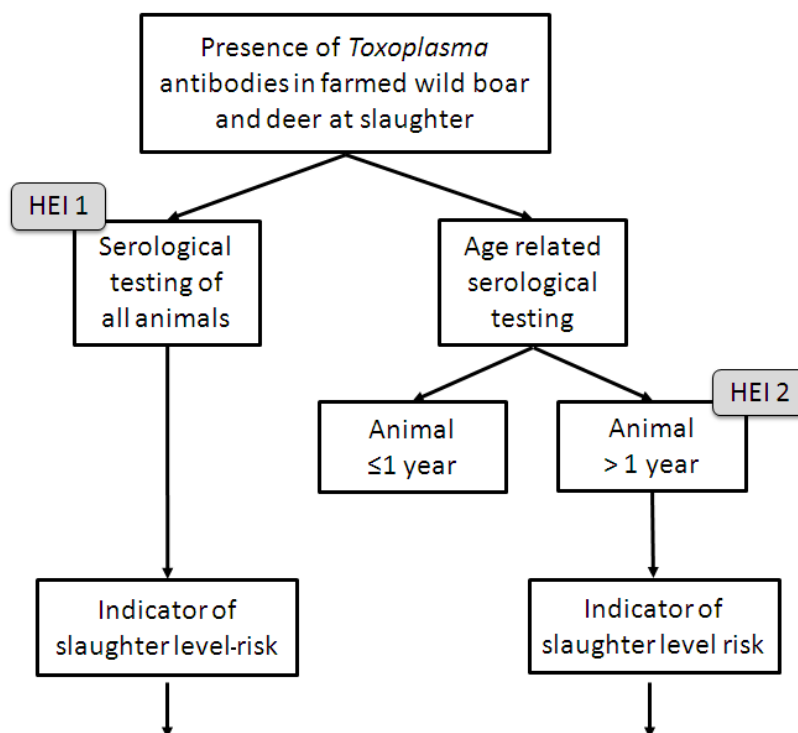
Figure 3: Schematic diagram illustrating the harmonised epidemiological indicators for *Toxoplasma* in farmed game

5.2.6. Harmonised monitoring requirements

Animal population

At slaughterhouse:

- All farmed deer or older animals (over one year) slaughtered.

- All farmed wild boar or older animals (over one year) slaughtered.

Stage of the food chain

The slaughterhouse for farmed deer and farmed wild boar.

Sampling

- HEI 1

  o Target population: all slaughtered farmed deer and farmed wild boar.

  o Epidemiological unit: the animal.

  o Sampling strategy: all animals—with the exception of very large slaughter batches: probability sampling by random or systematic sampling. However, most farms are small and sample size calculations are likely to include all animals.

  o Sample interval: all slaughter batches—with one exception: if the indicator is intended only for surveillance purposes rather than risk management purposes, batches or carcasses can be selected for sampling using probabilistic sampling methods and stratified on subpopulations, e.g. slaughterhouses or region of origin, as relevant for a surveillance objective in the country.

- HEI 2

  o Target population: farmed deer and farmed wild boar older than one year.

  o Epidemiological unit: the animal.

  o Sampling strategy: all animals older than one year.

  o Sample interval: All animals older than one year in the slaughter batches—with one exception: if the indicator is intended only for surveillance purposes rather than risk management purposes, batches or carcasses can be selected for sampling using probabilistic sampling methods and stratified on subpopulations, e.g. slaughterhouses or region of origin, as relevant for a surveillance objective in the country.

Type and details of samples

Meat juice samples are collected from muscle specimens of farmed deer and wild boar at slaughter. Meat juice samples are stored at –20 °C until serological testing. The pooling of samples should not be carried out.

Diagnostic/analytical methods

- Detection of antibodies to *T. gondii* in meat juice samples.

- Tests proposed are based on the enzyme-linked immunosorbent assay (ELISA) format:

  o ELISA using formalin-fixed whole tachyzoites as antigen (Gamble et al., 2000).

  o 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).

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

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

Case definition

Findings of *Toxoplasma* antibodies in a meat juice sample.

5.3. *Trichinella* in wild boar

5.3.1. Introduction

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. Four *Trichinella* species have been detected in various animal species in Europe so far. *T. spiralis* circulates mainly among domestic pigs (domestic cycle) but may also occur in wildlife (sylvatic cycle). *T. nativa* is resistant to freezing and circulates mainly among wild carnivores in Nordic countries. *T. britovi* is the most widespread species infecting mainly wild carnivores, and *T. pseudospiralis* is able to infect both mammals and birds (Pozio and Murrell, 2006; Merialdi et al., 2011). *T. spiralis* is highly infective to wild boar but *T. britovi* and *T. pseudospiralis* were detected at significantly lower numbers (Kapel, 2001). Although the freezing-resistant species *T. nativa* shows no relevant infectivity in domestic pigs, sporadic findings with a low larval burden have been reported from wild boar (Pozio and Kapel, 1999).

The domestic and sylvatic cycle of *Trichinella* can function either independently of each other or interactively (see Figure 4), and a switch from wild to farmed animals can occur when there is poor management in terms of segregating husbandry and wildlife (Gottstein et al., 2009). Therefore, meat inspection of domestic swine, wild boar and horses for *Trichinella*, as well as monitoring in wildlife (e.g. foxes and raccoon dogs), which plays an important role as the natural reservoir, are essential tools for assessing changes in disease prevalence as laid down in the current Regulation (EC) No 2075/2005.[12]
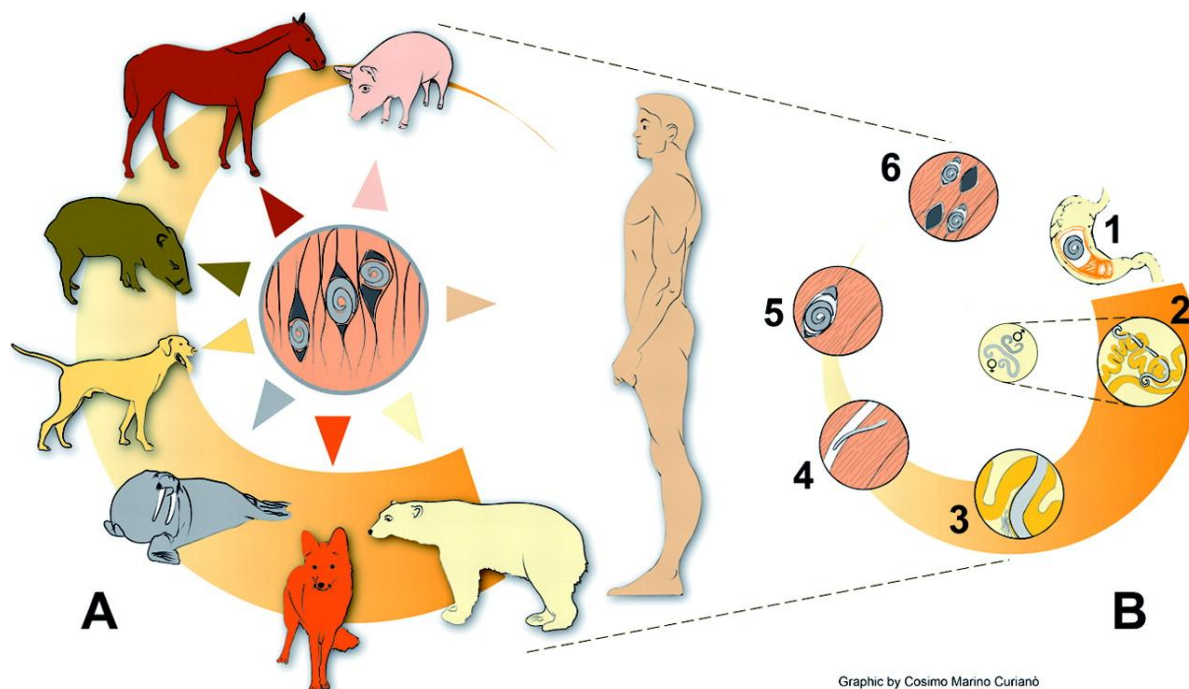
Graphic by Cosimo Marino Curianò

Note: (A) Main sources of *Trichinella* spp. infections in humans including pigs, horses, wild boar and, to a lesser extent, dogs, walruses, foxes and bears. (B) Enteral and parenteral phase of *Trichinella* development in the host body. In the enteral 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).

Source: International *Trichinella* Reference Centre (ISS, online).

Figure 4:   Life cycle of *Trichinella* spp.

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[12]   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, pp. 60–82.

Meat or meat products containing at least one *Trichinella* larva per gram are considered to induce a clinical infection in man. This would correspond to an infective dose of at least 150 larvae assuming an average meat consumption of 150 g (Nockler, 2003). 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, and itchy skin, 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).

5.3.2. Current situation and trends in the EU

In the framework of the annual data collection in accordance with Directive 2003/99/EC data on the occurrence of *Trichinella* in farmed wild boar have been submitted by MSs. These data originate from testing related to meat inspection and as such are representative of the situation in the EU. This information is summarised in Table 8.

Among the 151 *Trichinella* findings reported in farmed wild boar in 2007–2011 the proportion of *Trichinella*-positive animals varied between 0.003 % and 0.442 % and most positive cases are related to the year 2011. A minor proportion (14, 9.3 %) of *Trichinella* findings were further specified. Out of these, 10 were *T. pseudospiralis,* three *T. spiralis* and one *T. britovi* (EFSA, 2009c, 2010; EFSA and ECDC, 2011, 2012, 2013).

Table 8: Findings of *Trichinella* in farmed wild boar, 2007–2011

	2007			2008			2009			2010			2011		
	N	Pos	%	N	Pos	%	N	Pos	%	N	Pos	%	N	Pos	%
Trichinella spp.	6 615	2	0.03	31 791	1	0.003	27 591	7	0.025	36 871	26	0.071	25 996	115	0.442
T. pseudospiralis		0			0			5			4			1	
T. spiralis		2			1			0								
T. britovi		0			0			1								
Reporting MSs	6 MSs			10 MSs			9 MSs			8 MSs			9 MSs		

5.3.3. Wild boar meat as a source of infection for humans

Pork is an important source of human *Trichinella* infection both worldwide and in Europe, but meat of horses and wild boar have also played a significant role during the last three decades (Gottstein et al., 2009). Various preparations from wild boar meat such as goulash are popular in many European countries (ifood, online). There are specific regional habits of mixing different assortments of meat (especially pork and wild boar meat) for the production of raw products (such as raw sausages) which are a risk for consumers as *Trichinella* larvae are not killed under such conditions.

In the framework of the annual data collection in accordance with Directive 2003/99/EC, information on foodborne outbreaks caused by *Trichinella* is also collected. In the period 2007–2011, 23 foodborne *Trichinella* outbreaks caused by wild boar meat were reported from four MSs. These outbreaks included 323 human cases, and 57 % of the affected people were hospitalised (EFSA, 2009b, 2009c, 2010; EFSA and ECDC, 2011, 2012, 2013). As far as isolates could be obtained in conjunction with wild boar meat associated outbreaks, *T. spiralis* was identified as the causative agent. Various trichinellosis outbreaks due to consumption of raw products processed from wild boar meat have also been reported from several Eastern European countries such as Romania (Neghina et al., 2010).

