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SCIENTIFIC REPORT OF EFSA

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

European Food Safety Authority[2, 3]

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ABSTRACT

In this report harmonised epidemiological indicators are proposed for food-borne biological hazards to public health that are related to bovine animals and meat thereof and that can be addressed within meat inspection. These hazards include *Salmonella*, pathogenic verocytotoxin-producing *Escherichia coli*, *Cysticercus* (*Taenia saginata)* and mycobacteria. An epidemiological indicator is defined as the prevalence or the concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard that correlates with the human health risk caused by the hazard. The indicators can be used by the European Commission and the Member States to consider when adaptations to meat inspection methods may be required, and to carry out risk analysis to support such decisions. It is foreseen that the indicators will be used in the bovine carcase meat safety assurance system outlined in the EFSA Scientific Opinion, particularly to help categorise farms/herds and slaughterhouses according to the risk related to the hazards as well as setting appropriate specific hazard-based targets in/on bovine carcases and, when appropriate, in bovine farms/herds. 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. It is recommended that risk managers should define the harmonised requirements for controlled husbandry conditions of farms, and the requirements for food chain information. Member States are invited to organise training regarding the implementation of the indicators and the reporting of data generated by the implementation in accordance with Directive 2003/99/EC. The proposed indicators should be regularly reviewed in the light of new information and the data generated by their implementation.

KEY WORDS

Meat inspection, epidemiological indicators, bovine animals, *Salmonella*, VTEC, *Cysticercus*, mycobacteria.

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SUMMARY

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

In this report, harmonised epidemiological indicators are proposed for food-borne biological hazards to public health that are related to bovine animals and meat thereof and that can be addressed within meat inspection. These hazards include *Salmonella* and pathogenic verocytotoxin-producing *Escherichia coli* (VTEC), as well as *Cysticercus* (*Taenia saginata)* and mycobacteria, the last two being already covered by the current meat inspection process. An epidemiological indicator is defined as the prevalence or the concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard (such as audits) that correlates with the human health risk caused by the hazard. The epidemiological indicators can be used by the European Commission and the Member States to consider when adaptations to meat inspection methods may be required, and to enable the Member States to carry out risk analysis to support any such decisions. It is foreseen that the epidemiological indicators will be used in the bovine carcase meat safety assurance system outlined in the Scientific Opinion on the public hazards to be covered by inspection of meat from bovine animals, particularly to help to categorise farms/herds and slaughterhouses according to the risks related to particular hazards as well as setting appropriate specific hazard-based targets (hazard prevalence and/or concentration) in/on bovine carcases and, when appropriate, in bovine farms/herds.

Risk managers should decide on the most appropriate use of the epidemiological indicators at the European Union and national levels. Depending on the purpose and the epidemiological situation of the country, the indicators may be applied at national, regional, slaughterhouse and/or farm/herd level. The indicators can be used alone or in combination. For *Salmonella* and pathogenic VTEC, the proposed harmonised epidemiological indicators include microbiology-based indicators, which will give specific information on *Salmonella* and VTEC infection or contamination in the animal, hide or carcase. Harmonised epidemiological indicators based on audits at farm or transport conditions and visual inspection of bovine hide are also proposed, which will give a more general assessment of microbiological risk and, when used in combination with microbiological harmonised epidemiological indicators, will support assessment and knowledge of the *Salmonella*/VTEC risk.

The proposed indicators for *Salmonella*, pathogenic VTEC, *Cysticercus* (*Taenia saginata*) and mycobacteria may be applied to classify countries, regions, farms, slaughterhouses, slaughter batches and animals according to the infection status or risks related to the hazard. For *Salmonella* and pathogenic VTEC, some indicators may also be used to evaluate the measures taken in the slaughterhouses to control the hazard or to assess process hygiene. In the case of *Mycobacterium*, epidemiological indicators are suggested to enable surveillance for possible emergence of this rare biological hazard in European Union bovine animal production. The accumulated historical data from implementation of the harmonised epidemiological indicators will be particularly useful for the categorisation of farms and slaughterhouses and may be applied to justify reduction in the sampling frequencies for the harmonised epidemiological indicators.

Most of the epidemiological indicators are proposed for subpopulations of bovine animals or bovine carcases at the farm or slaughterhouse level using a variety of methods, such as visual, serological or bacteriological tests. Some indicators include auditing of the farms for controlled husbandry conditions or auditing of the transport of slaughter bovines, lairage conditions or slaughter methods. In the case of some of the biological hazards addressed it is accepted that there is a need for more research to clarify the factors that place bovine animals at risk of infection, and the role of bovine meat as a source of human infections.

Comparable data from the European Union Member States were available for mycobacteria. For each epidemiological indicator addressed, the key elements of minimum monitoring or inspection requirements are defined. This includes the animal population to be targeted, the stage of the food chain where the sampling should take place, sampling strategy, type and details of the specimen to be taken, diagnostic or analytical method to be used, and a case definition.

It is recommended that the European Commission and the Member States define the harmonised requirements for controlled husbandry conditions and the details of food chain information to be provided that are referred to in the epidemiological indicators.

The implementation of the proposed epidemiological indicators will generate additional data that will provide a more precise picture of the epidemiological situation in the European Union for these hazards, and these data may be used to update the indicators, when appropriate. It is recommended that the Member States report the data generated from the 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. The European Commission and the Member States are invited to organise training to ensure harmonised implementation of the minimum monitoring and inspection requirements of the epidemiological indicators.

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BACKGROUND AS PROVIDED BY THE COMMISSION

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

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

In accordance with Article 9(2) of Directive 2003/99/EC[4] 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, EFSA shall examine and publish a summary report on the trends and sources of zoonoses, zoonotic agents and microbiological resistance in the European Union based on reports transmitted by the Member States. In addition, EFSA has prepared several scientific reports on (harmonised) monitoring of food-borne infections. Prevalence data from the zoonoses monitoring are considered as relevant epidemiological criteria to carry out a risk analysis, however, such data may be limited in certain Member States or not sufficiently harmonised to compare the situation between Member States. It is, therefore, appropriate to lay down harmonised human health epidemiological criteria and their minimum requirements. Such criteria should provide a tool to be used by risk managers in case they consider the current methods for meat inspection disproportionate to the risk.

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

[4] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. OJ L 325, 12.12.2003, p. 31–40.

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

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

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

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

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

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

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

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

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

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

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

TECHNICAL SPECIFICATIONS

1. Introduction

There are a number of food-borne diseases affecting humans that can be related to consumption of bovine meat and traced back to live bovine animals. These hazards include parasites and bacteria.

According to the Scientific Opinion of EFSA's Panel on biological hazards (BIOHAZ), based on the limited data available and expert opinion, the bovine meat-borne biological hazards categorised as of high priority for meat inspection were *Salmonella* spp. and pathogenic verocytotoxin-producing *Escherichia coli* (VTEC). *Toxoplasma gondii* and extended-spectrum β-lactamase (ESBL)/AmpC gene-carrying *E. coli* were characterised as of 'undetermined' priority for bovine meat inspection because the data available were insufficient for conclusive ranking. Biological hazards categorised as low priority for bovine meat inspection were *Bacillus anthracis*, *Campylobacter* spp. (thermophilic), *Sarcocystis hominis* and *Taenia saginata* (EFSA BIOHAZ Panel, 2013).

According to the European Union Summary Report (EUSR) on Zoonoses and Food-borne Outbreaks in 2011 (EFSA and ECDC, 2013), there is strong evidence that 1.9 % of the reported food-borne outbreaks in the European Union (EU) (13 outbreaks in 2011) were caused by bovine meat and products thereof. This food vehicle was the fourteenth most frequently reported one. Of these 13 food-borne outbreaks linked to consumption of bovine meat and products thereof, eight were caused by *Salmonella* (six outbreaks due to *S.* Enteritidis; two due to *S.* Typhimurium), two were caused by VTEC (VTEC O157), and one each by *Clostridium perfringens*, *Campylobacter jejuni* and norovirus. Bovine meat and products thereof was reported as the fourth most frequently reported vehicle of *S.* Typhimurium outbreaks (6.9 %), after pig meat and products thereof (34.5 %), eggs and egg products (13.8 %), and unspecified meat and products thereof (13.8 %). The relevant hazards related to bovine meat and products thereof 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 the zoonotic hazards found in bovine animals. For example, zoonotic animal diseases such as cysticercosis, tuberculosis and brucellosis are directly targeted through the current meat inspection procedures for bovine animals (Regulation (EC) No 854/2004). However, biological hazards that are currently found in bovine animals and considered of high public health relevance, as mentioned above, are not specifically addressed by the meat inspection system in place in the EU (EFSA BIOHAZ Panel, 2013).

The Scientific Opinion of EFSA on the public health hazards to be covered by inspection of meat (bovine animals) (EFSA BIOHAZ Panel, 2013) proposes a new generic bovine carcase meat safety assurance system for biological hazards. It is foreseen that the harmonised epidemiological indicators will be used as part of this framework. Therefore, this report should be read in parallel with that Opinion.

It is possible to use the data on the prevalence or concentration of the biological hazards in animals, meat and humans as one aspect of the criteria when determining and ranking the human health importance of the hazards to be covered by meat inspection. These epidemiological criteria or indicators may be used by the risk managers when considering adaptations to current meat inspection methods for bovine animals. In the case of bovine animals, relevant prevalence data that could be used when designing the epidemiological indicators have been collected from the EU MSs within the framework of the annual reporting in accordance with Directive 2003/99/EC on the monitoring of zoonoses. Data on the incidence of food-borne diseases in humans are collected by the European

Centre for Disease Prevention and Control (ECDC) based on Decision 2119/98/EC on setting up a network for the epidemiological surveillance and control of communicable diseases in the EU.[10]

The Terms of Reference (ToRs) stated that, where relevant, and should available data so permit, distinction will be made when addressing the different ToRs between different: bovine species (*Bos taurus* (cattle), *Bubalis bubalis* (buffalo), *Bison bison* (bison)); animal production practices and slaughter procedures (e.g. dairy vs. beef; intensive vs. extensive farming; integrated vs. non-integrated farming; religious slaughter vs. non-religious slaughter); age (bovines younger and older than six weeks of age); and age-related current meat inspection practices. However, consistent with the EFSA Scientific Opinion, in the risk-based approach to meat safety assurance each of those aspects (i.e. species, age, farming system, slaughter system) were not considered as a stand-alone issue (i.e. in isolation) in the current document, but rather each was considered together with other risk-factors analysed within the Food Chain Information (FCI) and used for risk categorisation of incoming animals and/or slaughterhouses. Subsequently, the risk categories of incoming animals/slaughterhouses would determine the nature of meat safety assurance including meat inspection to be applied in a given situation. Age is not considered as a universal or unique factor, but it is addressed if relevant as a risk factor for specific hazards. This includes considerations given to bovines younger and older than six weeks of age (which was specified in the mandate).

2. Bovine farming practices in the EU

According to Eurostat[11] data, in 2011, the reported EU population of bovines amounted to approximately 86.2 million head, with the majority being cattle (i.e. *Bos taurus*), while less than 0.5 % are buffaloes (i.e. *Bubalus bubalis*). Buffaloes are reared mainly for milk production that is later processed into mozzarella cheese, while buffalo meat usually constitutes a secondary product. Only five EU MSs report the rearing of buffaloes: Italy (about 90 % of all EU reported buffalo production), Romania, Bulgaria, Germany and Hungary. Other farmed species of the family *Bovidae*, subfamily *Bovinae* (e.g. *Bison bison*, *Bos indicus*), are not significantly reared currently in the EU.

It should be noted that the distribution of bovines within the EU varies greatly. Thus, in 2010 and 2011, and based on the same Eurostat data source as above, seven EU MSs (France, Germany, the United Kingdom, Ireland, Italy, Spain and Poland) accounted for nearly 75 % of the overall EU cattle production.

Data at EU level regarding the distribution of the size of the holdings according to the number of cattle that they host are scarce. However, there are data available from Eurostat[12] on the number of cattle holdings in the MSs (the last complete dataset for all EU MSs was reported in 2007). Data available suggest that in the individual MSs there is not a direct correlation between the size of their cattle population and the number of cattle holdings. Thus, MSs with relatively small cattle population sizes report large numbers of holdings (e.g. Bulgaria, about 600 000 cattle in over 130 000 holdings; Poland, over 5 000 000 cattle in over 710 000 holdings), while others with relatively larger cattle populations report smaller numbers of holdings (e.g. the Netherlands , about 3 700 000 cattle in about 35 000 holdings; France, about 19 000 000 cattle in about 210 000 holdings).

The different cattle production systems present in the EU are classically divided into six main categories: (i) dairy farming, (ii) beef breeding herds, (iii) semi-intensive grazing systems, (iv) bobby calf production, (v) veal farming and (vi) intensive fattening units (i.e. feedlot production).

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

[11] Statistical database of EUROSTAT, Agriculture, Agricultural products, Animal Production, Livestock, Cattle population, annual data (apro_mt_lscatl). Units: 1 000 head (animals). Accessed on 20 June 2012.

[12] Statistical database of EUROSTAT, Agriculture, Agricultural products, Animal Production, Livestock, Cattle population, annual data (ef_olsaareg). Accessed on 20 June 2012.

Particular attention may be paid to pink veal calf farming. Pink veal calves are fed mainly roughage with concentrates. The production systems may also be different than that of white veal production, which uses a strict batch 'all-in-all-out' system and that has a rearing period of typically 20-28 weeks. In contrast, pink veal farms often have calves at different stages of production on the same farm and the rearing period is typically 35 weeks.

More details on EU bovine farming practices, and the global relationship between farming and meat inspection, and bovine slaughtering practices in the EU, can be found in the Annexes of the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

3. 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 husbandry conditions, transport, lairage and slaughter methods and whether these arrangements and activities are implemented effectively and are suitable to achieve the desired objectives.

Bovine animals: domestic animals of the species *Bos taurus*, *Bubalus bubalis* and *Bison bison* (Regulation (EC) No 853/2004).

Bovine meat: edible parts of bovine animals, including blood (Regulation (EC) No 853/2004).