5.3.4. Risk and risk-reducing factors

The risk of *Trichinella* infection in wild boar is closely associated with the presence of this parasite in wildlife (e.g. bear, lynx, raccoon dog, fox and wild boar). Wild boar gets infected with *Trichinella* through scavenging and cannibalism from animals harbouring this parasite. A close relationship was observed between the practice of hunters of leaving animal carcases in the field after skinning and the prevalence of *Trichinella* among wildlife (Pozio et al., 2001). Besides wild animals living in their natural habitat, rats were suspected to be an infection source for wild boar kept under farm conditions (Oivanen et al., 2000).

Consumption of raw or insufficiently treated products (e.g. semi-roasted ribs, raw sausages) processed from wild boar meat harbouring *Trichinella* larvae is the main risk factor for human trichinellosis.

A systematic *Trichinella* inspection in wild boar meat is an important risk-reducing measure. Thus, hunters must be aware of the risk of *Trichinella* infection from wild boar and the necessity of inspecting the carcase prior to private consumption and/or marketing. *Trichinella* can be inactivated by cooking meat which is the most reliable method for inactivation of muscle larvae. Although freezing of pork can be used as an alternative to meat inspection, this treatment is not appropriate for wild boar meat which can harbour freeze-resistant *Trichinella* species (Gamble et al., 2000).

5.3.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected for *Trichinella* in wild boar (Table 9 and Figure 5).

Table 9: Harmonised epidemiological indicators (HEIs) for *Trichinella* in wild boar

Indicators (animal/food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
HEI 1: *Trichinella* testing in all farmed wild boar	Slaughterhouse	Digestion	Meat

The scheme describing the food chain and related risk and risk-reducing factors as well as the evaluation of possible epidemiological indicators are presented in Appendix A.

The current production system for farmed wild boar is typically extensive. Based on the existing information, it seems difficult to audit biosecurity measures for controlling the access of farmed game to the areas of reservoir animals (e.g. foxes, raccoon dogs, rats). Therefore, no reliable controlled husbandry conditions can be identified for classifying farms according to *Trichinella* risk.

The systematic *Trichinella* testing of all slaughtered wild boar is proposed (HEI 1), which will provide data on the *Trichinella* status of the carcase and the slaughter batch. The data derived from testing of the carcases for *Trichinella* cannot be used to classify the farms of origin in the absence of the controlled husbandry conditions that could ensure maintaining the risk status of the farm.

Serological testing of serum and meat juice samples is not proposed as HEI. Serological testing for *Trichinella* antibodies by use of an indirect ELISA (based on excretory–secretory larval antigen) is considered a suitable tool for monitoring in swine herds (OIE, 2012) and commercial or in-house ELISA kits have been used for several serological studies in wild boar during recent years. However, serological testing for *Trichinella* antibodies has not been approved for monitoring purposes owing to a lack of standardisation in this ELISA method (OIE, 2012) and the high number of false-positives due to cross-reactions mainly in animals with outdoor access.

Trichinella
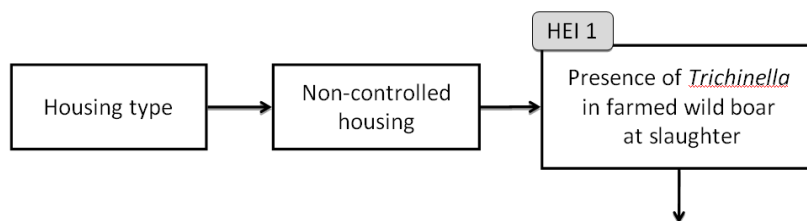
Figure 5: Schematic diagram illustrating the harmonised epidemiological indicators for *Trichinella* in farmed wild boar

5.3.6. Harmonised monitoring requirements

Animal population

All farmed wild boar slaughtered for human consumption.

Stage of the food chain

The slaughterhouse or facility where the farmed wild boar are slaughtered.

Sampling

- HEI 1
  - o Target population: all slaughtered farmed wild boar.
  - o Epidemiological unit: the carcase.
  - o Sampling strategy: all carcases.

Sample interval: all slaughter batches—with one exception: if the indicator is intended for surveillance purposes only rather than risk management purposes, batches or carcasses can be selected for sampling using probabilistic sampling methods and stratified on subpopulations, e.g. slaughterhouses or region of origin, as relevant for a surveillance objective in the country.

Type and details of sample

Muscle samples taken according to Commission Regulation (EC) No 2075/2005.

Diagnostic/analytical methods

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

Case definition

Finding of *Trichinella* spp. larvae from a meat sample.

5.4. *Mycobacterium* in deer and wild boar

5.4.1. Introduction

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 *M. bovis*, responsible for bovine tuberculosis. This agent is also capable of infecting a wide range of warm-blooded animals, including humans, wild boar and deer. 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, including wild boar, deer and humans (Erler et al., 2004; Rodriguez et al., 2009).

The main transmission routes of these agents 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 boar, badgers and the European bison are important hosts for *M. bovis* and may contribute to the spread and/or maintenance of *M. bovis* infection in cattle in some MSs (EFSA and ECDC, 2011).

Other *Mycobacteria* occasionally produce disease that is clinically indistinguishable from tuberculosis. *M. avium* complex (MAC) was recognised as the most common opportunistic bacterial infection in patients with acquired immune deficiency syndrome (AIDS) (Cook et al., 2000). MAC includes eight *Mycobacterium* species and several subspecies with different degrees of pathogenicity, a broad host range and environmental distribution in numerous biotopes including the soil, water, aerosols, etc. (Biet et al., 2005; Maeder et al., 2009; Alvarez et al., 2011). *M. avium* subsp. *avium* (MAA) is a potential zoonotic pathogen that belongs to MAC. MAC and other mycobacteria have been isolated from wild boar and deer (Glawischnig et al., 2006; Mackintosh et al., 2004; Santos et al., 2009).

Most wild boar rarely show clinical signs until disease is advanced, even in experimentally *M. bovis*-infected wild boar (Ballesteros et al., 2009). The frequent location of lesions in the head and neck lymph nodes (Bollo et al., 2000; Gortazar et al., 2003; Parra et al., 2006; Martin-Hernando et al., 2007) may arise from entry via either the respiratory route or ingestion, as these lymph nodes receive drainage from the nasal, oral and tonsillar regions. Some wild boar develop lesions in thoracic lymph nodes only, while others do so exclusively in mesenteric lymph nodes, indicating that either ingestion or inhalation of the agent may occur (Vicente et al., 2006; Martin-Hernando et al., 2007; Di Marco et al., 2008; Maeder et al., 2009).

Infected deer generally appear clinically healthy, even in advanced stages of the disease. Tuberculosis lesions in deer are similar to those observed in cattle, both grossly and histopathologically, although abscesses in deer tend to have a thinner wall containing pus with multiple bacilli, and minimal calcification or fibrosis. Deer, in particular, appear to develop severe granulomatous lesions or even abscesses as a result of *M. bovis* infections and those caused by some MAC, e.g. IS901 + *M. avium*. Gross lesions caused by *M. avium* subsp. *paratuberculosis* and *M. avium* in deer can be indistinguishable from lesions caused by *M. bovis*. Additionally, lesions caused by all of these agents may be small and not apparent during visual inspection, especially if they are located deep within the parenchyma of affected organs. Hence, at the slaughterhouse, these diseases can be overlooked or confused.

The frequent distribution of lesions in the head and neck lymph nodes indicates that inhalation and, perhaps, ingestion are the most common routes of infection in cervids. The tonsils are frequent sites of tuberculous infection, but do not always show gross lesions (Lugton et al., 1998; Rohonczy et al., 1996; Palmer et al., 2002). Purulent tonsillitis is observed in some cases. Tuberculous lesions have been recorded in other organs, such as spleen, liver, bones and others (Balseiro et al., 2009; Gavier-Widen et al., 2009).

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 et al., 2000). Although *M. avium* subsp. *hominisuis* can infect a wide variety of animals, including wild boar and deer, there is limited information on its prevalence in these species (Glawischnig et al., 2006; Domingos et al., 2009).

5.4.2.   Current situation and trends in the EU

The official reporting data in the EU Summary Report in 2010 (EFSA and ECDC, 2012) recorded 133 confirmed cases of human tuberculosis caused by *M. bovis* during the reporting year, mostly in people over 65 years old. This figure represents only 0.035 cases per 100 000 population, which has remained fairly stable over the period (2005–2009) that data has been submitted to EFSA and ECDC.

The monitoring data for farmed game in the EU Summary Reports (EFSA, 2005, 2006a, 2007a, 2009c, 2010; EFSA and ECDC, 2011, 2012) contains reports from several MSs for mycobacteria in farmed wild boar and farmed deer, although this information is rather sparse. During the period 2002–2010, only two MSs confirmed a low proportion of samples positive for *M. bovis*, *M tuberculosis* and atypical or unspecified mycobacteria in farmed wild boar, and five MSs confirmed a low proportion of samples positive for *M. bovis*, *M. tuberculosis*, *M. caprae*, MAC and atypical or unspecified mycobacteria in farmed deer. These figures probably do not reflect the true prevalence in the EU due to sampling biases and non-harmonisation of reporting.

During this same period, several MSs reported the presence of *M. bovis* and other mycobacteria species in hunted wild boar and several deer species (Wilson et al., 2009; EFSA and ECDC, 2013), which is supported by other published literature (Zanella et al., 2008; Pate et al., 2011; Garcia-Bocanegra et al., 2012b). Only one officially tuberculosis free (OTF) Member State reported such findings for *M. bovis*, supporting the view that wild animals may constitute a reservoir for *M. bovis*. Badgers and wild boar were considered to be the wildlife species posing the greatest potential risk to cattle and other domesticated livestock (Naranjo et al., 2008; Wilson et al., 2009). Indeed, reports of wild boar 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 most cases, deer are thought to be spill-over end hosts. Localised exceptions occur in some regions, where fallow deer live at high density and commonly interact with cattle, or where management practices and high population density suggest that red deer are probably maintenance hosts. The occurrence of *M. bovis* in wildlife and domestic animals other than cattle thus seems to reflect the status of the MSs regarding freedom from bovine tuberculosis.

Komijn et al. (2007) reported granulomatous lesions in 0.75 % of lymph nodes of slaughter pigs at two slaughterhouses in the Netherlands. However, these lesions were associated with the isolation of *Rhodococcus equi*. More recently, Miranda et al. (2012) detected granulomatous lesions in 2.15 % of 3 179 slaughtered pigs from four slaughterhouses in Portugal. They identified *Mycobacterium* spp. in 82 % of 50 lymph nodes that were examined by microbiological techniques (Miranda et al., 2012).

5.4.3.   Farmed wild boar and deer as a source of infection for humans

The genus *Mycobacterium* includes several species that cause tuberculous infections in humans and other animals. Although subclinical infections are more common than clinical infections, farmed wild boar and deer are unlikely to be a source of exposure for humans, based on the infrequent reporting of tuberculosis cases in these animals (see section 4.6.2) and the lack of documented evidence that farmed game meat is associated with human infection (EFSA BIOHAZ Panel, 2013a).