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

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

Calves: domestic animals of the bovine animals not exceeding a live weight of 300 kg, which do not yet have their second teeth (Decision 94/433/EC[13]).

Controlled husbandry conditions: a type of animal husbandry in which bovine animals are kept at all times and for their whole life under specific conditions that effectively exclude all relevant risk factors or maintains 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 and are specific for each hazard. Examples of proposed requirements to investigate for controlled housing conditions can be found in Appendix A.

Harmonised epidemiological indicator (HEI): 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 of farms) that correlates with 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). The establishment has to be approved by the competent authorities in accordance with Article 4 of Regulation (EC) No 853/2004 and Article 3 of Regulation (EC) No 854/2004.

[13] Commission Decision 94/433/EC of 30 May 1994 laying down detailed rules for the application of Council Directive 93/24/EEC as regards the statistical surveys on cattle population and production, and amending the said Directive. OJ L 179, 13.7.1994, p. 27–32.

4. Approach applied to select the epidemiological indicators

4.1. 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 (HEI) is, in this context, understood to mean the prevalence or concentration of the hazard at a certain stage of the food chain that correlates with the human health risk caused by the hazard. Indirect indicators of the hazards, such as audits of farms or transport, are also covered.

The purpose of the HEIs proposed in this report is to enable the European Commission (EC) and the MSs to consider whether adaptations to meat inspection methods may be implemented at the MS level, and to enable the MSs to carry out a risk analysis (or components thereof) to support decisions on any such adaptations to meat inspection methods. For those hazards identified in the complementary Scientific Opinion (EFSA BIOHAZ Panel, 2013) as the most relevant in the context of meat inspection, the epidemiological indicators provide information to be used in the bovine carcase meat safety assurance system proposed by the Opinion. This applies in particular to the process of classification of the farms/herds and slaughterhouses according to risk related to a particular hazard, as well as to the setting of appropriate specific hazard-based targets (hazard prevalence and/or concentration) in/on bovine carcases and, when appropriate, in bovine farms/herds. The indicators, either alone or in combination, may be used by risk managers at the national, regional, slaughterhouse or farm/herd level depending on the purpose.

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

- For each biological hazard, the prevalence of the agent at key points in the food chain, broken down by risk factors that may be used for risk-based sampling (e.g. type of production system, age of animals), is considered. The key points are those at which risk is first created, primarily on-farm, but also possibly points at which the hazard can enter the food chain (e.g. during transport and slaughter) and where the hazard reservoir is situated (e.g. wildlife).
- The key epidemiological indicator for a given hazard will almost always be the prevalence in the animal population or in the food.
- The identification of a range of risk factors is not, in itself, adequate. The 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 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 HEIs (the first ToR):

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

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

In the cases where 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 where 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 of how to choose the sampling strategy for each case has been presented in the EFSA's scientific report on HEIs for swine meat inspection (EFSA, 2011a).

4.2. The biological hazards addressed

The first ToR of the mandate for technical assistance from the EC asks for the HEIs 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 bovine animals, these hazards are *Cysticercus* (*Taenia saginata*), tuberculosis and brucellosis. However, as the mandate addresses specific public health hazards, brucellosis, which is mostly an occupational disease, and which usually presents with unspecific clinical signs and is therefore not usually detected during meat inspection, was not addressed in this document.

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 bovine animals (EFSA BIOHAZ Panel, 2013), which can be used to consider adaptations to meat inspection methodology, should be addressed as well. The EFSA Scientific Opinion identifies *Salmonella* spp. and pathogenic VTEC as such hazards.

5. Epidemiological indicators for the biological hazards

5.1. *Salmonella*

5.1.1. Biology and epidemiology

Salmonella has long been recognised as an important zoonotic pathogen of economic significance in animals and humans. The genus *Salmonella* is currently divided into two species: *S. enterica* and *S. bongori*. *S. enterica* is further divided into six sub-species, and most zoonotic *Salmonella* strains belong to the subspecies *S. enterica* subsp. *enterica*. Members of this subspecies have usually been named based on where the serovar or serotype was first isolated. In the following text, the organisms are identified by genus followed by serovar (e.g. *S* Typhimurium). More than 2 600 serovars of zoonotic *Salmonella* exist and the prevalence of the different serovars changes over time.

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, being sources of infection. Transmission often occurs when organisms are introduced in food preparation areas and are allowed to multiply in food, e.g. because of 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 humans or faecally contaminated environments.

In the EU, *S.* Enteritidis and *S.* Typhimurium are the serovars most frequently associated with human illness. Human *S.* Enteritidis cases are most commonly associated with the consumption of contaminated eggs and poultry meat, whereas cases caused by *S.* Typhimurium are mostly associated with the consumption of contaminated pig, poultry and bovine meat. However, there is one serovar showing a significant higher risk for invasive disease – *S.* Dublin.

Infection with this serovar has been reported to result in septicaemia in more than 20 % of all *Salmonella* infections in England and Wales (Threlfall et al., 1992), 40 % in the EU as a whole (Wollin, 2007) and 60 % in the USA (Jones et al., 2008), whereas septicaemia due to infection with the other serovars occurs in less than 2 % of cases in England and Wales and in the EU as whole, and

in about 7 % of cases in the USA. These apparent discrepancies may result from differences in health systems in Europe and the USA.

Amongst all human salmonellosis cases reported to TESSy for the period 2007 to 2011, 5.6 % included information on whether the patient was hospitalised: this information was not available for the remaining 94.4 % of the cases. Where information was available, 42 % resulted in hospitalisation. In the case of infection with *S.* Dublin, 83% resulted in hospitalisation. Furthermore, the same TESSy data showed a higher proportion of systemic infections (based on the isolation of the bacterium from blood) due to *S.* Dublin as compared to all *Salmonella* spp. (46 % vs 2 %).

Furthermore, the mortality after infection with *S.* Dublin is four times higher than for other serovars (Helms et al., 2003).

Numerous *Salmonella* serovars may cause clinical disease in bovines and *S.* Typhimurium is the most frequent serovar isolated from these outbreaks. However, other unadapted serovars may cause outbreaks and may originate from the use of *Salmonella*-contaminated feed. *Salmonella* may easily spread between bovines in a herd without detection and animals may become intermittent or persistent carriers. Infected cows may succumb to fever, diarrhoea and abortion. Within calf herds, *Salmonella* may cause outbreaks of diarrhoea and septicaemia with high mortality. Clinical signs are less common in pigs than in bovine animals, sheep and horses; goats and poultry usually show no signs of infection (EFSA and ECDC, 2012).

S. Dublin is considered host adapted to bovines (Wray and Sojka, 1977; Selander et al., 1992), which means that bovine animals are the most common host of *S.* Dublin and may be carriers. Other species, including pigs (Lawson and Dow, 1996), sheep (Ekdahl and Allan, 1966), rats (Hall, 1975) and humans (Fierer, 1983), have also been reported to become clinically ill from *S.* Dublin infection. The mechanisms of the host adaption are poorly understood, but most likely relate to genetic traits of both the bacteria and the host. Regulation of the pathogenesis relates to both host factors and bacterial factors, and much research has been and is currently being performed in this field to develop a better understanding of the importance of different factors and how they influence each other.

5.1.2. Current situation and trends in the EU

Salmonella species are responsible for many cases of human illness and in most developed countries, including the EU, are the second most common cause of bacterial gastrointestinal illness. A total of 94 878 confirmed cases of human salmonellosis were reported in EU in 2011 and the number of cases decreased by 4.2 % compared with 2010, continuing the statistically significant decreasing trend (EFSA and ECDC, 2013). It is assumed that the observed reduction of salmonellosis cases is mainly due to successful implementation of national *Salmonella* control programmes in poultry populations, but other control measures along the food chain may have also contributed to the reduction. In foodstuffs, *Salmonella* was most often detected in fresh broiler and pig meat, on average at levels of 6.0 % and 0.6 %, respectively. In the case of fresh bovine meat, 0.3 % of sampling units were positive. *Salmonella* was rarely detected in other foodstuffs, such as dairy products, fruit and vegetables (EFSA and ECDC, 2013).

An assessment of the incidence and severity of human salmonellosis cases in the EU can be found in the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

In Sweden, a national *Salmonella* control programme was established in 1953 after a large outbreak of salmonellosis with more than 90 000 human cases and more than 90 fatalities. The programme includes monitoring of *Salmonella* in feed, livestock, slaughter animals and food and products thereof. Positive cases are traced back to the source and eliminated. Finland implemented a similar programme to the Swedish model in the 1960s. Both countries have a very favourable situation with 0.15 % and 0.1 % positive findings in lymph nodes in slaughter cattle in 2011, respectively.

In Denmark, a voluntary *Salmonella* control programme was implemented in 2002. Since then, all cattle herds are categorised into three classes according to antibody level measures carried out on either bulk tank milk samples or on slaughter blood samples. Herds showing increasing antibody levels are retested using individual blood samples or bulk tank milk samples in order to establish the status of the herd. Carcase swabs are not used for the classification of the herd due to the low sensitivity of this procedure. The proportion of positive dairy herds has fell from 24 % in 2003 to 8 % in 2012 and 4 % in non-dairy herds. *Salmonella* was detected in 0.35 % of carcase swab samples in 2011.

5.1.3. Bovine meat as a source of infection for humans

There are several routes of transmission for salmonellosis, but the majority of the human infections are transmitted through consumption of contaminated food of animal origin. Contaminated bovine meat and products thereof have been implicated in a number of salmonellosis cases, and in 2011 bovine meat and products thereof were reported as the implicated food vehicle in 2.8 % (eight outbreaks) of the 284 strong evidence *Salmonella* outbreaks (EFSA and ECDC, 2013).

Bovine meat and products thereof were reported as the implicated food vehicle for 6.9 % of all *S.* Typhimurium outbreaks and in 3.2 % of all *S.* Enteritidis outbreaks (EFSA and ECDC, 2013). No *S.* Dublin outbreaks were reported. Available published data from source attribution studies of human salmonellosis on the role of bovine meat as a source of this biological hazard are presented in the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

5.1.4. Risk and risk-reducing factors

One of the main risk factors for introducing *Salmonella* into a herd is trading in *Salmonella*-infected bovine animals, i.e. buying in infected animals. Sharing of pastures and spreading of slurry is also known to be an important risk for introduction of *Salmonella* into a herd. Visitors (humans, cats, dogs, wildlife) to a herd may also pose a risk of introduction.

Stocking density, sectioned structure and hygienic management and calving procedures are of major importance to minimise the risk of *Salmonella* spread within a farm.

Carriers may start shedding bacteria if exposed to stressful conditions such as movement, transport or lairage. The duration and condition of transport and lairage can significantly increase the risk of *Salmonella* contamination of the hide of bovines due to cross-contamination.

Regulation (EC) No 853/2004 requires that "animals must be clean" when processed in slaughterhouses. Visual scoring of hide cleanliness before slaughter of bovine animals in practice varies in different countries. The aim of scoring is that excessively dirty animals are not sent from the farm to slaughter or that slaughtering is performed logistically (dirty animals slaughtered after clean animals), at slower line speed, with increased process hygiene controls applied more carefully. Visual cleanliness of bovine animals (currently assessed at *ante mortem* inspection) may be relevant for *Salmonella*-related risks. But, the sole information of the degree of visual cleanliness of the hide cannot be used as an indicator of absence or presence of the hazard in bovine animals. Nevertheless, for batches of bovine animals originating from *Salmonella*-positive farms, it could be assumed that animals dirtier with faecal material could present a higher risk for cross-contamination of the slaughterline environment, including the carcases (EFSA BIOHAZ Panel, 2013).

At the slaughterhouse, it is well established that the main sources of bovine carcase contamination with *Salmonella* are hides and intestinal contents. Contamination of carcases with this hazard occurs via numerous routes, including direct exposure during dehiding and evisceration and indirect contamination through contaminated equipment, tools, knives, aerosols, and manual handling during *post mortem* inspection. Bovine slaughterhouse operation-mediated meat contamination and cross-

contamination can be reduced through implementation of a range of general (Good Manufacturing Practices (GMP) /Good Hygiene Practices (GHP)) and more specifically defined (Hazard Analysis and Critical Control Points (HACCP)) measures, whilst *post mortem* inspection-mediated cross-contamination could be minimised by omission of related palpation/incision activities (EFSA BIOHAZ Panel, 2013).

5.1.5. Proposed harmonised epidemiological indicators (HEIs)

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

Table 1: Harmonised epidemiological indicators for *Salmonella* in bovine animals

Indicators (animal/ food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
HEI 1: Practices which increase the risk of introducing *Salmonella* into the farm (purchase policy, mixing with other herds, access to pasture, access to surface water)	Farm	Auditing	Not applicable
HEI 2: On-farm practices and conditions	Farm	Auditing	Not applicable
HEI 3: *Salmonella* status of the group(s) of bovine animals containing animals to be slaughtered within one month	Farm	Microbiology	Pooled faeces
HEI 4: Transport and lairage conditions	Transport and lairage	Auditing	Not applicable
HEI 5: Visual inspection of hide conditions of animals at lairage (clean animal scoring system)	Slaughterhouse	Visual inspection	Not applicable
HEI 6: *Salmonella* on incoming animals (after bleeding and before dehiding)	Slaughterhouse	Microbiology (detection and serotyping)	Hide swabs
HEI 7: *Salmonella* in incoming animals (evisceration stage)	Slaughterhouse	Microbiology (detection and serotyping)	Lymph nodes
HEI 8:. *Salmonella* on carcases pre-chilling	Slaughterhouse	Microbiology (detection and serotyping)	Carcase swabs
HEI 9: *Salmonella* on carcases post-chilling	Slaughterhouse	Microbiology (detection and serotyping)	Carcase swabs

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

The proposed HEIs include microbiology-based indicators, which will give specific information on *Salmonella* infection or contamination in the animal, hide or carcase as well as HEIs based on audits at farm or transport conditions and visual inspection of bovine hide, which will a give more general assessment of microbiological risk and, when used in combination with microbiological HEIs, will support assessment and knowledge of *Salmonella* risk.

Microbiological testing of either faeces, hide, mesenteric lymph nodes or carcase swabs is the analytical method proposed for those HEIs related to sampling of bovine animals or their carcases for

Salmonella infection or contamination. Bacteriological detection methods and typing of *Salmonella* spp. will provide data on specific new zoonotic serovars such as monophasic variants of *S.* Typhimurium and new emerging serovars which may go undetected if only serological surveillance systems were in place. Particular *Salmonella* clones of special public health significance (e.g. *S.* Dublin or clones with high virulence or resistance towards antimicrobials deemed critically important for treatment of human infections, but not necessarily related to particular serovars) may be identified. However, this requires all MSs to implement harmonised and standardised methods for identifying such clones.

Serological testing of serum or meat juice for detection of *Salmonella* antibodies is not proposed as an analytical method for HEIs for *Salmonella* in bovine animals. The monitoring of *Salmonella* antibodies is currently implemented in a few northern European countries and used for herd classification. For example, serology is currently used in Denmark to control *S.* Dublin infection in cattle. But the correlation between the infection status of bovine animals and level of antibodies is weak. In fact, even though *Salmonella* is present in the environment, the immunological status of cattle after primary, secondary and tertiary infection is unclear. Consequently, the relationship between seropositivity and food safety is questionable (Nielsen, 2013). Lastly, serology does not provide information on *Salmonella* serovars and clones.

HEI 1 focuses on evaluating the risk of introducing *Salmonella* infection into a farm. This relates to practices which may introduce *Salmonella* into the farm, including purchase policy for new stock, contact and mixing with other herds, access to open pasture and access to surface water. It should be used in combination with HEI 3. Examples of proposed requirements to investigate for controlled husbandry conditions can be found in Appendix A.

HEI 2 focuses on farm practices and conditions contributing to transmission of *Salmonella* within the farm. It should be used in combination with HEI 3. Examples of proposed requirements to investigate for controlled husbandry conditions can be found in Appendix A.

HEI 3 focuses on the provision of information on the occurrence of *Salmonella* and the serovars present on the farm in pre-slaughter bovines. Monitoring of trends in the *Salmonella* status of these bovines on farms will be enabled by regular sampling of pre-slaughter animals from the same farm. Information from bovines slaughtered within the last month may be used. The data derived from monitoring of HEI 3 may be used to set *Salmonella* hazard-based targets in bovine farms/herds as referred to in the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

HEI 4 focuses on conditions in the transport and lairage phases with particular emphasis on the length of time spent in each phase, vehicle and lairage cleanliness and cross-contamination, as these all have the potential to increase hide contamination of the animals. HEI 4 combined with HEI 3 and HEI 6 will provide information on the influence of transport and lairage conditions on the hide contamination of bovines.

HEI 5 focuses on the classification of animals on arrival at the abattoir based on a visual inspection of the condition and cleanliness of the bovine hide using a clean animal scoring system. It should be used in combination with information on *Salmonella* on hides generated in HEI 6.

HEI 6 focuses on the identification of the *Salmonella* level entering the slaughter process. The chosen sample will take account of the conditions on the farm and during transport and lairage. Serotyping and more detailed genotyping of isolates will give reliable information about hide contamination caused by transport and lairage.

HEI 7 focuses on assessing the presence of *Salmonella* in lymph nodes of slaughtered animals. This outcome relates to the status of bovine animals at the farm level. This HEI, together with HEI 3, will give information on the infection status of bovines at the farm level.

HEI 8 focuses on providing an indicator of the process hygiene on the slaughterline by measuring the presence of *Salmonella* on bovine carcase pre-chilling. Sampling is performed before chilling rather than after chilling as it is easier to recover and cultivate *Salmonella* bacteria at this point. By combining the results (including serotyping and genotyping) from HEI 6 and HEI 8 it will be possible to evaluate the effect of the slaughter process on the carcase contamination.

HEI 9 focuses on providing an indicator of the *Salmonella* status of the carcases after the entire slaughter process (including chilling) has been completed. However, it is recognised that there are difficulties with sampling of chilling carcases as there is active bacterial attachment to the carcase making it difficult to recover bacteria via swabbing and the bacteria may be stressed during chilling and in a viable but non-culturable state. The microbial levels found at this point in the process reflect the *Salmonella* contamination level entering the food chain from the slaughterhouse. The data derived from monitoring of HEI 9 may be used to set *Salmonella* hazard-based targets in/on bovine chilled carcases as referred to in the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

HEIs 1 to 5 deal with the live animals at various stages along the chain from farm to slaughter, while HEIs 6 to 9 deal with contamination of carcases.

The proposed HEIs give different types of information on the risk of *Salmonella* infection in bovine animals or contamination of the carcases and risk managers should choose the HEIs to be applied and then also interpret the available information in the appropriate way. The microbiological indicators (HEIs 3, 6, 7, 8 and 9) may be used alone or in different combinations and the more general HEIs 1, 2, 4 and 5 should be used to support the above microbiological HEIs and to see where correlations occur that may in time allow for a decrease of the sampling frequency for microbiological sampling or more risk-based microbiological sampling.

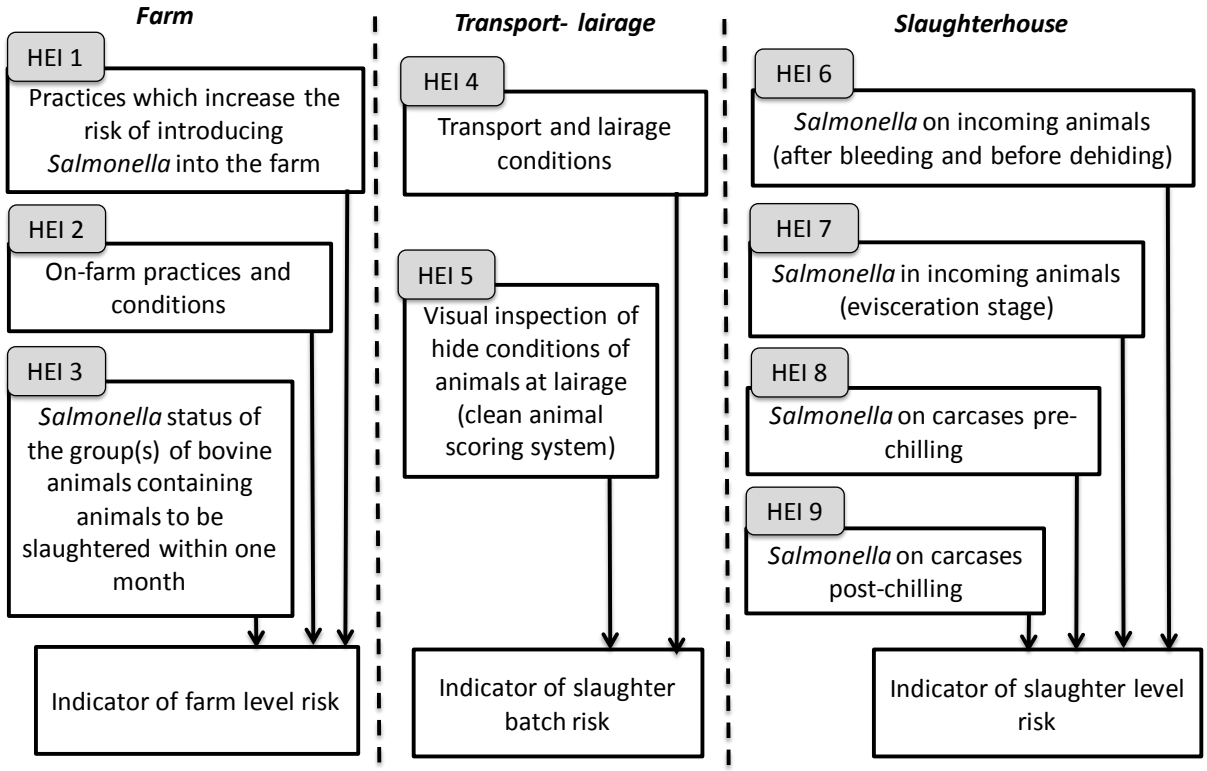


Figure 1: Schematic diagram illustrating the harmonised epidemiological indicators for *Salmonella* in bovine animals.

5.1.6. Harmonised monitoring requirements

Animal population

- Farms are subject to an audit of the production system standards to establish and verify controlled husbandry conditions and assess biosecurity (HEIs 1 and 2).
- Group of bovines at farm containing animals for slaughter (HEI 3).
- Transport conditions of bovines to the slaughterhouse and the lairage conditions at the slaughterhouse are subject to an audit of time between loading of bovines and slaughter, mixing from different herds and (re)use of pens at lairage (HEI 4).
- Bovines at slaughterhouse (HEIs 5, 6, 7, 8 and 9).

Stage of the food chain

- The farm for controlled husbandry conditions (HEIs 1 and 2).
- The farm for bovines (HEI 3).
- Transport and slaughterhouse for transport and lairage conditions (HEI 4).
- The slaughterhouse for bovines (HEIs 5, 6, 7, 8 and 9).

Sampling

HEI 1 and 2

- Target population: All farms claiming to operate under controlled husbandry conditions.
- Epidemiological unit: The farm.
- Sampling strategy: Census (all farms claiming to operate under controlled husbandry conditions should be audited).
- Audit interval: Repeated at a frequency (determined by risk managers) adequate to maintain confidence that farms continue to meet the controlled husbandry conditions.

HEI 3

- Target population: Bovines destined for slaughter.
- Epidemiological unit: The groups of bovine animals containing animals to be slaughtered within one month.
- Sampling strategy: For group(s) containing a large number of animals, a representative sample (random or systematic) of all bovines in the epidemiological unit(s). Samples from outdoor kept bovines may not be feasible to obtain prior to slaughter and information from previous slaughtered bovines can be used.
- Sample size: Adequate to assess the presence of *Salmonella*-infected bovine animals. On small farms, in order to achieve the required precision, it may be necessary to use a census sampling of all bovines.

HEI 4

- Target population: All batches of bovines sent for slaughter.
- Epidemiological unit: The slaughter batch.
- Sampling strategy: Census (all slaughter batches) or representative sample.

- Audit interval: Audit for every slaughter batch or repeated at a frequency (to be determined by risk managers) adequate to characterise the transport, mixing and lairage risks (in terms of the range of serotypes present).

HEI 5

- Target population: Bovine animals in lairage.
- Epidemiological unit: The slaughter batch.
- Sampling strategy: Census (all batches of animals pre-slaughter).

HEI 6

- Target population: Carcases after bleeding and before dehiding.
- Epidemiological unit: Slaughter batch.
- Sampling strategy: Representative sample (random or systematic).
- Sample size: Adequate to assess the *Salmonella* infection status of the hide of the incoming batch of bovines on the slaughter process, or to assess the difference in prevalence before and after processing.
- Survey interval: Initial survey,
repeated at a frequency to be determined by risk managers.

HEI 7

- Target population: Bovine carcases at the evisceration stage.
- Epidemiological unit: Slaughter batch.
- Sampling strategy: Representative sample (random or systematic).
- Sample size: Adequate to assess the *Salmonella* infection status of the incoming batch of bovines on the slaughter process, or to assess the difference in prevalence before and after processing.
- Survey interval: Initial survey,
repeated at a frequency (to be determined by risk managers) adequate to characterise the slaughterhouse risk (required particularly when procedures in the slaughterhouse change).

HEI 8

- Target population: Bovine carcases after the slaughter process, before chilling.
- Epidemiological unit: Slaughter batch.
- Sampling strategy: Representative sample (random or systematic).
- Sample size: Adequate to assess the *Salmonella* infection status of the carcases after processing (before chilling), or to assess the difference in prevalence before and after processing.
- Survey interval: Initial survey,
repeated at a frequency (to be determined by risk managers) adequate to characterise the slaughterhouse risk (required particularly when procedures in the slaughterhouse change).

HEI 9

- Target population: Bovine carcases after the slaughter process, and after chilling.

- Epidemiological unit: Slaughter batch
- Sampling strategy: Representative sample (random or systematic).
- Sample size: Adequate to assess the *Salmonella* infection status of the carcases leaving the slaughter process.
- Survey interval: Initial survey,
repeated at a frequency (to be determined by risk managers) adequate to characterise the prevalence of *Salmonella*-positive carcases entering the food chain.

Type and details of sample

- Questionnaire-based audit of farm procedures, including specific conditions for *Salmonella* (HEIs 1 and 2).
- Pooled faecal samples from the groups of bovine animals at the farm (HEI 3).
- Questionnaire-based audit of transport, mixing of herds and lairage conditions, including specific conditions for *Salmonella* (HEI 4).
- Visual inspection of animal coat and grading in line with clean animal scoring system with standardised system to score level of dirt /wetness of animal coat and a cut-off point where action is needed (HEI 5).
- Hide swab sample (site 400 cm^2) of the brisket area of the animal before hide removal (HEI 6).
- Mesenteric lymph nodes (HEI 7).
- Carcase surface samples of bovine carcases at the slaughterhouse according to Regulation (EC) 2073/2005[14] (HEIs 8 and 9).

Diagnostic/analytical methods

- Detection in accordance with ISO 6579:2002/Amd 1:2007 Annex D (ISO, 2007) (HEIs 3, 6, 7, 8 and 9).
- Serotyping of all *Salmonella* isolates (White–Kaufmann–Le Minor scheme).

Case definition

- Farms found not complying with the controlled husbandry conditions (HEIs 1 and 2).
- Transport and lairage not complying with the agreed conditions (HEI 4).
- Hide conditions not complying with the clean cattle policy (HEI 5).
- Findings of *Salmonella* in a sample (HEIs 3, 6, 7, 8 and 9).

[14] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26.