This conclusion is supported further by the lack of 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 the environment (direct contact/inhalation).

Also, assessments of the risk posed by meat from reactor-positive cattle concluded that such meat did not present any additional risks (EFSA BIOHAZ Panel, 2013a, 2013b).

5.4.4. Risk and risk-reducing factors

Wild boar and deer are susceptible to several mycobacterial infections, particularly *M. bovis* and MAC, which can be acquired from imported animals, wildlife or the environment, depending on the agent. Badgers and wild boar were considered to be the wildlife species posing the greatest potential risk of transmitting *M. bovis* to cattle and other domesticated livestock (Naranjo et al., 2008; Wilson et al., 2009). *M. caprae* has been reported in several wildlife species, but their role as reservoirs and modes of interspecies transmission have not been investigated extensively (Rodriguez et al., 2011).

Considering *M. bovis*, the main risk factor for farmed wild boar and farmed deer is purchase of infected animals and contact with wildlife (Wilson et al., 2009), particularly in regions/MSs that are not OTF (see section 4.6.2). Currently, the main farm-level risk-reducing factor consists of applying correct biosecurity measures (e.g. use of fences). A crucial adjunct to biosecurity measures would be the effective pre-slaughter detection of infection in live animals. For wild boar only limited information is available. Serological assays have been reported that show moderate sensitivity (73–77 %) and good specificity (96–97 %) (Aurtenetxe et al., 2008; Lyashchenko et al., 2008; Boadella et al., 2011) and an intradermal comparative test with variable sensitivity (43–75 %) and low specificity (48–77 %) (Jaroso et al., 2010a). However, handling of wild boar is dangerous, which compromises such inteventions. In deer, there are more data available and several immunological assays, including an assay to detect IFN-γ, have been reported for pre-slaughter detection of *M. bovis* infection in live animals (Lyashchenko et al., 2008; Waters et al., 2008; Buddle et al., 2010; Jaroso et al., 2010b; Queiros et al., 2012). A fuller assessment of many assays is required, but control programmes are in place that are supported by application of the single or comparative skin test, although false responses due to sensitisation to other mycobacteria may occur, hampering diagnostic specificity (Queiros et al., 2012). Further complications arise from age/sex interactions and influence of season (Jaroso et al., 2010a, 2010b) and interference from intercurrent *M. avium* infections or vaccination against *M. avium* subsp. *paratuberculosis* (Buddle et al., 2010).

An additional risk-reducing factor occurs at the slaughterhouse by the implementation of effective meat inspection to detect suspect (granulomatous) lesions. Because other pathogens can induce grossly similar lesions, it is important that suspect lesions are sampled for further laboratory investigations to confirm the presence of pathogenic mycobacteria and identify the species. Some data are available for meat inspection in deer (EFSA, 2008b) and indicates that sensitivity for meat inspection is rather low (62 %). The key factors affecting sensitivity are the number of animals inspected and the degree of detail and time during the inspection (i.e. larger numbers and less time per carcass during meat inspection than at necropsy (More et al., 2009)). There are few data available for wild boar, even though meat inspection is viewed as the only potential current source of information on the distribution and prevalence of tuberculosis (EFSA BIOHAZ Panel, 2013a).

The BIOMO questionnaire survey shows that all wild boar and deer farming systems in the MSs are extensive, with access to pasture, and usually sources of ground water. Consequently, animals are continually exposed to environmental mycobacteria (Glawischnig et al., 2006; Moser et al., 2011).

5.4.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected for zoonotic mycobacterium in farmed wild boar and farmed deer (Table 10 and Figure 6).

Table 10: Harmonised epidemiological indicators (HEIs) for mycobacterium in farmed wild boar and farmed deer

Indicators (animal/food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
HEI 1: Official bovine tuberculosis status	Farm/region/Member State	Official records, food chain information	Not applicable
HEI 2: Human pathogenic mycobacteria in farmed wild boar and deer at slaughter	Slaughterhouse	Visual meat inspection and microbiology	Suspected lesions

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

Although the currently recorded prevalence of zoonotic mycobacteria in wild boar and deer in the EU is very low, these animals are reared in outdoor systems with the potential to contact infected wildlife.

HEI 1 takes advantage of official data that establishes the OTF status of a Member State, zone (region) or even farm and supports the assessment of higher and lower risk for *M. bovis* in farmed deer and wild boar prior to slaughter.

HEI 2 focuses on inspection of all slaughtered animals at the slaughterhouse. This HEI is based on the Scientific Opinion's recommendation for meat inspection by visual inspection of all carcases at slaughter and confirmation of the presence and species of pathogenic mycobacteria in suspicious lesions by microbiological testing (EFSA BIOHAZ Panel 2013a). This measure would enable surveillance for detection of emergence of mycobacterial infections in wild boar and deer populations. However, many granulomatous lesions will not be detected by this approach and it may be advisable to enhance the inspection in some MSs/regions, e.g. where bovine tuberculosis is endemic or other mycobacteria are emerging.

Although immunological tests are available for the pre-slaughter diagnosis of *M. bovis* and *M. caprae* in wild boar and deer, such testing was not proposed as a HEI because of their current limitations, particularly in wild boar.

Mycobacteria
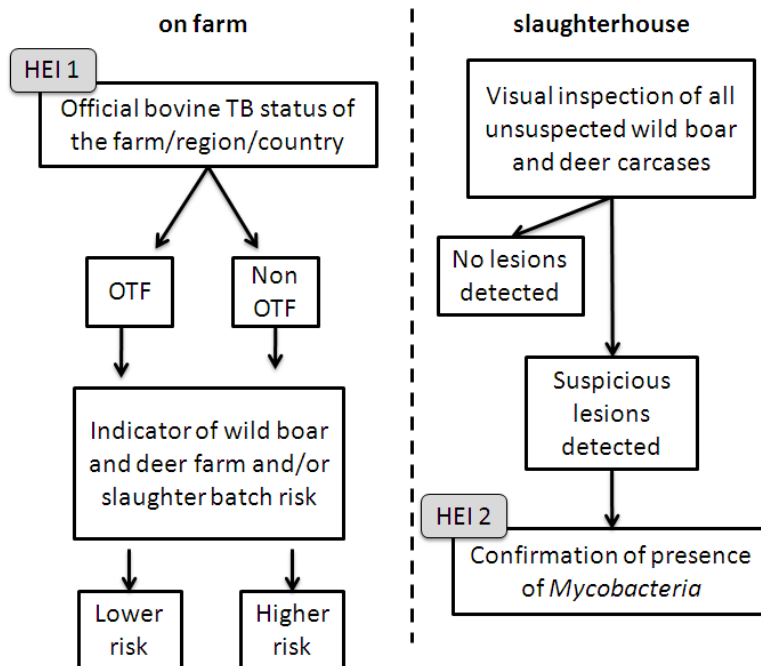
Figure 6: Schematic diagram illustrating the harmonised epidemiological indicators for mycobacteria in farmed wild boar and farmed deer

5.4.6. Harmonised monitoring requirements

Animal population

- Official records and food chain information: cattle populations in MSs/regions/farm to establish OTF status.

- All farmed wild boar and farmed deer at slaughter.

Stage of the food chain

The slaughterhouse.

Sampling

- HEI 1

  o Target population: cattle to establish the bovine tuberculosis status of the farm, region and Member State.

  o Epidemiological unit: mainly the Member State or region, but can be applied to individual herds/farms.

  o Sampling strategy: as defined in Council Directive 64/432/EEC[13] and prescribed by risk managers.

  o Sample interval: as prescribed by risk managers.

- HEI 2

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[13] Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine. OJ 121, 29.7.1964, pp. 1977–2012.

- o Target population: all wild boar and deer carcases at the slaughterhouse.

- o Epidemiological unit: the farm of origin.

- o Sampling strategy: visual inspection to detect suspect lesions for further laboratory investigation to confirm the presence and species of pathogenic mycobacteria in suspicious lesions by microbiological testing.

- o Sample interval: ongoing as all animals are inspected.

Type and details of sample

- Official data and records collected by MSs, provided in the food chain information.

- All suspected lesions observed during the visual meat inspection are sampled and sent to a diagnostic laboratory for subsequent investigation using bacterial culture and molecular identification of pathogenic mycobacteria.

Diagnostic/analytical methods

- Official data on occurrence of pathogenic mycobacteria are analysed by MSs.

- Pathogenic mycobacteria detected in suspect lesions by culture (most common) or PCR and the species confirmed by molecular procedures (PCR, sequencing).

Case definition

- OTF Member State/region/farm as defined in Council Directive 64/432/EEC.

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

6.   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, 2006b). The strategy should be aligned with the objectives of the surveillance (representative or risk based) and the population of interest, as well as the constraints of the environment in which sampling is to be done. General guidance on the choice of appropriate sampling strategies as well as for calculating appropriate sample sizes for the harmonised epidemiological indicators are given in the scientific report on technical specifications for harmonised epidemiological indicators for public health hazards to be covered by meat inspection of swine (EFSA, 2011).

7. Comparable data on the harmonised epidemiological indicators

In the case of *Trichinella* in farmed wild boar, comparable data from the EU MSs on the proposed indicator (HEI 1) are available from the mandatory testing of wild boar carcasses during meat inspection (see Table 11). This data is reported in accordance with the Directive 2003/99/EC and published in the EU Summary Report 2011 (EFSA and ECDC, 2013).

Table 11: Findings for *Trichinella* in farmed wild boar, 2011

Country	Description	Species (no of isolates)	Sample unit	N	Pos	% Pos
Austria	Official and industry sampling, surveillance, census		Animal	743	0	0
Bulgaria	Official sampling, surveillance, unspecified		Slaughter batch	87	0	0
Denmark	Official sampling, objective sampling, census		Animal	1 599	0	0
Finland	Surveillance, census	*T. pseudospiralis*	Animal	486	1	0.2
France	Official sampling, surveillance, objective sampling		Animal	3 553	0	0
Italy	Official sampling, unspecified, census		Animal	527	0	0
			Slaughter batch	3	0	0
Lithuania	Official and industry sampling, surveillance, objective sampling	*Trichinella* spp.	Animal	18 208	114	0.6
Portugal	Official and industry sampling, surveillance		Animal	28	0	0
United Kingdom	Official sampling, surveillance, census		Animal	852	0	0
Total (9 MSs in 2011)			Animal	25 996	115	0.4
			Slaughter batch	90	0	0

CONCLUSIONS AND RECOMMENDATIONS

ToR 1 Define harmonised epidemiological criteria for specific hazards already covered by current meat inspection (trichinellosis, tuberculosis, cysticercosis, etc.) 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 foodborne biological hazards related to farmed game and meat thereof in the context of the scientific opinion on public health hazards to be covered by inspection of meat from farmed game (EFSA BIOHAZ Panel, 2013a). These hazards include *Trichinella* and *Mycobacterium*, which are already covered by meat inspection of farmed wild boar and deer, as well as *Salmonella* in wild boar and *Toxoplasma* in wild boar and deer, which were identified by the scientific opinion. The epidemiological indicators proposed in this report will provide relevant information to the risk managers (i.e. the EC and the MSs), in order to consider whether adaptations in meat inspection methods may be relevant and to enable the MSs to carry out a risk analysis to support such decisions. It is also envisaged that the epidemiological indicators will be used in the revised meat inspection system for farmed game as proposed by the scientific opinion. Thus, the indicators can facilitate the development and implementation of risk-based meat inspection.

- 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 for *Salmonella* can be used in the classification of slaughter batches according to the infection status related to the hazard and to evaluate the measures taken in the slaughterhouses to control the hazard or to guarantee process hygiene.

- The epidemiological indicator for *Trichinella* will provide information on the infection status of animals, whe*reas the ep*idemiological indicator for *Toxoplasma* will enable the classification of animals into low or high risk at the slaughterhouse.

- In cases of rare biological hazards in EU farmed game production, epidemiological indicators are suggested to enable surveillance for possible emergence of such hazards. This is the case for mycobacterium.

- The data accumulated from the implementation of the HEIs will provide for historical information over time of the infection status of regions and countries. The history of test results could inform the future testing frequency applied for harmonised epidemiological indicators.

- The epidemiological indicators are suggested for farmed wild boar or deer at the farm or for their carcasses at slaughterhouse.

- Husbandry conditions in the current production systems of farmed wild boar and deer were not considered effective in preventing and controlling the risks related to the biological hazards addressed. Therefore auditing of farms was of limited value and no harmonised epidemiological indicators based on husbandry conditions are proposed.

- The proposed harmonised epidemiological indicators are listed in Table 12.

Recommendations

- The proposed epidemiological indicators will generate data that will provide information on the epidemiological situation in the EU, and these data can be used to update the epidemiological indicators, when appropriate. It is recommended that the MSs 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 the light of new information and the data generated from monitoring of them.

- The opportunity to implement more risk-based indicators at farm level could be considered if the production systems of farmed game change in the future.

ToR 2 Provide a summary of comparable data from MSs based on the above-defined harmonised epidemiological criteria, if they exist (e.g. from ongoing monitoring in humans, food or animals).

Conclusions

- Comparable data from the EU MSs were available only for *Trichinella* in farmed wild boar, where such data were provided by annual reporting on zoonotic agents under Directive 2003/99/EC. These data are summarised in chapter 7 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 MSs.

Conclusions

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

- If the Commission or MSs need further advice on the sampling schemes to be applied for the harmonised epidemiological indicators, EFSA can be requested to provide technical assistance in the formulation of such schemes.

Table 12: Proposed harmonised epidemiological indicators for meat inspection of farmed wild boar and deer

Indicators (animal/food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
Salmonella			
HEI 1: *Salmonella* in farmed wild boar before slaughter	Farm	Microbiology (detection, isolation and serotyping)	Pooled faeces sample
HEI 2: *Salmonella* in/on farmed wild boar carcasses after slaughter but before chilling	Slaughterhouse	Microbiology (detection, isolation and serotyping)	Carcass swabs
Toxoplasma			
HEI 1: Detection of *Toxoplasma* antibodies in all farmed deer and wild boar	Slaughterhouse	Serology	Meat juice
HEI 2: Detection of *Toxoplasma* antibodies in the older animals (over one year) of farmed deer and wild boar	Slaughterhouse	Serology	Meat juice
Trichinella			
HEI 1: *Trichinella* testing in all farmed wild boar	Slaughterhouse	Digestion	Meat
Mycobacterium			
HEI 1: Official bovine tuberculosis status	Farm/region/ Member State	Official records, food chain information	Not applicable
HEI 2: Human pathogenic mycobacteria in farmed wild boar and deer at slaughter	Slaughterhouse	Visual meat inspection and microbiology	Suspected lesions

REFERENCES

Alvarez J, Castellanos E, Romero B, Aranaz A, Bezos J, Rodriguez S, Mateos A, Dominguez L and de Juan L, 2011. Epidemiological investigation of a *Mycobacterium avium* subsp. hominissuis outbreak in swine. Epidemiology and Infection, 139, 143–148. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20653991.

Antolova D, Reiterova K and Dubinsky P, 2007. Seroprevalence of *Toxoplasma gondii* in wild boars (Sus scrofa) in the Slovak Republic. Annals of Agricultural and Environmental Medicine, 14, 71–73. Available online: http://www.ncbi.nlm.nih.gov/pubmed/17655180.

Aubert D, Ajzenberg D, Richomme C, Gilot-Fromont E, Terrier ME, de Gevigney C, Game Y, Maillard D, Gibert P, Darde ML and Villena I, 2010. Molecular and biological characteristics of *Toxoplasma gondii* isolates from wildlife in France. Veterinary Parasitology, 171, 346–349. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20417034.

Aurtenetxe O, Barral M, Vicente J, de la Fuente J, Gortazar C and Juste RA, 2008. Development and validation of an enzyme-linked immunosorbent assay for antibodies against *Mycobacterium bovis* in European wild boar. BMC Veterinary Research, 4, 43. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18976491.

Avagnina A, Nucera D, Grassi MA, Ferroglio E, Dalmasso A and Civera T, 2012. The microbiological conditions of carcasses from large game animals in Italy. Meat Science, 91, 266–271. Available online: http://www.ncbi.nlm.nih.gov/pubmed/22386324.

Ballesteros C, Garrido JM, Vicente J, Romero B, Galindo RC, Minguijon E, Villar M, Martin-Hernando MP, Sevilla I, Juste R, Aranaz A, de la Fuente J and Gortazar C, 2009. First data on Eurasian wild boar response to oral immunization with BCG and challenge with a *Mycobacterium bovis* field strain. Vaccine, 27, 6662–6668. Available online: http://www.ncbi.nlm.nih.gov/pubmed/19747578.

Balseiro A, Oleaga A, Orusa R, Robetto S, Zoppi S, Dondo A, Goria M, Gortazar C, Marin JF and Domenis L, 2009. Tuberculosis in roe deer from Spain and Italy. Veterinary Record, 164, 468–470. Available online: http://www.ncbi.nlm.nih.gov/pubmed/19363229.

Bartova E, Sedlak K and Literak I, 2006. Prevalence of *Toxoplasma gondii* and *Neospora caninum* antibodies in wild boars in the Czech Republic. Veterinary Parasitology, 142, 150–153. Available online: http://www.ncbi.nlm.nih.gov/pubmed/16876948.

Bartova E, Sedlak K, Pavlik I and Literak I, 2007. Prevalence of Neospora caninum and Toxoplasma gondii antibodies in wild ruminants from the countryside or captivity in the Czech Republic. Journal of Parasitology, 93, 1216–1218. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18163361.

BDFPA (The British Deer Farms and Parks Association) online. Available online: http://www.bdfpa.org/.

Berger-Schoch AE, Bernet D, Doherr MG, Gottstein B and Frey CF, 2011. *Toxoplasma gondii* in Switzerland: a serosurvey based on meat juice analysis of slaughtered pigs, wild boar, sheep and cattle. Zoonoses Public Health, 58, 472–478. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21824348.

Biet F, Boschiroli ML, Thorel MF and Guilloteau LA, 2005. Zoonotic aspects of *Mycobacterium bovis* and *Mycobacterium avium*-intracellulare complex (MAC). Veterinary Research, 36, 411–436. Available online: http://www.ncbi.nlm.nih.gov/pubmed/15845232.

Billinis C, 2013. Wildlife diseases that pose a risk to small ruminants and their farmers. Small Ruminant Research, 110, 67–70. Available online: http://www.smallruminantresearch.com/article/S0921-4488(12)00465-8/fulltext.

Blagojevic B AD, Ducic M and Buncic S, 2011. Ratio between carcass-and skin-microflora as an abattoir process hygiene indicator. Food Control, 22, 186–190.

Boadella M, Lyashchenko K, Greenwald R, Esfandiari J, Jaroso R, Carta T, Garrido JM, Vicente J, de la Fuente J and Gortazar C, 2011. Serologic tests for detecting antibodies against *Mycobacterium bovis* and *Mycobacterium avium* subspecies *paratuberculosis* in Eurasian wild boar (*Sus scrofa scrofa*). Journal of Veterinary Diagnosis and Investigation, 23, 77–83. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21217031.

Bollo E, Ferroglio E, Dini V, Mignone W, Biolatti B and Rossi L, 2000. Detection of *Mycobacterium tuberculosis* complex in lymph nodes of wild boar (Sus scrofa) by a target-amplified test system. Journal of Veterinary Medicine B Infectious Diseases and Veterinary Public Health, 47, 337–342. Available online: http://www.ncbi.nlm.nih.gov/pubmed/10900824.

Bolton DJ, Sheridan JJ and Doherty AM, 2000. HACCP for Irish beef slaughter. Teagasc—The National Food Centre, Dublin, Ireland.

Booth WD, 1995. Wild boar farming in the United Kingdom. Journal of Mountain Ecology, 3, 245–248.

Brown J and Tollison JW, 1979. Influence of pork consumption on human infection with *Mycobacterium avian*-intracellulare. Applied Environmental Microbiology, 38, 1144–1146. Available online: http://www.ncbi.nlm.nih.gov/pubmed/526017.

Buddle BM, Wilson T, Denis M, Greenwald R, Esfandiari J, Lyashchenko KP, Liggett S and Mackintosh CG, 2010. Sensitivity, specificity, and confounding factors of novel serological tests used for the rapid diagnosis of bovine tuberculosis in farmed red deer (*Cervus elaphus*). Clinical Vaccine Immunology, 17, 626–630. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20164247.

CALU (Use The Centre for Alternative Land), 2007. Wild boar production, online. Available online: http://www.openfields.org.uk/topics/livestock-and-dairy/minority-species-as-livestock/wild-boar-production.html.

Cenci-Goga BT, Rossitto PV, Sechi P, McCrindle CM and Cullor JS, 2011. Toxoplasma in animals, food, and humans: an old parasite of new concern. Foodborne Pathogens and Diseases, 8, 751–762. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21486145.

Chen XG, Gong Y, Hua L, Lun ZR and Fung MC, 2001. High-level expression and purification of immunogenic recombinant SAG1 (P30) of *Toxoplasma gondii* in *Escherichia coli*. Protein Expression and Purification, 23, 33–37. Available online: http://www.ncbi.nlm.nih.gov/pubmed/11570843.

Choi WY, Nam HW, Kwak NH, Huh W, Kim YR, Kang MW, Cho SY and Dubey JP, 1997. Foodborne outbreaks of human toxoplasmosis. Journal of Infectious Diseases, 175, 1280–1282. Available online: http://www.ncbi.nlm.nih.gov/pubmed/9129105.

Cook AJ, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, Foulon W, Semprini AE and Dunn DT, 2000. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. BMJ, 321, 142–147. Available online: http://www.ncbi.nlm.nih.gov/pubmed/10894691.

Cowled BD, Ward MP, Laffan SW, Galea F, Garner MG, MacDonald AJ, Marsh I, Muellner P, Negus K, Quasim S, Woolnough AP and Sarre SD, 2012. Integrating survey and molecular approaches to better understand wildlife disease ecology. PLoS One, 7, e46310. Available online: http://www.ncbi.nlm.nih.gov/pubmed/23071552.