5.2. Pathogenic verocytotoxin-producing *Escherichia coli*

5.2.1. Biology and epidemiology

Pathogenic VTEC can cause serious illness in humans, with symptoms including diarrhoea ranging from mild to bloody (haemorrhagic colitis), haemolytic-uremic syndrome (HUS) and thrombocytopenia. VTEC strains are characterised by the production of potent verocytotoxins (VT) and are a genetically diverse group of *E. coli*, of which only a subset are considered to be pathogenic to humans. VTEC O157, O26, O103, O145, O111 and O104 are the serogroups which have been most commonly linked to severe HUS illness in Europe, but illness has also been reported in individuals infected with a broad range of other VTEC serogroups. Pathogenicity of VTEC is related to the presence of the verocytotoxin gene in combination with other virulence related genes but, according to a recent scientific Opinion of the EFSA's BIOHAZ Panel (2013a), there is no single or combination of marker(s) that can now fully define a 'pathogenic' VTEC. However, in this Opinion it is concluded that any *E. coli* strains positive for verocytotoxin gene (*vtx*) in combination with *eae* (intimin production), or *aaiC* (secreted protein of enteroaggregative *E. coli* (EAEC)) plus *aggR* (plasmid-encoded regulator) genes pose a risk of human VTEC infection. For the purpose of this report the term pathogenic VTEC is used to refer to those strains that cause disease in humans.

Bovines are reservoirs of a diverse range of VTEC, and their potential as human pathogens can be assessed by screening isolates for the above combination of virulence genes. Animals can be exposed to VTEC via faecally contaminated grass, feed, water, other animals, environment, etc. Most information on VTEC colonisation of bovines relates to VTEC O157, and it is known that this serogroup can pass through the ruminant stomachs and colonise the distal colon at a specific site called the recto-anal junction (RAJ). However, colonisation does not always occur following exposure, and three distinct patterns of VTEC O157:H7 carriage in cattle have been reported. Firstly, following exposure animals can shed the pathogen for a short duration of a few days, do not colonise the RAJ and are considered passive shedders. In the second situation, cattle are colonised and shed the bacteria for an average of one month and typically not longer than two months, and during this time the animal will shed the pathogen at intermittent times and in different concentrations in the faeces. In the third, relatively rare, situation, animals are colonized for a long duration and shed the bacteria for 3 to 12 months or longer. The reasons for this difference in patterns of carriage are not well understood, but it may be related to specific genotypes of VTEC O157 or other factors at the RAJ colonisation site. When colonised, cattle display no clinical symptoms of illness although in young unweaned calves VTEC colonisation can cause scouring/diarrhoea. Shedding is also usually longer and more intense in calves than in adult cattle, and increases after weaning. Some animals, deemed 'super-shedders', excrete an exceptionally high number of the pathogens (> 10 000 colony-forming units (CFU)/g) in their faeces (Naylor et al., 2003). The risk factors unpinning the different shedding patterns are poorly understood and knowledge in this area is also focused primarily on VTEC O157.

Transmission of VTEC from bovines to humans can occur by direct contact (hand to mouth) with contaminated faeces or indirectly via consumption of contaminated meat or contact with contaminated environment such as water courses or soil or fresh produce grown or harvested in a contaminated setting. The relative importance of these transmission routes for human disease is unknown.

5.2.2. Current situation and trends in the EU

The case classification of a confirmed human case is defined in Decision No 2012/506/EU[15] and detection of VTEC is highly dependent on the methods applied to clinical specimens. Such methods

[15] 2012/506/EU: Commission Implementing Decision of 8 August 2012 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. OJ L 262, 27.9.2012, p. 1–57

vary markedly between different EU MSs, and VTEC O157 is more readily detected than non-O157 VTEC. Thus, data relating to non-O157 VTEC probably represent a substantive underestimation of its true incidence, both for the EU as a whole and particularly for those MSs where molecular detection methods are not yet fully utilised.

A total of 4 000 confirmed human VTEC cases were reported from 25 EU MSs in 2010 through The European Surveillance System (TESSy), and the EU notification rate of confirmed human VTEC cases was 0.83 cases per 100 000 population (EFSA and ECDC, 2012). In 2011, as a result of the O104:H4 outbreak (EFSA, 2011b), a large increase was observed and 9 485 confirmed VTEC cases were reported from 26 MSs (EFSA and ECDC, 2013). The overall EU notification rate of VTEC was 1.9 cases per 100 000 population in 2011. Full serotype data on VTEC isolates were reported for 32 % and 7.2 % of confirmed infections in 2010 and 2011, respectively. In 2010, almost half of the reported O serogroups were O157 (41.1 %). In 2011, the most commonly reported O serogroups was O157 (41.2 %) followed by O104 (20.1 %). The latter was due to the O104:H4 outbreak. Only two cases of serogroup O104 infection were reported in 2010.

An assessment of the incidence and severity in humans of VTEC cases in the EU can be found in the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

5.2.3. Bovine meat as a source of infection for humans

VTEC rarely causes disease in animals, and ruminants are recognised as their main natural reservoir. Bovine animals are considered to be the major animal source of VTEC that are virulent to humans. However, not all the VTEC strains carried by bovines are demonstrated to cause disease in humans, only the subset with particular combinations of virulence markers as described above. The ecology of VTEC O157 in bovines has been extensively studied (Caprioli et al., 2005), but there is less information on other serogroups.

According to EUSR on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011 (EFSA and ECDC, 2013), in 2011, eight MSs reported testing of 4 347 fresh bovine meat units (from investigations of 25 or more samples) of which 1.4 % were found to be VTEC positive and 0.3 % VTEC O157 positive.

Regarding the other important pathogenic VTEC serogroups (O26, O91, O111, O103 and O145), in 2011, serogroups O26, O103, O111 and O145 were detected in bovine meat by Belgium, but overall very little information on the serogroups was provided by MSs.

In 2011, 12 MSs reported a total of 63 food-borne outbreaks caused by pathogenic VTEC, which was 1.1 % of the total number of reported food-borne outbreaks in the EU. Seventeen pathogenic VTEC outbreaks (27.0 %) were supported by strong evidence, of which two were linked to bovine meat or products thereof (EFSA and ECDC, 2013). The Scientific Opinion presents a review of data available on source attribution studies of VTEC (EFSA BIOHAZ Panel, 2013).

5.2.4. Risk and risk-reducing factors

Many different factors can impact on the carriage and shedding of pathogenic VTEC in the faeces of bovine animals. The number of pathogenic VTEC O157 organisms shed in faeces is also variable, with some animals excreting very high numbers (up to > 10 000 CFU/g). It has been estimated that such super-shedding animals contribute up to 80 % of all VTEC transmitted on the farm and during transport, lairage and slaughter operations (Matthews et al., 2006).

It is reported that contact with cattle from outside the herd as a result of the purchase of new stock, taking animals off the farm to visit agriculture shows or fairs and the use of common grazing pasture increase the risk of cattle being exposed to VTEC from faeces of other shedding animals (Cernicchiaro

et al., 2009). Gunn et al. (2007) showed that the risk of VTEC shedding was significantly higher in farms classed as "buy in" than in those classed as "breeding only".

The presence of VTEC O157 in faeces is influenced by the age of the animals and is higher in postweaned calves than in very young calves (less than three months) or older animals (older than 24 months) (EFSA, 2009). In veal production, the prevalence of VTEC O157 in white veal[16] (calves less than five months old) is significantly lower than in older pink or rose veal calves (~ eight months) (Shouten et al., 2005; Berends et al., 2008). The type of farm can have an impact on VTEC prevalence, with some studies reporting significantly high prevalence in beef herds than in dairy herds (Gunn et al., 2007), while others have reported a higher prevalence in dairy cattle than in beef feedlot systems (Hancock et al., 1998). Season also impacts on carriage, and the prevalence of VTEC O157 in cattle peaks in the summer (EFSA, 2007).

When VTEC are shed in the faeces of cattle, they can survive well in the farm environment, including water, organic agricultural materials (i.e. animal manure and slurry), feed and farm surfaces. Measures to control the spread of VTEC on the farm include good hygiene, clean and dry bedding, appropriate stocking rates, well-ventilated housing with good floor drainage and practising a closed herd policy (Vidovic and Korber, 2006; Ellis-Iversen et al., 2007; VLA, 2008). Clean and dry bedding in particular is reported to prevent heavy soiling of the animal's brisket, and keeping cattle clean is helpful in the control of carcase contamination at slaughter. Keeping young cattle in the same group throughout rearing is also important in VTEC O157 control. While the exclusion of animals other than livestock from access to cattle feed and water is best practice and may have additional benefits, the effects of wildlife exclusion on VTEC O157 prevalence in livestock have not been documented.

While feed troughs have been reported as a source of VTEC cross-contamination on the farm (Shere et al., 1998; Van Donkersgoed et al., 2001), limited studies on VTEC in commercially produced feed as a source of the pathogen show its presence at very low levels, 0.2 % of feed components and 0.4 % of feed mill samples (Davies et al, 2003) or absent in feed ingredients (Ge et al., 2013)

Transmission of VTEC O157:H7 and other VTEC serogroups can occur rapidly in groups of co-housed bovines on farms, in transport and in lairage, with cross-contamination from hides of cohort animals and the environment. The grooming behaviour of bovines plays an important role in the transmission of VTEC among co-housed animals (McGee et al., 2004). Significant cross-contamination from animal to animal can occur during transport to the factory and in lairage, and mixing of animals from different farms and herds will impact on this. The cleanliness and operation of transport vehicles and lairage arrangements influence the cleanliness and dryness of animals on arrival and in the pre-slaughter period. Fasting associated with prolonged transportation may result in an increased level of faecal shedding of VTEC prior to slaughter (Callaway et al., 2009) with cross-contamination in transport and lairage also playing a role in transmission of the pathogen (Arthur et al., 2007).

Regulation (EC) No 853/2004 requires that "animals must be clean" when processed in slaughterhouses. Visual scoring of hide cleanliness before slaughter of bovine animals in practice varies between countries.

At slaughter, the bovine hide represents a key source of VTEC contamination into slaughter plants (EFSA, 2007). A number of studies have investigated if there is a correlation between visual cleanliness of the hide and contamination with pathogens such as VTEC. While studies have found a

[16] The majority of veal calves in Europe are produced in Italy, France and the Netherlands (Sans and de Fontguyon, 2009). Rearing systems are similar in these countries. Calves, typically two weeks old, are raised in specialised fattening units under intensive rearing conditions. White veal is a product of a low iron dietary supply. In contrast, calves used to produce pink or rose veal have no iron restriction. White veal calves are fed a diet that consists mainly of milk replacer with a modest supplement of roughage and/or concentrates.

positive relationship between hide cleanliness and total viable counts (TVCs) occurring on the carcases (McEvoy et al., 2000), other studies have shown no correlation with pathogens or VTEC (McCleery et al., 2008; Thomas et al., 2012), though methodology may have an impact here with difficulty in swabbing and recovery of VTEC from heavily compacted soiled hides. Nonetheless, it is good hygiene to control the amount of faecal matter going into the abattoir, and many countries implement a clean cattle policy involving visual inspection of hides for level of faecal material and dry/wet condition. The animal is then classified and those in dirtier condition can be subject to logistic slaughter.

In the USA meat sector, antimicrobial treatments are routinely applied to bovine hide but are mainly based on the use of chemicals: cetylopyridium chlorine (Bosilevac et al., 2004), sodium hydroxide (Bosilevac et al., 2005) and hypobromous acid (Schmidt et al., 2012). Environmental disposal issues linked to these chemicals are well known, and they are not used in slaughter facilities in the EU. Bacteriophage can also be used as an animal hide decontaminant and are licensed in the USA, but EU regulations do not permit such application.

To a lesser extent gut contents and faeces are a source of carcase contamination, but careful evisceration techniques with effective sealing of the oesophagus and rectum before removal of the stomach and intestines will reduce this risk. Personnel and equipment may also play a role in carcase contamination.

Carcase dressing operations which may reduce the number of VTEC organisms include trimming of visibly dirty areas of carcases, carcase washing (hot water at 74 °C (165 °F) for 5.5 seconds) (Bosilevac et al., 2006; EFSA Panel on Biological Hazards (BIOHAZ), 2010) and steam pasteurisation. Treating carcases with decontaminants (organic acids) can yield a reduction of up to 1-2 log CFU/cm^2 (Dormedy et al., 2000).

The prevalence of pathogens on carcases is generally lower on carcase following chilling for 24 hours; however, the impact of chilling on the micro-flora is extremely variable because the industry does not refrigerate carcases in a uniform manner, with differences noted in temperature, air speed and relative humidity and resultant water activity (Sheridan, 2004).

5.2.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected for VTEC in bovines (Table 2).

Table 2: Harmonised epidemiological indicators for pathogenic VTEC in bovine animals

Indicators (animal/ food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
HEI 1: Practices which increase the risk of introducing pathogenic VTEC into the farm (purchase policy, mixing with other herds, access to pasture, access to surface water)	Farm	Auditing	Not applicable
HEI 2: On-farm practices and conditions	Farm	Auditing	Not applicable
HEI 3: Pathogenic VTEC status of the group(s) of bovine animals containing animals to be slaughtered within one month	Farm	Microbiology	Pooled faeces or floor samples
HEI 4: Transport and lairage conditions	Transport and lairage	Auditing	Not applicable
HEI 5: Visual inspection of hide conditions of animals at lairage (clean animal scoring system)	Slaughterhouse	Visual inspection	Not applicable
HEI 6: Pathogenic VTEC on incoming animals (after bleeding and before dehiding)	Slaughterhouse	Microbiology	Hide swabs
HEI 7: Pathogenic VTEC on carcases pre-chilling	Slaughterhouse	Microbiology	Carcase swabs
HEI 8: Pathogenic VTEC on carcases post-chilling	Slaughterhouse	Microbiology	Carcase swabs

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

The proposed HEIs include microbiology-based indicators, which will give specific information on VTEC infection or contamination in the animal, hide or carcase as well as HEIs based on audits at farm or transport conditions and visual inspection of bovine hide, which will a give more general assessment of microbiological risk and, when used in combination with microbiological HEIs, will support assessment and knowledge of VTEC risk.

It should be noted that there is a very large data gap on occurrence of pathogenic VTEC in bovines at both farm and slaughterhouse level. Microbiological analyses at key points in the chain conducted by MSs using harmonised and standardised sampling and testing methodologies, together with serotyping and virulotyping of isolated VTEC, will provide essential data on the occurrence of pathogenic VTEC (*E. coli* O157 and emerging serogroups) in bovines. Such microbiological data should in time allow for historical risk ranking of farms or regions and strengthen the value of HEIs based on audits, potentially allowing them to be used independently or to focus microbiological sampling.