De Craeye S, Speybroeck N, Ajzenberg D, Darde ML, Collinet F, Tavernier P, Van Gucht S, Dorny P and Dierick K, 2011. *Toxoplasma gondii* and *Neospora caninum* in wildlife: common parasites in Belgian foxes and Cervidae? Veterinary Parasitology, 178, 64–69. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21236577.

Di Marco V MC, Russo M, Aronica V, Bollo E, Amedeo S, Valenza F, Capucchio MT, 2008. Black wild pigs of Nebrodi Park in Sicily: evidence of their possible role as reservoir of *Mycobacterium*

tuberculosis complex infection. Proceedings of the Proceedings of the 26th meeting of European Society of Veterinary Pathology, 17–21 September, Dubrovnik, Croatia.

Domingos M, Amado A and Botelho A, 2009. IS1245 RFLP analysis of strains of Mycobacterium avium subspecies hominissuis isolated from pigs with tuberculosis lymphadenitis in Portugal. Veterinary Record, 164, 116–120. Available online: http://www.ncbi.nlm.nih.gov/pubmed/19168882.

Dubey JP, Jenkins MC, Kwok OC, Zink RL, Michalski ML, Ulrich V, Gill J, Carstensen M and Thulliez P, 2009. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in white-tailed deer (Odocoileus virginianus) from Iowa and Minnesota using four serologic tests. Veterinary Parasitology, 161, 330–334. Available online: http://www.ncbi.nlm.nih.gov/pubmed/19285809.

EFSA (European Food Safety Authority), 2005. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial resistance in the European Union in 2004. The EFSA Journal 2005, 310,1–280.

EFSA (European Food Safety Authority), 2006a. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the European Union in 2005. The EFSA Journal 2006, 94, 1-288.

EFSA (European Food Safety Authority), 2006b. Report of the Task Force on Zoonoses Data Collection on Guidance Documents on Good Practices for Design of Field Surveys, The EFSA Journal 2006, 93,1–24.

EFSA (European Food Safety Authority), 2007a. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the European Union in 2006. The EFSA Journal 2007, 130, 1–352.

EFSA (European Food Safety Authority), 2007b. Scientific Opinion of the Panel on Biological Hazards on surveillance and monitoring of Toxoplasma in humans, food and animals. The EFSA Journal 2007, 583, 1–64.

EFSA (European Food Safety Authority), 2008a. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of Salmonella in slaughter pigs, in the EU, 2006–2007 [1]—Part A: Salmonella prevalence estimates. The EFSA Journal 2008, 135,1–111.

EFSA (European Food Safety Authority), 2008b. Scientific report on ''Tuberculosis testing in deer''. Panel on Animal Health and Animal Welfare. The EFSA Journal 2008, 645, 1–34.

EFSA (European Food Safety Authority), 2009a. Analysis of the baseline survey on the prevalence of Salmonella in holdings with breeding pigs in the EU, 2008—Part A: Salmonella prevalence estimates. EFSA Journal 2009; 7(12):1377, 93 pp. doi:10.2903/j.efsa.2009.1377

EFSA (European Food Safety Authority), 2009b. The Community Summary Report on Food-Borne Outbreaks in The European Union in 2007. The EFSA Journal 2009,271, 1–128.

EFSA (European Food Safety Authority), 2009c. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. The EFSA Journal 2009, 223, 1–320.

EFSA (European Food Safety Authority), 2010 The Community Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008 EFSA Journal 2010;8(1):1496, 410 pp. doi:10.2903/j.efsa.2010.1496

EFSA (European Food Safety Authority), 2011. Technical specifications on harmonised epidemiological indicators for public health hazards to be covered by meat inspection of swine EFSA Journal 2011;9(10):2371, 125 pp. doi:10.2903/j.efsa.2011.2371

EFSA (European Food Safety Authority), 2012. Technical hearing on the hazards to be covered by inspection of meat from farmed game EFSA Journal, EN-376 [24 pp].

EFSA and ECDC (European Food Safety Authority, European Centre for Disease Prevention and Control), 2011. The European Union Summary Report on Trends and sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009. EFSA Journal 2011;9(3):2090, 378pp. doi:10.2903/j.efsa.2011.2090

EFSA and ECDC (European Food Safety Authority, European Centre for Disease Prevention and Control), 2012. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. EFSA Journal 2012;10(3):2597, 442pp. doi:10.2903/j.efsa.2012.2597

EFSA and ECDC (European Food Safety Authority, European Centre for Disease Prevention and Control), 2013. The European Union Summary Report on Trends and sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011. EFSA Journal 2013;11(4):3129, 250 pp. doi:10.2903/j.efsa.2013.3129

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013a. Scientific Opinion on the public health hazards to be covered by inspection of meat from farmed game. EFSA Journal 2013;11(6):3264, 181 pp. doi:10.2903/j.efsa.2013.3264

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013b. Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine animals). EFSA Journal 2013;11(6):3266, 261pp. doi:10.2903/j.efsa.2013.3266

EFSA Panel on Biological Hazards (BIOHAZ), 2011. Scientific Opinion on the public health hazards to be covered by inspection of meat (swine). EFSA Journal 2011;9(10):2351, 198 pp. doi:10.2903/j.efsa.2011.2351

Erler W, Martin G, Sachse K, Naumann L, Kahlau D, Beer J, Bartos M, Nagy G, Cvetnic Z, Zolnir-Dovc M and Pavlik I, 2004. Molecular fingerprinting of *Mycobacterium bovis* subsp. caprae isolates from central Europe. Journal of Clinical Microbiology 42, 2234–2238. Available online: http://www.ncbi.nlm.nih.gov/pubmed/15131199.

Foyle KL, Delahay RJ and Massei G, 2010. Isolation of *Mycobacterium bovis* from a feral wild boar (Sus scrofa) in the UK. Veterinary Record, 166, 663–664. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20495174.

Gamarra JA, Cabezon O, Pabon M, Arnal MC, Luco DF, Dubey JP, Gortazar C and Almeria S, 2008. Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain. Veterinary Parasitology, 153, 152 156. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18316161.

Gamble HR, Bessonov AS, Cuperlovic K, Gajadhar AA, van Knapen F, Noeckler K, Schenone H and Zhu X, 2000. International Commission on Trichinellosis: recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption. Veterinary Parasitology, 93, 393–408. Available online: http://www.ncbi.nlm.nih.gov/pubmed/11099850.

Garcia-Bocanegra I, Simon-Grife M, Dubey JP, Casal J, Martin GE, Cabezon O, Perea A and Almeria S, 2010. Seroprevalence and risk factors associated with Toxoplasma gondii in domestic pigs from Spain. Parasitology International, 59, 421-426. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20601110.

Garcia-Bocanegra I, Cabezon O, Pabon M, Gomez-Guillamon F, Arenas A, Alcaide E, Salas-Vega R, Dubey JP and Almeria S, 2012a. Prevalence of *Toxoplasma gondii* and *Neospora caninum* antibodies in Spanish ibex (C*apra pyrenaica hispanica*). Veterinary Journal, 191, 257–260. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21159529.

Garcia-Bocanegra I, Perez de Val B, Arenas-Montes A, Paniagua J, Boadella M, Gortazar C and Arenas A, 2012b. Seroprevalence and risk factors associated to *Mycobacterium bovis* in wild artiodactyl species from southern Spain, 2006-2010. PLoS One, 7, e34908. Available online: http://www.ncbi.nlm.nih.gov/pubmed/22523563.

Gauss CB, Dubey JP, Vidal D, Ruiz F, Vicente J, Marco I, Lavin S, Gortazar C and Almeria S, 2005. Seroprevalence of *Toxoplasma gondii* in wild pigs (Sus scrofa) from Spain. Veterinary Parasitology, 131, 151–156. Available online: http://www.ncbi.nlm.nih.gov/pubmed/15927398.

Gauss CB, Dubey JP, Vidal D, Cabezon O, Ruiz-Fons F, Vicente J, Marco I, Lavin S, Gortazar C and Almeria S, 2006. Prevalence of *Toxoplasma gondii* antibodies in red deer (*Cervus elaphus*) and other wild ruminants from Spain. Vet Parasitol, 136, 193–200. Available online: http://www.ncbi.nlm.nih.gov/pubmed/16359801.

Gavier-Widen D, Cooke, M.M., Gallagher, J., Cahmbers, M.A., Gortázar, C, 2009. A review of infection of wildlife hosts with *Mycobacterium bovis* and the diagnostic difficulties of the no visible lesion presentation. New Zealand Veterinary Journal, 57, 122–131. Available online: http://www.ncbi.nlm.nih.gov/pubmed/?term=gavier+widen+chambers+ma+tuberculosis.

Glawischnig W, Steineck T and Spergser J, 2006. Infections caused by *Mycobacterium avium* subspecies avium, hominissuis, and paratuberculosis in free-ranging red deer (*Cervus elaphus hippelaphus*) in Austria, 2001–2004. Journal of Wildlife Diseases, 42, 724–731. Available online: http://www.ncbi.nlm.nih.gov/pubmed/17255438.

Gortazar C, Vicente J and Gavier-Widen D, 2003. Pathology of bovine tuberculosis in the European wild boar (*Sus scrofa*). Veterinary Record, 152, 779780. Available online: http://www.ncbi.nlm.nih.gov/pubmed/12846290.

Gottstein B, Pozio E and Nockler K, 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clinical Microbiological Reviews, 22, 127–145, table of contents. Available online: http://www.ncbi.nlm.nih.gov/pubmed/19136437.

Grimont PAD and Weill F (WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur), 2007. Antigenic formulae of the *Salmonella* serovars, 9th edition [167 pp].

Halova D, Mulcahy G, Rafter P, Turcekova L, Grant T and de Waal T, 2013. *Toxoplasma gondii* in Ireland: seroprevalence and novel molecular detection method in sheep, pigs, deer and chickens. Zoonoses Public Health, 60, 168–173. Available online: http://www.ncbi.nlm.nih.gov/pubmed/22697578.

Holec-Gasior L, Kur J, Hiszczynska-Sawicka E, Drapala D, Dominiak-Gorski B and Pejsak Z, 2010. Application of recombinant antigens in serodiagnosis of swine toxoplasmosis and prevalence of *Toxoplasma gondii* infection among pigs in Poland. Polish Journal of Veterinary Science, 13, 457–464. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21033559.

ifood (ifood Culinary Encyclopedia), online. Wild boar meat. Available online: http://www.ifood.tv/network/wild_boar_meat.

ISO (International Organization for Standardization), 2002. ISO 6579:2002—Microbiology of food and animal feeding stuffs—Horizontal method for the detection of Salmonella spp.

ISO (International Organization for Standardization), 2007. ISO 6579:2002/Amd 1:2007. Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.

ISS (Istituto Superiore di Sanità), online. Figure on *Trichinella* life cycle. Available online: http://www.iss.it/site/Trichinella/index.asp

Jaroso R, Vicente J, Fernandez-de-Mera IG, Aranaz A and Gortazar C, 2010a. Eurasian wild boar response to skin-testing with mycobacterial and non-mycobacterial antigens. Preventive Veterinary Medicine, 96, 211–217. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20633938.