HEI 1 focuses on evaluating the risk of introducing bovine animals infected with pathogenic VTEC onto a farm. This relates to practices which may introduce pathogenic VTEC into the farm including policy for the purchase of new stock, contact and mixing with other herds, access to open pasture and access to surface water. It should be used in combination with HEI 3. Examples of proposed requirements to investigate for controlled husbandry conditions can be found in Appendix A.

HEI 2 focuses on farm practices and conditions contributing to transmission of pathogenic VTEC within the farm. It should be used in combination with HEI 3. Examples of proposed requirements to investigate for controlled husbandry conditions can be found in Appendix A.

HEI 3 focuses on the provision of information on the occurrence of pathogenic VTEC and the serogroups present on the farm in pre-slaughter bovines. Monitoring of trends in the pathogenic VTEC status of these bovines on farms will be enabled by regular sampling of pre-slaughter animals from the same farm. Use of information from bovines slaughtered within the last month may be used. The data derived from monitoring of HEI 3 may be used to set pathogenic VTEC hazard-based targets in bovine farms/herds as referred to in the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

HEI 4 focuses on the transport and lairage conditions of the bovines. It covers specific aspects such as duration of transport and lairage, animal density, mixing of animals from different farms during transport or in lairage pens and the sanitary conditions of transport vehicle and lairage pens. Data from this HEI should be used in combination with information from VTEC on the farm (HEI 3) and on bovine hide (HEI 5) and will provide information on the influence of transport and lairage conditions on VTEC carriage in bovines.

HEI 5 focuses on classifying animals on arrival at the abattoir based on a visual inspection of the condition and cleanliness of the bovine hide using a clean animal scoring system. It should be used in combination with information on VTEC on hides generated in HEI 6. Over time, correlation of data from this HEI and HEI 6 will allow a better understanding of the impact of hide cleanliness on VTEC contamination.

HEI 6 provides information on the level of VTEC present on the bovine hide and is an indicator of the VTEC status of bovines entering the slaughter process. Because of the time delay in obtaining a result, this indicator will give data most relevant for surveillance purposes and when linked to HEI 4 and HEI 5 will also build up evidence of VTEC contamination occurring during transport and lairage and hide cleanliness.

HEI 7 measures the presence of VTEC on the bovine carcase pre-chilling. Sampling is performed prior to chilling rather than after chilling as it is easier to recover and cultivate VTEC bacteria at this point. Due to the time delay in obtaining a result, this indicator will give data most relevant for surveillance purposes. Combining the results from HEI 6 and HEI 7 will assess the ability of the slaughter process to influence VTEC contamination of the carcases.

HEI 8 focuses on providing an indicator of the VTEC status of the carcases after the entire slaughter process (including chilling) has been completed. However, there may also be methodology difficulties with recovery of bacteria from chilled carcases as the bacteria may be sublethally injured by the combination of chilling and reduced water activity rendering them non-cultivable. During chilling, some bacteria may become firmly attached to the meat or embedded into the meat tissue and thus not be readily recoverable by swabbing (Warriner et al., 2001). The microbial levels found at this point in the process reflect the VTEC contamination level entering the food chain from the slaughterhouse. The data derived from monitoring of HEI 8 could be used to set pathogenic VTEC hazard-based targets in/on bovine chilled carcases as referred to in the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

HEIs 1 to 5 deal with the live animals at various stages along the chain from farm to slaughter, while HEIs 6 to 8 deal with contamination of carcases.

The proposed HEIs give different types of information on the risk of pathogenic VTEC infection in bovines or contamination of the carcases and risk managers should choose the HEIs to be applied and then also interpret the available information in the appropriate way. The microbiological indicators (HEIs 3, 6, 7 and 8) may be used alone or in different combinations and the more general HEIs 1, 2, 4 and 5 should be used to support the microbiological HEIs and to determine where correlations occur that may in time allow for a decrease of the sampling frequency for microbiological sampling or more risk-based microbiological sampling.

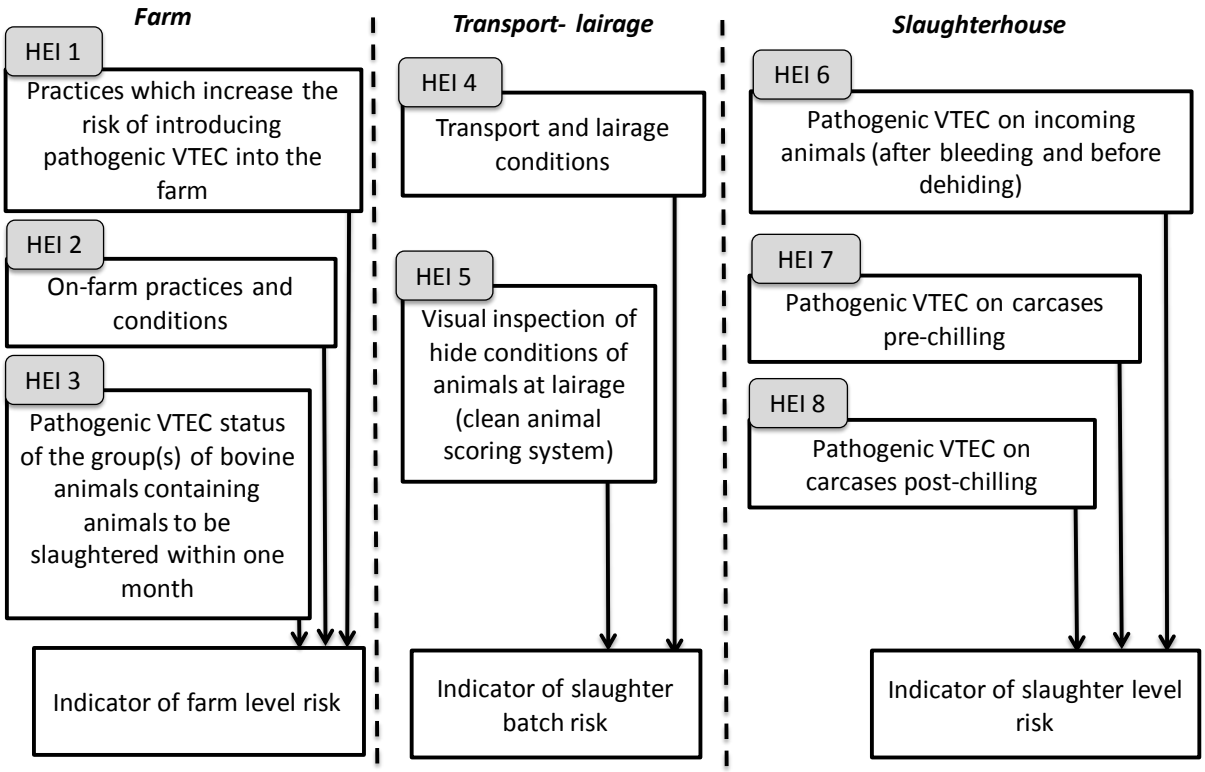


Figure 2: Schematic diagram illustrating the harmonised epidemiological indicators for pathogenic VTEC in bovine animals.

5.2.6. Harmonised monitoring requirements

Animal population

- Farms are subject to an audit of the production system standards to establish and verify controlled husbandry conditions and assess biosecurity (HEIs 1 and 2).
- Group of bovines at farm containing animals for slaughter (HEI 3).
- Transport conditions of bovines to the slaughterhouse and the lairage conditions at the slaughterhouse are subject to an audit of time between loading of bovines and slaughter, mixing from different herds and (re)use of pens at lairage (HEI 4).
- Bovines at slaughterhouse (HEIs 5, 6, 7 and 8).

Stage of the food chain

- The farm for controlled husbandry conditions (HEIs 1 and 2).
- The farm for bovines (HEI 3).
- Transport and slaughterhouse for transport and lairage conditions (HEI 4).
- The slaughterhouse for bovines (HEIs 5, 6, 7 and 8).

Sampling

HEI 1 and 2

- Target population:	All farms claiming to operate under controlled husbandry conditions.
- Epidemiological unit:	The farm.
- Sampling strategy:	Census (all farms claiming to operate under controlled husbandry conditions should be audited).
- Audit interval	Repeated at a frequency (determined by risk managers) adequate to maintain confidence that farms continue to meet the controlled husbandry conditions.

HEI 3

- Target population:	Bovines destined for slaughter.
- Epidemiological unit:	The groups of bovine animals containing animals to be slaughtered within one month.
- Sampling strategy:	For group(s) containing a large number of animals, a representative sample (random or systematic) of all bovines in the epidemiological unit(s). Samples from outdoor kept bovines may not be feasible to obtain prior to slaughter and information from previous slaughtered bovines can be used.
- Sample size:	Adequate to assess the presence of pathogenic VTEC-infected bovine animals. On small farms, in order to achieve the required precision, it may be necessary to use a census sampling of all bovines.

HEI 4

- Target population:	All batches of cattle sent to slaughter.
- Epidemiological unit:	The slaughter batch.
- Sampling strategy:	Census (all slaughter batches) or representative sample.

- Audit interval: Audit for every slaughter batch or repeated at a frequency (to be determined by risk managers) adequate to characterise the transport, mixing and lairage risks.

HEI 5

- Target population: Bovine animals in lairage.
- Epidemiological unit: The slaughter batch.
- Sampling strategy: Census (all animals pre-slaughter).

HEI 6

- Target population: Carcases after bleeding and before dehiding.
- Epidemiological unit: Slaughter batch.
- Sampling strategy: Representative sample (random or systematic).
- Sample size: Adequate to assess the pathogenic VTEC infection status of the hide of the incoming batch of bovines on the slaughter process, or to assess the difference in prevalence before and after processing.
- Survey interval: Initial survey,
repeated at a frequency to be determined by risk managers.

HEI 7

- Target population: Bovine carcases after the slaughter process, before chilling.
- Epidemiological unit: Slaughter batch.
- Sampling strategy: Representative sample (random or systematic).
- Sample size: Adequate to assess the pathogenic VTEC infection status of the carcases after processing (before chilling), or to assess the difference in prevalence before and after processing.
- Survey interval: Initial survey,
repeated at a frequency (to be determined by risk managers) adequate to characterise the slaughterhouse risk (required particularly when procedures in the slaughterhouse change).

HEI 8

- Target population: Bovine carcases after the slaughter process, and after chilling.
- Epidemiological unit: Slaughter batch.
- Sampling strategy: Representative sample (random or systematic).
- Sample size: Adequate to assess the pathogenic VTEC infection status of the carcases leaving the slaughter process.
- Survey interval: Initial survey,
repeated at a frequency (to be determined by risk managers) adequate to characterise the prevalence of pathogenic VTEC-positive carcases entering the food chain.

Type and details of sample

- Questionnaire-based audit of farm procedures, including specific conditions for pathogenic VTEC (HEIs 1 and 2).
- Pooled faecal samples either from groups of bovine animals or from the floor at the farm (HEI 3).
- Questionnaire-based audit of transport, mixing of herds and lairage conditions, including specific conditions for pathogenic VTEC (HEI 4).
- Visual inspection of animal coat and grading in line with clean animal scoring system with standardised system to score level of dirt /wetness of animal coat and a cut-off point at which action is needed (HEI 5).
- Hide swab sample (site 400 cm^2) of the brisket area of the animal before hide removal as outlined in EFSA's technical monitoring plan (EFSA, 2009) (HEI 6).
- Carcase surface samples of bovine carcases at the slaughterhouse in accordance with to Regulation (EC) 2073/2005 (HEIs 7 and 8).

Diagnostic/analytical methods

- Qualitative detection of selected serogroups as described in ISO16654:2001 (ISO, 2001a) and ISO/Technical Specification 13136:2012 (ISO, 2001b).
- Virulotyping of recovered isolates to assess human virulence potential in accordance with the EFSA Scientific Opinion on VTEC seropathotypes (EFSA Panel on Biological Hazards (BIOHAZ), 2013).

Case definition

- Farms found not complying with the controlled husbandry conditions (HEIs 1 and 2).
- Transport and lairage not complying with agreed conditions (HEI 4).
- Hide conditions not complying with the clean cattle policy (HEI 5).
- Findings of pathogenic VTEC in a sample (HEIs 3, 6, 7 and 8).

5.3. *Cysticercus* (*Taenia saginata*)

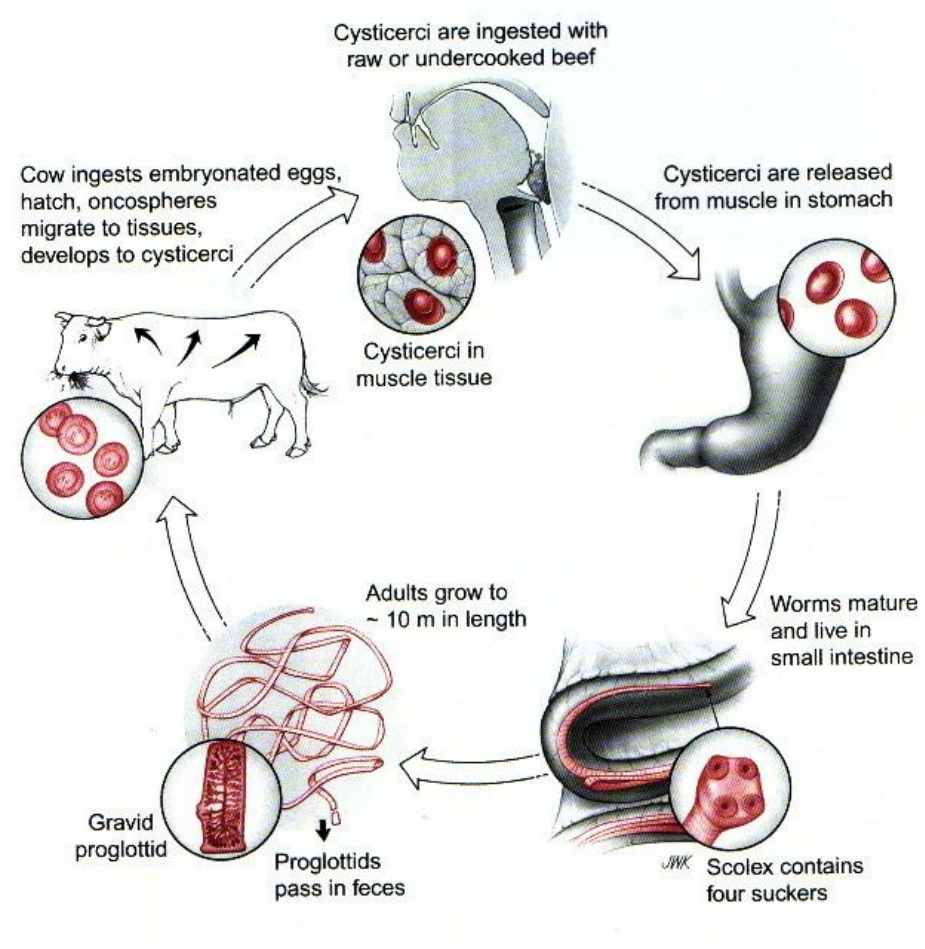
5.3.1. Biology and epidemiology

Taenia saginata (*T. saginata*, the beef tapeworm) is one of the three species causing taeniasis in humans. It has a universal distribution and is a common parasite in industrialised as well as in developing countries. It has an obligate two-host life cycle. Humans are the only final host while taurine and zebu bovines as well as buffaloes act as the intermediate hosts.

The parasite has little clinical importance. In humans, the presence of an adult tapeworm, which can grow up to a length of 12 metres, can cause abdominal discomfort, weight loss and anal pruritis. Rare severe cases are mainly caused by intestinal obstructions. Generally, no clinical symptoms are observed when the bovine intermediate host is infected, although in experimental infections light fever and inappetence have been observed. The importance of bovine cysticercosis is mainly economic. A detailed description of both human *T. saginata* infection and bovine *T. saginata* cysticercosis as regards condition and relevance in EU can be found in the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

The life cycle of *T. saginata* (beef tapeworm) is shown in Figure 3:

- The adult worm lives in the small intestine of humans.
- Gravid proglottids leave the host by active migration through the anus or in the stools.
- Eggs that are eliminated by the human final host within proglottids or in the stools contain a larva (oncosphere) and are infective for the intermediate host (bovines) immediately after release from the human host.
- Bovines acquire the infection by accidental ingestion of the eggs while grazing, through contaminated feed or by drinking from infected water sources. Following release, oncospheres penetrate the mucous layer of the digestive tract and enter the blood circulation of the host. The oncospheres do not multiply in the bovine.
- Following migration in the animal's body, the oncospheres establish in the muscles and organs, such as lung and liver, and develop into the infective *Cysticercus* (a pea-sized, fluid filled cyst containing the metacestode larval stage) after 8 to 10 weeks. Cysticerci remain viable for several months/years, after which they will degenerate, calcify and eventually disappear.
- On average, in an infected bovine, 23 % of the cysticerci will establish in the so-called predilection sites consisting of heart, masseter muscles, tongue, oesophagus and diaphragm, which are examined by routine meat inspection as required by Regulation (EC) No 854/2004. In 68 % of infected carcases of bovines cysticerci are found in these predilection sites (Walther and Koske, 1980; Kyvsgaard et al., 1990).
- In the EU, more than 90 % of cysticercosis cases are light infections, i.e. only one or a few cysticerci are found at meat inspection. Heavy infections occur only occasionally (Dorny and Praet, 2007).
- Human infection occurs trough consumption of raw or undercooked meat containing cysticerci. After ingestion, the digestive enzymes break down the cysticercal wall, releasing the larva; the inverted scolex will evaginate and attach to the host's intestine. The adult tapeworm will develop in the host's small intestine and will reach maturity within two to three months. An adult tapeworm can measure 3-12 metres and will release gravid proglottids that contain between 30 000 and 80 000 eggs. The daily egg production can be as high as 150 000 (Murrell, 2005). Usually, only one tapeworm will develop in the human's intestine (*solitary worm*).



Source: http://www.microbeworld.org/images/stories/twip/t_saginata_cycle.jpg

Figure 3: Life cycle of *Taenia saginata*

5.3.2. Current situation and trends in the EU

No information is available at EU level on the incidence of human *T. saginata*, as taeniasis is not a notifiable disease. But the true incidence of taeniasis can be estimated from the sale of taenicidal drugs. In Europe prevalence rates between 0.01 % and 10 % have been reported, with Slovakia and Turkey reporting the highest values (Cabaret et al., 2002). The prevalence of bovine cysticercosis in Europe is mostly based on meat inspection reports and ranges from 0.007 % to 6.8 % with a wide variation between countries, regions and abattoirs (Cabaret et al., 2002). The rates of detection from UK meat inspection data from 2008-2011 are 0.0075 % (15 out of 190 493) and 0.035 % (2 674 out of 8 484 371) for slaughtered calves and adult cattle, respectively. The prevalence of cysticercosis is likely to be underestimated as a result of the low sensitivity of the current meat inspection method. Detection rate of carcases with light infestation (1-10 cysts) of *T. saginata* cysticerci is believed to be low (27 %), rising to 43 % for animals with 11-20 cysts and 78 % when 20 or more cysts are present (EFSA, 2004). By adding additional cuts to the inspection of the heart, the number of cases detected was increased by twofold (Eichenberger et al., 2011).

In the EUSRs on zoonoses (EFSA and ECDC, 2011, 2012) only a few MSs have provided information on cysticercosis in bovine animals, with no or very rare positive findings (0.001 % or lower). Previously, a scientific report was submitted to EFSA concerning the development of harmonised schemes for the monitoring and reporting of *Cysticercus* in animals and foodstuffs in EU (Dorny et al., 2010). This scientific report concluded, from 17 MSs where information was available, that a rare

occurrence of bovine cysticercosis was recorded, covering MSs from all regions of EU. Data reported showed that there was an obvious disparity in the number of cases detected in the different MSs.

A further description of bovine *T. saginata* cysticercosis as regards the prevalence in EU can be found in the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

5.3.3. Bovine meat as a source of infection for humans

Bovine meat is the only source for acquiring *T. saginata* taeniasis in humans. Other tapeworm species are acquired by eating pork (*T. solium*, *T. asiatica*), fish (*Diphyllobothrium latum*) or by faeco-oral transmission (*Hymenolepis nana*). Experimental infection with *T. saginata* in reindeer has been described (Blazek et al., 1986), but as humans are the only final hosts, a sylvatic cycle is very unlikely.

Heating, freezing and pickling in common salt will destroy the cysticerci. The time and temperature combinations required to ensure the death of cysticerci are 15 days at -5 °C, 9 days at -10 °C and 6 days at -15 °C or lower (Hilwig et al., 1978).

5.3.4. Risk and risk-reducing factors

Taeniasis in humans is associated with the consumption of raw or undercooked bovine meat. Cysticerci do not resist high temperatures and dietary habits and culinary practices affect transmission. Taeniasis is more common in populations/age groups that consume raw or undercooked bovine meat (Murrell, 2005). Meat inspection has a low sensitivity and is likely to miss most cases, especially in lightly infected carcases (Dorny and Praet, 2007). According to the Regulation (EC) No 854/2004, all bovines of over six weeks of age have to be individually inspected for cysticercosis by visual observation and cuts in the masseter muscles and heart, and by visual inspection of the tongue, oesophagus and diaphragm. If an animal has a generalised infection, the carcase and offal are declared unfit for human consumption. If the infection is localised, the carcase has to be stored at a temperature not exceeding -10 °C for > 14 days before being released for human consumption.

T. saginata is not an animal health concern as it does not appear to cause clinical disease in bovines. Heavy cysticercosis infections in bovines are rather uncommon. Light infections are much more common and they are the result of accidental ingestion of eggs that are disseminated in the environment. The farm is mainly associated with the following risk factors, as described by Adonajto et al. (1976) and Ilsøe et al. (1990):

- the presence of a tapeworm carrier on the farm or the indiscriminate defecation associated with camping and tourism;
- the illegal application of sludge from septic tanks on pasture or crops;
- grazing on pastures in close proximity to municipal sewage treatment effluents likely to play a role in the dissemination of the eggs (Kyvsgaard et al., 1991) or after flooding;
- free access of bovines to surface water and the proximity of wastewater effluent, which were reported to be significant explanatory variables for bovine cysticercosis in a herd (Boone et al., 2007);
- demographic pressure as a result of higher population density, as this can increase the risk of bovine cysticercosis (Boone et al., 2007).

There are no risk factors associated during transport and in the slaughterhouse.

5.3.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected for *T. saginata* in bovines (Table 3).

Table 3: Harmonised epidemiological indicators for *Taenia saginata* in bovines

Indicators (animal/ food category/other)	Food chain stage	Analytical/ diagnostic method	Specimen
HEI 1: Audit of farming practices	Farm	Auditing	Not applicable
HEI 2: Prevalence of *T. saginata* cysticerci-positive slaughter animals (excluding white veal calves)	Slaughterhouse	Serology. At individual level. Direct method to detect circulating parasite antigens	Blood
HEI 3: *T. saginata* cysticerci in suspected lesions from all types of farms (excluding white veal calves)	Slaughterhouse	Visual meat inspection and polymerase chain reaction (PCR) for confirmation of *Taenia* DNA in the lesion	Suspect lesion (meat)

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

HEI 1 aims to audit husbandry conditions at the farm that could contribute to avoid the contact of livestock with possible sources of infection. This HEI could be used in low-prevalence areas combined with risk-based targeted surveillance through any of the other HEIs. Based on the risk-reducing factors, it is reasonable to assume that calves raised under controlled conditions without outdoor access, housed on wooden slats without bedding material and fed with milk and/or concentrates (white veal calves which in the EU are mainly reared in France, Italy and the Netherlands) are the least likely to be infected with *T. saginata.* Therefore, testing of bovines under these husbandry conditions is not selected as an indicator due to expected low prevalence. Examples of proposed requirements to investigate for controlled husbandry conditions can be found in Appendix A.

HEI 2 focuses on the prevalence of positive animals detected by serology. Serological methods include direct and indirect methods. Direct methods aim at detecting circulating parasite antigens by monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (ELISA) (Harrison et al., 1989; Brandt et al., 1992; Dorny et al., 2000). They only detect the presence of metabolically active (viable) cysticerci. Sensitivity and specificity are in the order of 91 % and 96 %, respectively (Gabriël et al., 2012). Using a monoclonal antibody-based ELISA increased the sensitivity of detection of bovine cysticercosis with viable cysts by 10-50 fold (Dorny et al., 2000; Allepuz et al., 2012).

Indirect methods aim at detecting the hosts' antibody response to cysticercus infection, mainly by ELISA. These methods measure both active and past infection and are rather an indication of exposure and, consequently, are likely to overestimate current infections. Both native and recombinant/synthetic antigens can be used in ELISA. The sensitivity and specificity of the HP6-2 synthetic peptide in ELISA using serum from experimentally infected and parasite naive cattle were calculated to be 100 % and 98 %, respectively (Abuseir et al., 2007), but are expected to be much lower when used on a sample of naturally infected/uninfected animals. Full validation of serological methods is very difficult as cysticerci may develop anywhere in the muscles and full carcase dissection would be needed.

For differential diagnosis with other visual lesions (e.g. abscess, sarcocysts), HEI 3 focuses on confirmation of *T. saginata* cysticerci in suspected lesions by using PCR (Geysen et al., 2007).

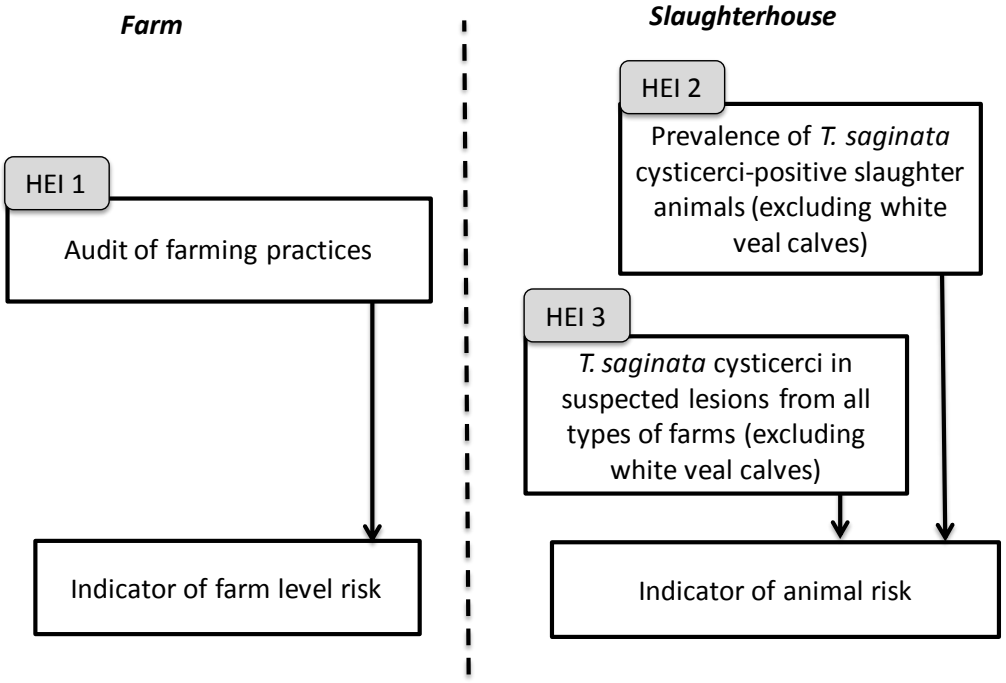


Figure 4: Schematic diagram illustrating the harmonised epidemiological indicators for *Taenia saginata* in bovine meat.

5.3.6. Harmonised monitoring requirements

Animal population

- Farms are subject to an audit of the production system standards to establish and verify controlled husbandry conditions and assess biosecurity (HEI 1).
- Bovines, except white veal calves (HEIs 2 and 3).

Stage of the food chain

- The farm for controlled husbandry conditions (HEI 1).
- The slaughterhouse for bovines (HEIs 2 and 3).