Jaroso R, Vicente J, Martin-Hernando MP, Aranaz A, Lyashchenko K, Greenwald R, Esfandiari J and Gortazar C, 2010b. Ante-mortem testing wild fallow deer for bovine tuberculosis. Veterinary Microbiology, 146, 285–289. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20605378.

Jokelainen P, Nareaho A, Knaapi S, Oksanen A, Rikula U and Sukura A, 2010. *Toxoplasma gondii* in wild cervids and sheep in Finland: north-south gradient in seroprevalence. Veterinary Parasitology, 171, 331–336. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20434266.

Jokelainen P, Nareaho A, Halli O, Heinonen M and Sukura A, 2012. Farmed wild boars exposed to *Toxoplasma gondii* and *Trichinella* spp. Veterinary Parasitology, 187, 323–327. Available online: http://www.ncbi.nlm.nih.gov/pubmed/22244535.

Kapel CM, 2001. Sylvatic and domestic *Trichinella* spp. in wild boars; infectivity, muscle larvae distribution, and antibody response. Journal of Parasitology, 87, 309–314. Available online: http://www.ncbi.nlm.nih.gov/pubmed/11318560.

Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, Eskild A and Eng J, 1996. Risk factors for Toxoplasma gondii infection in pregnancy. Results of a prospective case-control study in Norway. American Journal of Epidemiology, 144, 405–412. Available online: http://www.ncbi.nlm.nih.gov/pubmed/8712198.

Kijlstra A and Jongert E, 2008. Control of the risk of human toxoplasmosis transmitted by meat. International Journal of Parasitology, 38, 1359–1370. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18694755.

Kimbita EN, Xuan X, Huang X, Miyazawa T, Fukumoto S, Mishima M, Suzuki H, Sugimoto C, Nagasawa H, Fujisaki K, Suzuki N, Mikami T and Igarashi I, 2001. Serodiagnosis of *Toxoplasma gondii* infection in cats by enzyme-linked immunosorbent assay using recombinant SAG1. Veterinary Parasitology, 102, 35–44. Available online: http://www.ncbi.nlm.nih.gov/pubmed/11705650.

Koutsoumanis K and Sofos JN, 2004. Microbial contamination. In: Jensen WK, Devine C, Dikeman M (eds), Encyclopaedia of Meat Sciences. Elsevier, Oxford, pp. 727–737.

Lopes AP, Sargo R, Rodrigues M and Cardoso L, 2011. High seroprevalence of antibodies to *Toxoplasma gondii* in wild animals from Portugal. Parasitology Research, 108, 1163–1169. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21104273.

Lugton IW, Wilson PR, Morris RS and Nugent G, 1998. Epidemiology and pathogenesis of *Mycobacterium bovis* infection of red deer (*Cervus elaphus*) in New Zealand. New Zealand Veterinary Journal, 46, 147–156. Available online: http://www.ncbi.nlm.nih.gov/pubmed/16032039.

Lyashchenko KP, Greenwald R, Esfandiari J, Chambers MA, Vicente J, Gortazar C, Santos N, Correia-Neves M, Buddle BM, Jackson R, O'Brien DJ, Schmitt S, Palmer MV, Delahay RJ and Waters WR, 2008. Animal-side serological assay for rapid detection of *Mycobacterium bovis* infection in multiple species of free-ranging wildlife. Veterinary Microbiology, 132, 283–292. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18602770.

Mackintosh CG, de Lisle GW, Collins DM and Griffin JF, 2004. Mycobacterial diseases of deer. New Zealand Veterinary Journal, 52, 163–174. Available online: http://www.ncbi.nlm.nih.gov/pubmed/15726126.

Maeder S, Hars J, Rambaud T, Game Y, Boschiroli M-L 2009. Rôle du sanglier (*Sus scrofa*) dans l épidémiologie de la tuberculose dans la forêt de Brotonne (France). Résultats de l'enquête épidémiologique 2006–2007. Épidémiologie et Santé Animale, 53, 129–144. Available online: http://aeema.vet-alfort.fr/public/php/article.php?numero=53&lang=1&article=13.

Magnino SFM, Fabbi M, Bianchi A, Grazia Zanoni M, Merialdi G, Ludovica Pacciarini, Gaffuru A, 2011. The monitoring of selected zoonotic diseases of wildlife in Lombardy and Emilia-Romagna, northern Italy. In: Paulsen et al. (eds), Game meat hygiene in focus. Wageningen, Academic Publisher, pp. 223-243.

Malmsten J, Jakubek EB and Bjorkman C, 2011. Prevalence of antibodies against *Toxoplasma gondii* and Neospora caninum in moose (Alces alces) and roe deer (*Capreolus capreolus*) in Sweden.

Veterinary Parasitology, 177, 275–280. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21232869.

Martin-Hernando MP, Hofle U, Vicente J, Ruiz-Fons F, Vidal D, Barral M, Garrido JM, de la Fuente J and Gortazar C, 2007. Lesions associated with *Mycobacterium tuberculosis* complex infection in the European wild boar. Tuberculosis (Edinb), 87, 360–367. Available online: http://www.ncbi.nlm.nih.gov/pubmed/17395539.

Mentaberre G PM, Navarro-Gonzalez N, Serrano E, Dominguez L, Lavin S, 2013. Cattle drive *Salmonella* infection in the wildlife-livestock interfere. Zoonoses Public Health. In press..

Merialdi G, Bardasi L, Fontana MC, Spaggiari B, Maioli G, Conedera G, Vio D, Londero M, Marucci G, Ludovisi A, Pozio E and Capelli G, 2011. First reports of *Trichinella pseudospiralis* in wild boars (*Sus scrofa*) of Italy. Veterinary Parasitology, 178, 370–373. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21296503.

Miranda C, Matos M, Pires I, Correia-Neves M, Ribeiro P, Alvares S, Vieira-Pinto M and Coelho AC, 2012. Diagnosis of *Mycobacterium avium* complex in granulomatous lymphadenitis in slaughtered domestic pigs. Journal of Comparative Pathology, 147, 401–405. Available online: http://www.ncbi.nlm.nih.gov/pubmed/22784782.

Montagnaro S, Sasso S, De Martino L, Longo M, Iovane V, Ghiurmino G, Pisanelli G, Nava D, Baldi L and Pagnini U, 2010. Prevalence of antibodies to selected viral and bacterial pathogens in wild boar (*Sus scrofa*) in Campania Region, Italy. Journal of Wildlife Diseases 46, 316–319. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20090052.

More SJ, Cameron AR, Greiner M, Clifton-Hadley RS, Rodeia SC, Bakker D, Salman MD, Sharp JM, De Massis F, Aranaz A, Boniotti MB, Gaffuri A, Have P, Verloo D, Woodford M and Wierup M, 2009. Defining output-based standards to achieve and maintain tuberculosis freedom in farmed deer, with reference to MSs of the European Union. Preventive Veterinary Medicine, 90, 254–267. Available online: http://www.sciencedirect.com/science/article/pii/S0167587709000798.

Moser I, Schettler E, Hotzel H, Herzog S and Frolich K, 2011. Mycobacterial infections in free-living cervids in Germany (2002-2006). Journal of Wildlife Diseases 47, 999–1004. Available online: http://www.ncbi.nlm.nih.gov/pubmed/22102674.

Naranjo V, Gortazar C, Vicente J and de la Fuente J, 2008. Evidence of the role of European wild boar as a reservoir of *Mycobacterium tuberculosis* complex. Veterinary Microbiology, 127, 1–9. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18023299.

Neghina R, Neghina AM, Marincu I, Moldovan R and Iacobiciu I, 2010. Evidence of *Trichinella spiralis* in Timis County, Romania: a report of a winter trichinellosis outbreak in 2008 due to consumption of contaminated pork. Vector Borne Zoonotic Diseases, 10, 931–933. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20370432.

Nockler K, 2003. *Trichinella* prevalence in the domestic and sylvatic cycle and its importance as foodborne pathogen. Helminthologia, 40, 103–108.

Nogareda F, Beauflis P, Le Hello S, Thos AL, Roy G, Robert M, Thill E, Moury F, Weill FX and Jourdan- Da Silva, N, 2011. Gastroenteritis outbreak due to *Salmonella* Enteritidis following the consumption of wild boar meat in central France, February 2011. Abstract book of European Scientific Conference on Applied Infectious Disease Epidemiology, Stockholm, Sweden, 6-8 November 2011.

Offermann U, Bodmer T, Audige L and Jemmi T, 1999. [The prevalence of salmonella, yersinia and mycobacteria in slaughtered pigs in Switzerland]. Schweizer Archiv fur Tierheilkunde, 141, 509–515. Available online: http://www.ncbi.nlm.nih.gov/pubmed/10596271.

OIE (World Organisation for Animal Health), 2012. Manual of diagnostic tests and vaccines for terrestrial animals, 7th edition.

Oivanen L, Mikkonen T and Sukura A, 2000. An outbreak of trichinellosis in farmed wild boar in Finland. APMIS, 108, 814–818. Available online: http://www.ncbi.nlm.nih.gov/pubmed/11252814.

Opsteegh M, Swart A, Fonville M, Dekkers L and van der Giessen J, 2011. Age-related *Toxoplasma gondii* seroprevalence in Dutch wild boar inconsistent with lifelong persistence of antibodies. PLoS One, 6, e16240. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21283764.

Palmer MV, Whipple DL, Butler KL, Fitzgerald SD, Bruning-Fann CS and Schmitt SM, 2002. Tonsillar lesions in white-tailed deer (Odocoileus virginianus) naturally infected with *Mycobacterium bovis*. Veterinary Record, 151, 149–150. Available online: http://www.ncbi.nlm.nih.gov/pubmed/12199435.

Parra A, Garcia A, Inglis NF, Tato A, Alonso JM, Hermoso de Mendoza M, Hermoso de Mendoza J and Larrasa J, 2006. An epidemiological evaluation of *Mycobacterium bovis* infections in wild game animals of the Spanish Mediterranean ecosystem. Research in Veterinary Science, 80, 140–146. Available online: http://www.ncbi.nlm.nih.gov/pubmed/16045949.

Pate M, Zolnir-Dovc M, Kusar D, Krt B, Spicic S, Cvetnic Z and Ocepek M, 2011. The first report of *Mycobacterium celatum* isolation from domestic pig (*Sus scrofa domestica*) and roe deer (*Capreolus capreolus*) and an overview of human infections in Slovenia. Veterinary Medicine International, 2011, 432954. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21647336.

Paulsen P, Smulders FJM and Hilbert F, 2012. *Salmonella* in meat from hunted game: a Central European perspective. Food Research International, 45, 609–616. Available online: http://www.sciencedirect.com/science/article/pii/S0963996911004376

Pozio E and Darwin Murrell K, 2006. Systematics and epidemiology of *Trichinella*. Advances in Parasitology, 63, 367–439. Available online: http://www.ncbi.nlm.nih.gov/pubmed/17134656.

Pozio E and Kapel CM, 1999. *Trichinella nativa* in sylvatic wild boars. Journal of Helminthology, 73, 87–89. Available online: http://www.ncbi.nlm.nih.gov/pubmed/10431373.

Pozio E, Casulli A, Bologov VV, Marucci G and La Rosa G, 2001. Hunting practices increase the prevalence of *Trichinella* infection in wolves from European Russia. Journal of Parasitology, 87, 1498–1501. Available online: http://www.ncbi.nlm.nih.gov/pubmed/11780851.

Queiros J, Alvarez J, Carta T, Mateos A, Ortiz JA, Fernandez-de-Mera IG, Martin-Hernando MP and Gortazar C, 2012. Unexpected high responses to tuberculin skin-test in farmed red deer: implications for tuberculosis control. Preventive Veterinary Medicine, 104, 327–334. Available online: http://www.ncbi.nlm.nih.gov/pubmed/22197268.

Richomme C, Afonso E, Tolon V, Ducrot C, Halos L, Alliot A, Perret C, Thomas M, Boireau P and Gilot-Fromont E, 2010. Seroprevalence and factors associated with *Toxoplasma gondii* infection in wild boar (*Sus scrofa*) in a Mediterranean island. Epidemiology and Infection, 138, 1257–1266. Available online: http://www.ncbi.nlm.nih.gov/pubmed/20096148.

Robert-Gangneux F and Darde ML, 2012. Epidemiology of and diagnostic strategies for toxoplasmosis. Clinical Microbioly Reviews, 25, 264–296. Available online: http://www.ncbi.nlm.nih.gov/pubmed/22491772.

Rodriguez E, Sanchez LP, Perez S, Herrera L, Jimenez MS, Samper S and Iglesias MJ, 2009. Human tuberculosis due to *Mycobacterium bovis* and *M. caprae* in Spain, 2004-2007. International Journal of Tuberculosis and Lung Diseases 13, 1536–1541. Available online: http://www.ncbi.nlm.nih.gov/pubmed/19919773.

Rodriguez S, Bezos J, Romero B, de Juan L, Alvarez J, Castellanos E, Moya N, Lozano F, Javed MT, Saez-Llorente JL, Liebana E, Mateos A, Dominguez L, Aranaz A, Spanish Network on S and Monitoring of Animal T, 2011. *Mycobacterium caprae* infection in livestock and wildlife, Spain. Emerging Infectious Diseases, 17, 532–535. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21392452.

Rohonczy EB, Balachandran AV, Dukes TW, Payeur JB, Rhyan JC, Saari DA, Whiting TL, Wilson SH and Jarnagin JL, 1996. A comparison of gross pathology, histopathology, and mycobacterial culture for the diagnosis of tuberculosis in elk (*Cervus elaphus*). Canadian Journal of Veterinary Research, 60, 108–114. Available online: http://www.ncbi.nlm.nih.gov/pubmed/8785715.

Ross RD, Stec LA, Werner JC, Blumenkranz MS, Glazer L and Williams GA, 2001. Presumed acquired ocular toxoplasmosis in deer hunters. Retina, 21, 226–229. Available online: http://www.ncbi.nlm.nih.gov/pubmed/11421011.

Santos N, Correia-Neves M, Ghebremichael S, Kallenius G, Svenson SB and Almeida V, 2009. Epidemiology of *Mycobacterium bovis* infection in wild boar (*Sus scrofa*) from Portugal. Journal of Wildlife Diseases, 45, 1048–1061. Available online: http://www.ncbi.nlm.nih.gov/pubmed/19901381.

ScottishVenison, online. Deer farming in Scotland. Available online: http://www.scottish-venison.info/index.php?page=Deer-farming-in-Scotland

Teagasc (Ireland's Agriculture and Food Development Authority), online. Deer farming. Available online: http://www.teagasc.ie/ruraldev/deer/

Thakur S, Sandfoss M, Kennedy-Stoskopf S and DePerno CS, 2011. Detection of *Clostridium difficile* and *Salmonella* in feral swine population in North Carolina. Journal of Wildlife Diseases, 47, 774–776. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21719851.

van Ingen J HW, Dekhuijzen PN, Boeree MJ, van Soolingen D., 2010. The changing pattern of clinical *Mycobacterium avium* isolation in the Netherlands. International Journal of Tuberculosis and Lung Diseases, 14, 1176–1180.

Vengust G, Valencak Z and Bidovec A, 2006. A serological survey of selected pathogens in wild boar in Slovenia. Journal of Veterinary Medicine B Infectious Diseases and Veterinary Public Health,, 53, 24–27. Available online: http://www.ncbi.nlm.nih.gov/pubmed/16460352.

Vicente J, Hofle U, Garrido JM, Fernandez-De-Mera IG, Juste R, Barral M and Gortazar C, 2006. Wild boar and red deer display high prevalences of tuberculosis-like lesions in Spain. Veterinary Research, 37, 107–119. Available online: http://www.ncbi.nlm.nih.gov/pubmed/16336928.

Vieira-Pinto M, Morais L, Caleja C, Themudo P, Torres C, Igrejas G, Poeta P and Martins C, 2011. *Salmonella* sp. in game (*Sus scrofa* and *Oryctolagus cuniculus*). Foodborne Pathogens and Diseases, 8, 739–740. Available online: http://www.ncbi.nlm.nih.gov/pubmed/21254910.

Wacheck S, Fredriksson-Ahomaa M, Konig M, Stolle A and Stephan R, 2010. Wild boars as an important reservoir for foodborne pathogens. Foodborne Pathogens and Diseases, 7, 307–312. Available online: http://www.ncbi.nlm.nih.gov/pubmed/19899962.

Waddell LA, Rajic A, Sargeant J, Harris J, Amezcua R, Downey L, Read S and McEwen SA, 2008. The zoonotic potential of *Mycobacterium avium* spp. *paratuberculosis*: a systematic review. Canadian Journal of Public Health, 99, 145–155. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18457292.

Wahlstrom H, Tysen E, Olsson Engvall E, Brandstrom B, Eriksson E, Morner T and Vagsholm I, 2003. Survey of *Campylobacter* species, VTEC O157 and *Salmonella* species in Swedish wildlife. Veterinary Record, 153, 74–80. Available online: http://www.ncbi.nlm.nih.gov/pubmed/12892266.

Waters WR, Palmer MV, Thacker TC, Orloski K, Nol P, Harrington NP, Olsen SC and Nonnecke BJ, 2008. Blood culture and stimulation conditions for the diagnosis of tuberculosis in cervids by the Cervigam assay. Veterinary Record, 162, 203–208. Available online: http://veterinaryrecord.bmj.com/content/162/7/203.abstract.

Wilson G, Broughan J, Chambers M, Clifton-Hadley R, Crawshaw T, de la Fuente J, Delahay R, Gavier-Widen D, Gortazar C, Hewinson G, Jackson V, Martín-Hernando MP, Neimanis A, Salguero FJ, Vicente J, Ward A and McDonald R, 2009. Scientific Review on Tuberculosis in

Wildlife in the EU. Technical Report submitted to EFSA. Available online: http://www.efsa.europa.eu/en/supporting/pub/12e.htm.

Zanella G, Duvauchelle A, Hars J, Moutou F, Boschiroli ML and Durand B, 2008. Patterns of lesions of bovine tuberculosis in wild red deer and wild boar. Veterinary Record, 163, 43–47. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18621995.

Zanetti S, Bua A, Molicotti P, Delogu G, Mura A, Ortu S and Sechi LA, 2008. Identification of mycobacterial infections in wild boars in Northern Sardinia, Italy. Acta Veterinaria Hungarica, 56, 145–152. Available online: http://www.ncbi.nlm.nih.gov/pubmed/18669241.

Zottola T, Montagnaro S, Magnapera C, Sasso S, De Martino L, Bragagnolo A, D'Amici L, Condoleo R, Pisanelli G, Iovane G and Pagnini U, 2013. Prevalence and antimicrobial susceptibility of *Salmonella* in European wild boar (*Sus scrofa*); Latium Region—Italy. Comparative Immunological and Microbiological Infectious Diseases, 36, 161–168. Available online: http://www.ncbi.nlm.nih.gov/pubmed/23253890.

APPENDIX

Appendix A. Food chain, risk and risk-reducing factors, possible harmonised epidemiological indicators and their evaluation

Salmonella

1.   Identification of potential epidemiological indicators

Table 13:   Potential epidemiological indicators for *Salmonella* in farmed wild boar

	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 *Salmonella* infection in farmed wild boar on-farm before the slaughter	Data available	Possible to gather	Presence of *Salmonella* in farmed wild boar. Microbiology testing of pooled faecal samples
			Presence of antibodies in wild boar. Serological testing of blood samples before slaughter
Risk factor 2 Replacement animals from *Salmonella* negative/positive herds)	Data on *Salmonella* in breeding animals can be available	Possible to gather	Audit on-farm
Risk factor 3 *Salmonella* contaminated feed and water	It is possible to generate such data?	Yes	*Salmonella* presence in feed. Audit on feed management practices
Risk factor 4 Contact of feed with wildlife, especially rodents and birds		Yes	Audit for husbandry conditions
Risk factor 5 Evisceration carried out on-farm—cross-contamination of meat	No	Yes	Audit of evisceration process, cleanliness of animals
Transport to slaughterhouse			
Risk factor 1 Contamination during loading and transport	No	No	Audit of cleanliness of vehicle and carcasses, time of transport, temperature during transport, mixing of carcasses during transport

Table continued overleaf.

Table 13 (continued): Potential epidemiological indicators for *Salmonella* in farmed wild boar

	Availability of prevalence data	Data availability to divide population into groups between which the risk varies	Suggested epidemiological indicator (HEI)
Slaughterhouse			
Risk factor 1 Faecal contamination during dehiding	It is possible to generate such data?	Surveys on surface samples from carcasses can easily carried out Limited data available to show differences between slaughterhouses	Microbiological testing of *Salmonella* carcass swabs
Risk factor 2 Faecal contamination during evisceration			
Risk factor 3 Cross-contamination during the slaughter process			
Processing of meat and products thereof			
Risk factor 1 Boning/mincing/further processing Cross-contamination due to operatives, poor procedures and dirty equipment	No	Yes	Microbiological testing—end product testing In accordance with Regulation (EC) No 2075/2005[a]
Retail			
Risk factor 1 Temperature abuses			
Risk factor 2 Cross-contamination			
Consumer			
Risk factor 1 Eating of raw or undercooked meat			
Risk factor 2 Temperature abuses		Temperature of refrigerator	
Risk factor 3 Cross-contamination			

(a): Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, pp. 1–26.