Sampling

HEI 1

- Target population: All farms claiming to operate under controlled husbandry conditions to control cysticercosis infections.
- Epidemiological unit: The farm.
- Sampling strategy: Census (all farms claiming to operate under controlled husbandry conditions to control cysticercosis infections should be audited).
- Audit interval: Repeated at a frequency (to be determined by risk managers) adequate to maintain confidence that farms continue to meet the controlled husbandry standards.

HEI 2

- Target population: All bovines more than six weeks old, except white veal calves.
- Epidemiological unit: The batch from the same farm or individual animal.
- Sampling strategy: Representative sample (random or systematic)
- Survey interval: Ongoing inspection as part of routine meat inspection.

  Periodic (e.g. annual) assessment of prevalence to be compared with threshold.

  Prevalence values determined by risk managers.

HEI 3

- Target population: All bovines more than six weeks old, except white veal calves.
- Epidemiological unit: The batch from the same farm or individual animal.
- Sampling strategy: Initial visual inspection:
  - all bovines, except white veal calves, at the slaughterline, by meat inspector in accordante with Rgeulation (EC) No 854/2004,
  - Suspected lesions: all suspect lesions followed up with further investigation.
- Survey interval: Ongoing inspection as part of routine meat inspection:

  Periodic (e.g. annual) assessment of prevalence to be compared with threshold.

  Prevalence values determined by risk managers.

Type and details of sample

- Questionnaire-based audit of farm practices contributing to the risk of introducing *T. saginata* into the herd, including purchase policy, access to pasture, surface water, flooding of pastures, vicinity of camping place, highway car park, hiking or biking trail, railway, or other tourist activity (HEI 1), vicinity of water treatment plant, tapeworm carrier on the farm.
- Blood samples are collected at slaughter and the blood is stored at room temperature to allow the blood to clot, then serum is separated and stored at -20 °C until the serological test. The pooling of samples should not be carried out (HEI 2).
- Suspected lesion/cyst (viable, degenerated or calcified), isolated from host tissue. The sample is to be stored at -20 °C or in ethanol 70 % (HEI 3).

Diagnostic/analytical methods

- Blood sample: antigen detection method on serum samples. This method will indicate only infection with viable cysticerci. Sensitivity and specificity are in the order of 91 % and 96 %, respectively, for detecting viable cysticerci (Gabriël et al., 2012) (HEI 2).
- Suspect lesions: confirmation and identification by molecular methods (PCR-restriction fragment length polymorphism (RFLP) or multiplex PCR) of *Taenia* species (HEI 3).
  - Preparation of specimen in the laboratory: DNA extraction (Boom extraction or commercial kit).

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

The above tests (ELISA and PCR) are not officially validated at the EU level.

Case definition

- Farms found not complying with the controlled husbandry conditions (HEI 1).
- Finding of animal positive to antigen detecting test (indication of viable *Cysticercus*) (HEI 2).
- Detection of the parasitic DNA in a suspected lesion (HEI 3).

5.4. Mycobacteria

5.4.1. Biology and epidemiology

Mycobacterium tuberculosis complex

Tuberculosis is a serious disease of humans and animals caused by the bacterial species of the family *Mycobacteriaceae*, more specifically by species of the *Mycobacterium tuberculosis* complex (MTC). This group includes *Mycobacterium bovis* (*M. bovis*), causing bovine tuberculosis. Bovine tuberculosis, which is a highly contagious disease that can easily spread from one cow to another, is a chronic, mainly respiratory, infectious disease of bovine animals. The causative agent is capable of infecting a wide range of warm-blooded mammals such as badgers, deer, goats, pigs, camelids, dogs and cats. In humans, infection with *M. bovis* causes a disease that is indistinguishable from that caused by infections with *M. tuberculosis*, the primary agent of human tuberculosis. Furthermore, the recently defined *M. caprae* also causes tuberculosis among animals, and to a limited extent in humans.

Transmission of *M. bovis* can occur between animals, from animals to humans and, more rarely, from humans to animals and between humans (Fritsche et al., 2004). The main transmission route of *M. bovis* to humans is through unpasteurised milk from infected animals or through unpasteurised milk products from infected animals. But as pasteurization kills *M. bovis*, cases of transmission of this bacterium to humans are extremely rare. *M. bovis* can also be transmitted to humans through direct contact with infected animals, notably by inhaling the bacteria shed by infectious animals in respiratory and other secretions.

Several wildlife animal species, such as deer, wild boars, badgers and the European bison, might contribute to the spread and/or maintenance of *M. bovis* infection in bovines (EFSA and ECDC, 2012). An overview of the main wildlife species from which *M. bovis* was isolated and their possible role as maintenance or spill-over hosts in the transmission of bovine tuberculosis to livestock can be found in Humblet et al. (2009).

Bovine tuberculosis is characterised by the formation of lesions (tubercles) where bacteria have localised. In bovines, tubercles are found in the lymph nodes, particularly those of the head and thorax. They are also common in the lung, spleen, liver and the surfaces of body cavities. In generalised cases, multiple small lesions may be found in numerous organs. The primary infection complex is observed in retropharyngeal, submandibular and mediastinal lymph nodes. Lesions in the mesenteric lymph nodes are less frequent. Some tubercles inside lymph nodes are small enough to be missed by the naked eye, even when the predilection lymph nodes are cut during *post mortem* inspection. Owing to the early detection of infection as a result of disease surveillance, which is currently the case in the EU, infected bovines typically have few, if any, visible lesions at *post mortem* examination.

In animals, latent infections are more common than clinical infections (Boschiroli and Thorel, 2010). During the early stages of infection of bovines with *M. bovis*, animals will often show no signs of

disease. In the later stages, common signs include progressive emaciation, a low-grade fluctuating fever, weakness and inappetence. Some animals will exhibit a moist cough that is worse in the morning, in cold weather or during exercise. In the terminal stages, animals may become extremely emaciated and develop acute respiratory distress. In some animals, the retropharyngeal or other lymph nodes enlarge and may rupture and drain. Greatly enlarged lymph nodes can also obstruct blood vessels, airways or the digestive tract. If the digestive tract is involved, intermittent diarrhoea and constipation may be seen.

It is unlikely that animals showing the above signs will be slaughtered for human consumption. Normally those animals will not reach the slaughterhouse because they will not be considered either fit to travel or fit to be slaughtered for human consumption. In the rare event that those animals are transported to the slaughterhouse, they will be identified at *ante mortem* inspection as not fit to be slaughtered for human consumption.

Non-tuberculous mycobacteria (NTM)

Numerous other mycobacteria species occasionally produce disease that is clinically indistinguishable from tuberculosis (personal communication from Maria Laura Boschiroli, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), 2013). Such disease is usually detected by investigation following identification of visible lesions on carcases at the slaughterhouse or a positive skin test in cattle delivered to the slaughterhouse. *Mycobacterium avium* complex (MAC) was recognised as the most common opportunistic bacterial infection in cattle and in patients with acquired immunodeficiency syndrome (AIDS) (Cook, 2010). MAC comprises eight mycobacteria species and several subspecies with different degrees of pathogenicity, host preference and environmental distribution (Álvarez et al., 2011).

Mycobacterium avium subsp. *avium* (MAA) is a potential zoonotic pathogen that belongs to MAC with wild avifauna as the reservoir, currently. As demonstrated by many experimental studies, inoculation of cattle by this pathogen usually does not cause any active visible lesion. However, it can cause mild transient hyperplasia of the lymph nodes, especially those that drain the digestive tract (Lucas and Gayot, 1967) and, in a small number of animals, tuberculosis-like lesions in these lymph nodes (Dvorska et al., 2004).

M. avium subsp. *hominisuis* (MAH) can infect a wide variety of animals. It is an environmental bacterium (e.g. water, soil, dust, straw, sawdust) that is rarely if ever pathogenic to birds. However, it is an opportunistic pathogen in mammals, including pigs, in which it is responsible for the majority of tuberculoid lesions discovered at the slaughterhouse (Matlova et al., 2005). This bacterium is, like MAA, commonly isolated from cattle (Dvorska et al., 2004; Möbius et al., 2006; Radomski et al., 2010; Boschiroli, 2013), but, unlike MAA, less frequently detectable in lesions (Dvorska et al., 2004; Boschiroli, 2013).

Considering NTM other than *Mycobacterium avium* subspecies *paratuberculosis* (MAP), various mycobacteria species can be implicated, but at a low level, and the species most frequently isolated from skin test bovine reactors for subsequent investigation at the slaughterhouse are from the MAC (MAA and MAH). In general, NTM other than MAP are ubiquitous and present in the environment or in wild avifauna (MAA).

NTM infections are acquired from environmental (water, soil) reservoirs and are not transmitted between humans or between animals and humans.

In humans NTM infection progression to clinical disease requires one or more predisposing host conditions. Pulmonary NTM disease (outside the context of AIDS) usually occurs in patients who are not obviously immunosuppressed but who almost always have pre-existing, underlying lung abnormalities (Cook, 2010). Lymphadenitis due to 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 species (e.g. *M. kansasii*, *M. xenopi*, *M. malmoense*, *M. avium* subsp. *hominisuis*) can cause NTM infections (Cook, 2010).

Considering their epidemiology, NTM species are not considered in this document, despite the fact that all suspected lesions observed during visual meat inspection are sampled and sent to a diagnostic laboratory for subsequent investigation and characterization. In addition, macroscopic lesions of NTM are often indistinguishable from true MTC lesions. This is why meat inspection rules are the same for *M. bovis* infection and for NTM.

5.4.2. Current situation and trends in the EU

Tuberculosis due to *M. bovis* is rare in humans in the EU, with 132 confirmed human cases reported in 2011 (EFSA and ECDC, 2013). The case numbers reported over recent years are fairly constant, with no observed trend in any MSs or at the EU level. There is no clear association between a country's status as Officially Tuberculosis Free (OTF) and notification rates in humans. This could be because infected cattle are sometimes also detected in OTF MSs and, on average, more than half of the cases in OTF MSs occur in individuals who have immigrated to the country; and, thus, might have acquired the infection in their country of origin.

Fifteen MSs have OTF status, and five of these reported infected cattle herds: Belgium, Germany, Poland and the Netherlands detected only very few positive herds, while France found 173 such herds. However, owing to the low numbers of infected herds compared with the numbers of officially free herds, their status as OTF countries was retained.

The proportion of infected or positive herds in the 12 non-OTF MSs slightly increased in 2011. Three of the 12 non-OTF MSs reported no infected cattle herds in 2011. Of the nine non-OTF MSs reporting herds infected with or positive for *M. bovis*, the prevalence of bovine tuberculosis remained at a level comparable to 2010 or decreased, except in the United Kingdom, which reported an increase in the prevalence of bovine tuberculosis and accounted for the highest proportion of positive herds. This was the third consecutive year that the United Kingdom reported an increase in bovine tuberculosis. No statistically significant trend was observed in the grouped weighted prevalence for the three co-financed non-OTF MSs, Italy, Portugal and Spain, during 2004-2011 (EFSA and ECDC, 2013).

In MSs where infection is still prevalent, the slaughterhouse plays a substantial role in confirmation of *M. bovis* infection through detection of characteristic lesions and collection of samples for mycobacterial isolation, and efficient *post mortem* examination of specified lymph nodes and of the lungs represents an important element of national bovine tuberculosis eradication programmes within the EU (EFSA, 2003). Furthermore, routine meat inspection at slaughterhouse of bovines from bovine tuberculosis-free herds contributes to the detection of a significant fraction of the total new bovine tuberculosis breakdowns in non-OTF zones, as shown by data from Ireland, the United Kingdom and Catalonia (EFSA BIOHAZ Panel, 2013). The role of the current slaughterhouse meat inspection in bovine tuberculosis surveillance is, however, of great relevance for the surveillance programmes of the infection in herds and animals (EFSA BIOHAZ Panel, 2013). Its value as a method of demonstrating continuous freedom should not be underestimated in those countries which are OTF. Also, besides direct surveillance, meat inspection would also indirectly enable prevention of human exposure in the farms of origin.

5.4.3. Bovine meat as a source of infection for humans

The risk of transmission of *M. bovis* to humans by meat consumption is reviewed in the EFSA Scientific Opinion, and it is currently considered as negligible owing to the non meat-borne nature of the agent (EFSA BIOHAZ Panel, 2013). Human infections occur via exposure to other foods (i.e. milk) or the animal environment (direct contact/inhalation).

5.4.4. Risk and risk-reducing factors

The main bovine tuberculosis risk factors classified into animal, herd and region/country levels are presented in Figure 5 (Humblet et al., 2009).