2. Evaluation of suggested indicators

Table 14: Suggested epidemiological indicators for *Salmonella* in farmed wild boar

Weighting factor				30 %	40 %	15 %	15 %	
Indicators (animal/food category)	**Food chain stage**	**Analytical/ diagnostic method**	**Specimen**	**Quality of indicator[a] (0, 1, 2)[e]**	**Appropriateness of indicator[b] (0, 1, 2)[e]**	**Data availability[c] (0, 1, 2)[e]**	**Feasibility[d] (0, 1 ,2)[e]**	**Total points**
Presence of *Salmonella* in farmed wild boar—infection status on-farm	Farm	Microbiological testing	Faecal samples	2	2	2	1	1.85
Presence of *Salmonella* antibodies in farmed wild boar	Farm	Serological testing	Blood samples	0	0	1	0	0.15
Audit on-farm for husbandry conditions	Farm	Auditing	n.a.	0	1	1	1	0.70
Replacement animals from *Salmonella* negative/positive herds)	Farm	Auditing	n.a.	0	1	0	0	0.40
Salmonella presence in feed	Farm	Microbiological testing	Feed samples	2	0	1	1	0.90
Audit of evisceration process, cleanliness of animals	Farm	Auditing	n.a.	1	0	1	0	0.45
Audit of cleanliness of vehicle and carcasses, time of transport, temperature during transport, mixing of carcasses during transport	Slaughterhouse	Auditing	n.a.	1	1	1	1	1.0
Presence of *Salmonella* by carcass swabs before chilling	Slaughterhouse	Microbiological testing	Swabs	2	2	2	2	2.0
Presence of *Salmonella* in meat	Cutting or processing plant	Microbiological testing	Swabs or meat samples	1	1	0	1	0.85

(a): Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
(b): Appropriateness of indicator = how well the indicator correlates with the human health risk caused by the hazard and the possibility/need to amend the meat inspection method.
(c): Data availability = are there data already available or is it easy to get the data needed?
(d): Feasibility = how laborious is the sampling and testing procedure?
(e): 0 = bad, 1 = moderate, 2 = good.

Toxoplasma

1. Identification of potential epidemiological indicators

Table 15: Potential epidemiological indicators for *Toxoplasma* in farmed deer and farmed wild boar

	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 Presence of cats at the farm	Available in scientific literature. Only limited data are available	Yes	Audit on the farm
Risk factor 2 Age of animals (older animals at higher risk)	Available in scientific literature. Only limited data are available	Yes	Detection of *Toxoplasma* antibodies in the older animals (at slaughterhouse)
Risk factor 3 *Toxoplasma*-infected animals	Available in scientific literature. Only limited data are available	It is possible to generate such data?	Detection of *Toxoplasma* antibodies in the animals (at slaughterhouse)
Risk factor 4 Inefficient rodent control (wild boar)	–	Yes	Audit on the farm, rodent control
Risk factor 5 Use of contaminated surface water and feed	–	It is possible to generate such data?	Audit on the farm, water and feed
Transport to slaughterhouse			
Risk factor 1	–	–	
Slaughterhouse			
Risk factor 1	–	–	

Table continued overleaf.

Table 15 (continued): Potential epidemiological indicators for *Toxoplasma* in farmed deer and farmed wild boar

	Availability of prevalence data	Data availability to divide population into groups between which the risk varies	Suggested epidemiological indicator (HEI)
Processing of meat and products thereof			
Risk factor 1	–	–	
Retail			
Risk factor 1	–	–	
Consumer			
Risk factor 1 Seronegative pregnant women (fetus)	Human surveillance data		
Risk factor 2 Immunosuppressed persons	Available in scientific literature		
Risk factor 3 Consuming raw or undercooked game	Available in scientific literature		

2. Evaluation of suggested indicators

Table 16: Suggested epidemiological indicators for *Toxoplasma* in farmed deer and farmed wild boar

Weighting factor				30 %	40 %	15 %	15 %	
Indicators (animal/food category/other)	Food chain stage	Analytical/ diagnostic method	Specimen	Quality of indicator[a] (0, 1, 2)[e]	Appropriateness of indicator[b] (0, 1, 2)[e]	Data availability[c] (0, 1, 2)[e]	Feasibility[d] (0, 1 ,2)[e]	Total points
Audit on the farm on husbandry conditions (access of cats, rodent control, contamination of water and feed)	Farm	Auditing	n.a.	0	1	1	1	0.70
Detection of *Toxoplasma* antibodies in the older animals (more than one year)	Slaughterhouse	Serology	Meat juice	2	1	2	2	1.60
Detection of *Toxoplasma* antibodies in all animals	Slaughterhouse	Serology	Meat juice	1	2	2	2	1.70

(a): Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
(b): Appropriateness of indicator = how well the indicator correlates with the human health risk caused by the hazard and the possibility/need to amend the meat inspection method.
(c): Data availability = are there data already available or is it easy to get the data needed?
(d): Feasibility = how laborious is the sampling and testing procedure?
(e): 0 = bad, 1 = moderate, 2 = good.

Trichinella

1.   Identification of potential epidemiological indicators

Table 17:   Potential epidemiological indicators for *Trichinella* in farmed wild boar

	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 of wild animals and ingestion of their flesh	Some from annual reporting on zoonoses; a few from literature	Very few, indication from a serological studies that older animals are at higher risk of infection	Presence of *Trichinella* spp. infection in: farmed wild boar wild animals Audit of farm
Risk factor 2 Ingestion of flesh from rats	A few from literature	Yes	Presence of *Trichinella* spp. infection in rats Audit of farm
Risk factor 3 Cannibalism	A few from literature	Yes	Presence of *Trichinella* spp. infection in farmed wild boar Audit of farm
Slaughterhouse			
Risk factor 1 No or insufficient meat inspection for *Trichinella* larvae in wild boar meat	n.a.	n.a.	
Processing of meat and products thereof			
Risk factor 1	–	–	–
Retail			
Risk factor 1	–	–	–
Consumer			
Risk factor 1 Consumption of raw or undercooked wild boar meat or products thereof	Human data from zoonoses report; some from literature	Higher exposure in hunters due to consumption behaviour	

Evaluation of suggested indicators

Table 18: Suggested epidemiological indicators for *Trichinella* in farmed wild boar

Weighting factor				30 %	40 %	15 %	15 %	
Indicators (animal/food category/other)	**Food chain stage**	**Analytical/ diagnostic method**	**Specimen**	**Quality of indicator[a] (0, 1, 2)[e]**	**Appropriateness of indicator[b] (0, 1, 2)[e]**	**Data availability[c] (0, 1, 2)[e]**	**Feasibility[d] (0, 1 ,2)[e]**	**Total points**
Presence of *Trichinella* spp. larvae in muscle samples of wildlife (wild carnivores) and rats	Environment	Digestion	Muscle sample	1	1	1	0	0.85
Presence of *Trichinella* spp. antibodies in wild boar	Slaughterhouse	Serology[f]	Meat juice	1	1	2	1	1.15
Presence of *Trichinella* spp. larvae in muscle samples of wild boar	Slaughterhouse	Digestion	Muscle sample	2	2	2	2	2.0
Audit of husbandry conditions (access of wild animals, feed, rodent control, garbage disposal)	Farm	Auditing	n.a.	0	1	1	1	0.7

(a): Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
(b): Appropriateness of indicator = how well the indicator correlates with the human health risk caused by the hazard and the possibility/need to amend the meat inspection method.
(c): Data availability = are there data already available or is it easy to get the data needed?
(d): Feasibility = how laborious is the sampling and testing procedure?
(e): 0 = bad, 1 = moderate, 2 = good.
(f): ELISA antibody detection for monitoring purposes only, not for diagnosis. Possible cross-reaction with other pathogens.

Mycobacterium

1. Identification of potential epidemiological indicators

Table 19: Potential epidemiological indicators for *Mycobacterium* in farmed wild boar and farmed deer

	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 OTF status of the Member State/region/farm	Official data on tuberculosis in cattle collected by MSs, *ad hoc* reports in the literature	Prevalence data are available to identify affected regions/MSs/farms and to substantiate freedom from disease as well as maintenance of the status	Bovine tuberculosis status of the region/country/farm of origin
Risk factor 2 Presence of infection in wildlife	Limited data are available to identify the range of affected wildlife species		Presence of pathogenic mycobacteria, particularly *M. bovis*, in wildlife
Risk factor 3 Production system—most wild boar and deer are raised in outdoor production systems with limited biosecurity (mixed grazing, access of wildlife to feed and water provided for farmed livestock)	Limited prevalence data available	It is possible to obtain such data? Because of contacts with wildlife and the rooting behaviour of wild boar, higher prevalence might be expected in boar than deer	Level of biosecurity at farm
Risk factor 4 Failure to detect infected animals	Only *ad hoc* reports in the literature	It is possible to obtain such data?	Clinical disease in farmed wild boar and deer Detection of *M. bovis* in live wild boar and deer by immunological tests
Transport to slaughterhouse			
Risk factor 1	–	–	–
Slaughterhouse			
Risk factor 1 Failure to recognise and report granulomatous lesions for further investigation			Detection of *Mycobacterium* spp. in suspected lesions in all slaughtered animals
Processing of meat and products thereof			
Risk factor 1	–	–	–
Retail			
Risk factor 1	–	–	–
Consumer	–		
Risk factor 1	–	–	–

2. Evaluation of suggested indicators

Table 20: Suggested epidemiological indicators for *Mycobacterium* in farmed wild boar and farmed deer

Weighting factor				30 %	40 %	15 %	15 %	
Indicators (animal/food category/other)	Food chain stage	Analytical/ diagnostic method	Specimen	Quality of indicator[a] (0, 1, 2)[e]	Appropriateness of indicator[b] (0, 1, 2)[e]	Data availability[c] (0, 1, 2)[e]	Feasibility[d] (0, 1 ,2)[e]	Total points
Official tuberculosis (*M. bovis*) status	Member State/region/farm	Food chain information	Not applicable	1	1	2	2	1.3
Presence of tuberculosis in wildlife	Member State/region	Monitoring	Carcasses and organs	0	0	0	1	0.15
Production system and level of biosecurity	Farm	Audit (of records)	Not applicable	0	0	1	2	0.45
Infection with *M. bovis* in live wild boar and deer	Farm	Clinical observations	Live WB[f] and deer	0	0	0	1	0.15
Infection with *M. bovis* in live wild boar and deer	Farm	Immunological tests, e.g. serology, skin test, or γ-interferon test	Live WB or deer, blood	0 for WB 1 for deer	1	0	0 for WB 1 for deer	WB 0.4 deer 0.85
Detection of pathogenic mycobacteria in wild boar and deer at slaughter	Slaughterhouse	Visual inspection and microbiology	Suspect lesions from carcase	2	1	1	2	1.45

(a): Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
(b): Appropriateness of indicator = how well the indicator correlates with the human health risk caused by the hazard and the possibility/need to amend the meat inspection method.
(c): Data availability = are there data already available or is it easy to get the data needed?
(d): Feasibility = how laborious is the sampling and testing procedure?
(e): 0 = bad, 1 = moderate, 2 = good.
(f): WB= wild boar.

ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
BIOHAZ	Biological Hazards
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
HEI	harmonised epidemiological indicator
IFN-γ	interferon-γ
ISO	International Organization for Standardization
MAA	*Mycobacterium avium* subsp. *avium*
MAC	*Mycobacterium avium* complex
MSs	Member States
MTC	*Mycobacterium tuberculosis* complex
OTF	officially tuberculosis free
NTM	Non-tuberculous *Mycobacteria*
PCR	polymerase chain reaction
ToR	Term of Reference