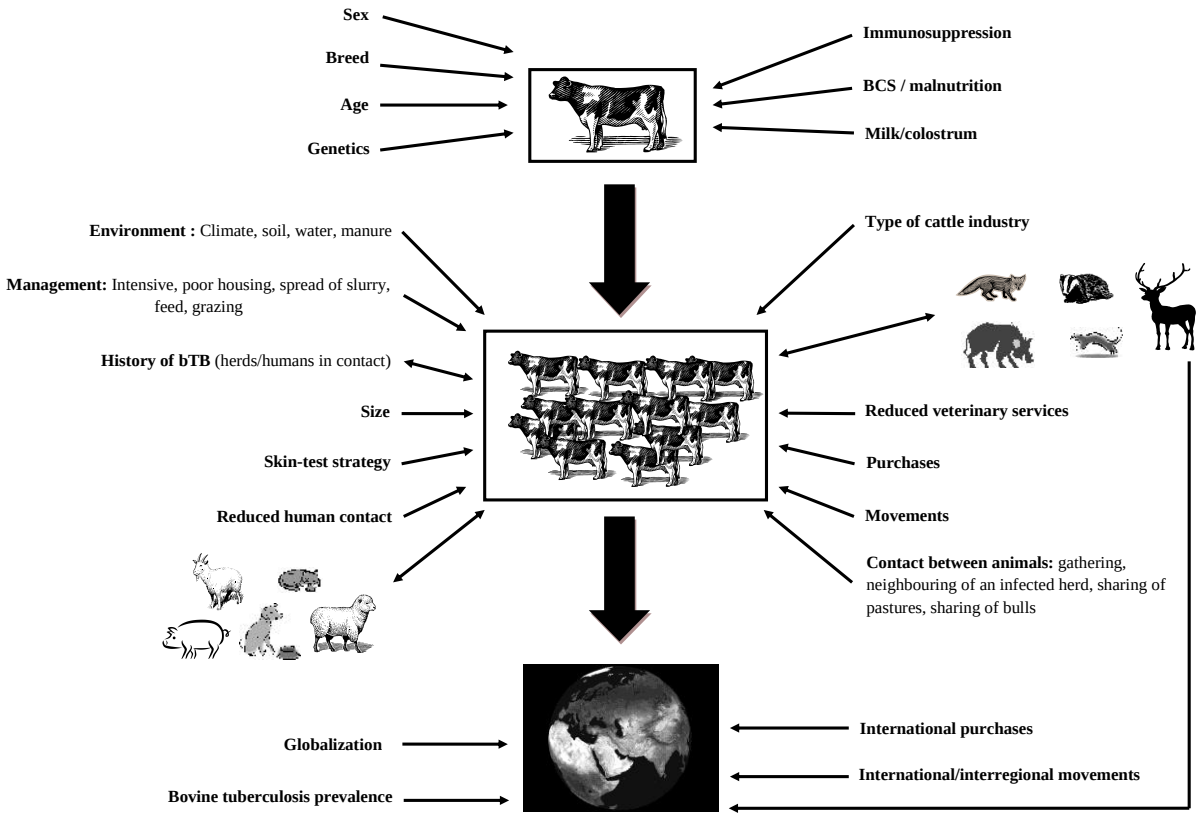


Figure 5. Bovine tuberculosis risk factors classified into animal, herd and region/country levels

Bovine tuberculosis is mainly a respiratory disease and is transmitted between bovines by air (breathing in the *M. bovis* bacteria). This usually happens when the density of animals is high, animals are in close contact with each other, and the air quality is poor (e.g. high ammonia levels weaken the respiratory mucosa and make it more likely that the animal will become infected). Bacteria released into the air through coughing and sneezing spread to uninfected animals. Direct transmission can occur, for example, through nose-to-nose contact. There is evidence that indirect transmission is possible through contact with saliva, urine, faeces, pus from abscesses, etc. Bovine tuberculosis is transmitted from bovine to bovine. Several wildlife animal species, such as deer, wild boars, badgers and the European bison, might contribute to the spread and/or maintenance of *M. bovis* infection in bovines (EFSA and ECDC, 2012).

Considering the current status of MSs regarding *M. bovis* (see section 5.4.2), the main risk factors at farm level for the introduction of bovine tuberculosis in non-infected herds are the purchase of infected animals; the proximity of infected neighboring herds; or herds grazing on pastures adjacent to those grazed by infected herds or inhabited by a wide range of infected wildlife (badgers, deer, etc.) that could contaminate feed through their excretions. Possible reactivation of a latent infection in the herd itself should be also taken into account. These situations are more likely in non-OTF MSs.

The main preventative measures consist in applying correct biosecurity measures, in no particular order of priority:

- keeping animal identification and movement records accurate and up to date to be able to trace bovine movements between herds and carrying out pre-movement testing;

- protect bovines from neighboring livestock, e.g. using perimeter fencing and gateways to prevent nose-to-nose contact and sharing of water troughs;

- proving good ventilation in livestock housing and not overcrowding stock either when housed or at grass;

- protecting bovines from wild animals susceptible to infection with *M. bovis*, e.g. making sure buildings are secure or preventing access through the use of electric fencing;

- prevent access of wildlife to animal feed by covering the face of silage clamps, protecting areas for storage, feeders, troughs and salt licks and cleaning up feed spillages.

At the slaughterhouse there are also key factors that affect the sensitivity of the meat inspection procedures and its effectiveness in detecting bovine tuberculosis. Issues related to the non-perfect sensitivity of meat inspection for detecting *M. bovis* are discussed in the Animal Health and Welfare Appendix of the EFSA Scientific Opinion (EFSA BIOHAZ Panel, 2013).

Those factors relate to the facilities (e.g. level of lighting at inspection points and slaughterhouse line speed), and the performance, training and experience of official inspectors. These factors have an effect on the ability to detect tuberculosis lesions in infected bovines that do not travel with any FCI indicating that they are reactors or that they have been in contact with reactors or that they are likely to be infected with bovine tuberculosis.

The probability of detecting lesions increases with the number and frequency of animals from different herds sent to the slaughterhouse. This is even more important in MSs where slaughterhouse surveillance is a key element of the bovine tuberculosis surveillance programme in both OTF and non-OTF regions. A high slaughtering rate from individual herds increases herd turnover and number of animals being purchased into the herd, thus increasing the rate of tuberculin skin tests.

The correct *post mortem* inspection decisions and removal of infected organs/carcases and their disposal as the adequate animal by-product categories is another slaughterhouse risk factor that stops infected tissues further spreading bovine tuberculosis.

5.4.5. Proposed harmonised epidemiological indicators (HEIs)

The following epidemiological indicators have been selected mycobacteria in bovines (Table 4).

Table 4: Harmonised epidemiological indicators for mycobacteria in bovines

Indicators (animal/ food category/other)	Food chain stage	Analytical /diagnostic method	Specimen
HEI 1: Official status of bovine herd as regards bovine tuberculosis (OTF status)	Farm	Food chain information	Not applicable
HEI 2: Human pathogenic mycobacteria in bovines at slaughter (identification of tuberculosis-like lesions through visual *post mortem* inspection and microbiology of suspect lesions)	Slaughterhouse	Visual meat inspection and microbiology[(a)]	Suspected lesions

(a): Detection of the human pathogenic mycobacteria from lesions detected through visual inspection.

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

HEI 1 focuses on assessing the official status of bovine herd as regards bovine tuberculosis. In the current meat inspection system for bovine animals, FCI is of particular importance relative to bovine tuberculosis, bovine spongiform encephalopathy (BSE) and brucellosis. Concerning bovine tuberculosis, even though *M. bovis* is not included in the list of meat-borne pathogens identified, because of its prominence in the current meat inspection system and for historical reasons, the following is presented for clarification as to how it is addressed. EU MSs/regions are designated either OTF or non-OTF. In non-OTF regions, animals suspected of being affected by tuberculosis (i.e. based on clinical evidence or the results of diagnostic tests on-farm) travel to the slaughterhouse accompanied by FCI which includes their tuberculosis status; those animals are required to be segregated in the lairage and undergo separate slaughter and dressing under hygienic operational conditions in order to minimize the likelihood of cross-contamination of other animals or carcases. Thus, where available, complete and reliable FCI enables differentiation of batches of bovines posing higher or lower risk of being affected by bovine tuberculosis. Such differentiation is a basis for decisions to pay particular attention to higher risk batches during *ante* and *post mortem* examinations and to apply specific measures to ensure that affected carcases or organs are disposed of as an animal by-product and those animals and their farms of origin are identified for animal health controls.

HEI 2 is based on visual inspection of bovine carcases at slaughter and confirmation of the presence of the bacteria in suspicious lesions by microbiological testing. This HEI covering surveillance of all slaughtered bovines at the slaughterhouse is proposed in the light of the very low to rare prevalence of *M. bovis* in bovine animals in the EU. It would enable surveillance for detection of emergence of *M. bovis* infections in the bovine animal populations that permits countries/ regions to demonstrate their OTF status.

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

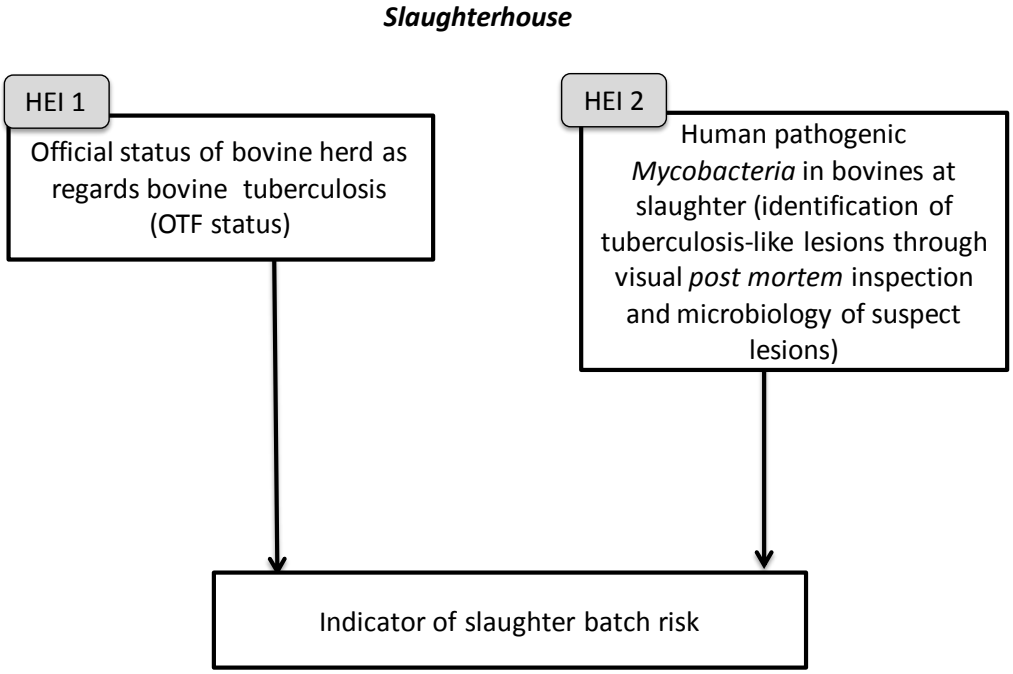


Figure 6: Schematic diagram illustrating the harmonised epidemiological indicators for mycobacteria in bovine meat.

5.4.6. Harmonised monitoring requirements

Animal population

- All bovines (HEI 1).
- Suspect tuberculosis-like lesions in bovine animals presented for slaughter (HEI 2).

Stage of the food chain

- The farm (HEI 1).
- The slaughterhouse (HEI 2).

Sampling

HEI 1

- Target population: All bovine farms from areas with high prevalence of *M. bovis* infection.
- Epidemiological unit: The herd.
- Sampling strategy: All bovines from areas with high prevalence of tuberculosis.
- Survey interval: Every time bovines are moved from the farm or when sent to slaughter.

HEI 2

- Target population: All suspect tuberculosis-like lesions identified through *post mortem* inspection.
- Epidemiological unit: All bovines showing suspect tuberculosis-like lesions.
- Sampling strategy: All suspect tuberculosis-like lesions in bovines presented for slaughter.

- Survey interval: Every time suspect tuberculosis-like lesions are identified.

Type and details of samples

- All suspected lesions from the target population, such as relevant lymph nodes (retropharyngeal, bronchial and mediastinal) and organs (lungs and udder), observed during the visual meat inspection, are sampled and sent to a diagnostic laboratory for subsequent investigation (HEI 2).

Diagnostic / analytical methods

- Microscopy, Ziehl-Neelsen staining, culture and molecular characterization for epidemiological purposes, such as RFLP, spoligotyping and/or mycobacterial interspersed repetitive unit-variable-number tandem repeat (MIRU-VNTR) (HEI 2).

Case definition

- OTF Member State/ region /farm as defined in Council Directive 64/432/EEC[17] (HEI 1).
- Suspect lesions containing confirmed *Mycobacterium* species known to be a human pathogen (HEI 2).

[17] 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, p. 1977–2012.

6. Combined sampling and audits for the epidemiological indicators

HEIs including sampling or audits at farm and at slaughterhouse have been proposed for *Salmonella*, pathogenic VTEC and *Cysticercus* in this report. It may be possible to combine the sampling for some of these HEIs and a proposal for this is presented in Table 5.

Table 5: Proposed combined sampling for the epidemiological indicators in bovine animals

Indicators (animal/ food category/other)	Hazard (related HEI)	Food chain stage	Type of sample	Combined sampling
Practices which increase the risk of introducing the pathogen into the farm	*Salmonella* (HEI 1) Pathogenic VTEC (HEI 1) *Cysticercus* (HEI 1)	Farm	Not applicable	Same audit session
On-farm practices and conditions	*Salmonella* (HEI 2) Pathogenic VTEC (HEI 2) *Cysticercus* (HEI 1)	Farm	Not applicable	Same audit session
Pathogen status of the group(s) of bovine animals containing animals to be slaughtered within one month	*Salmonella* (HEI 3) Pathogenic VTEC (HEI 3)	Farm	Pooled faeces	Same sample or same sampling session
Transport and lairage conditions	*Salmonella* (HEI 4) Pathogenic VTEC (HEI 4)	Transport and lairage	Not applicable	Same audit session
Visual inspection of hide conditions of animals at lairage (clean animal scoring system)	*Salmonella* (HEI 5) Pathogenic VTEC (HEI 5)	Slaughterhouse	Not applicable	Same visual session
Pathogen on incoming animals (after bleeding and before dehiding)	*Salmonella* (HEI 6) Pathogenic VTEC (HEI 6)	Slaughterhouse (after bleeding and before dehiding)	Hide swabs	Same sample or sampling session
Pathogen on carcases pre-chilling	*Salmonella* (HEI 8) Pathogenic VTEC (HEI 7)	Slaughterhouse (before chilling)	Carcase swabs	Same sample or sampling session
Pathogen on carcases post-chilling	*Salmonella* (HEI 9) Pathogenic VTEC (HEI 8)	Slaughterhouse (after chilling)	Carcase swabs	Same sample or sampling session

7. Comparable data on the harmonised epidemiological indicators

Comparable data on the proposed harmonised epidemiological indicators from the EU MSs are available only for mycobacteria, notably *M. bovis*, for the proposed farm-level indicator HEI 1 and for the proposed animal-level indicator HEI 2. These can be found in the "Bovine and swine diseases, 2011 Annual report" (EC, 2012); and are displayed in Table 6.

Table 6. Total number of bovine herds, total number of OTF bovine herds and total number of slaughtered bovine animals positive in bacteriological examination for tuberculosis, in the EU, 2011

MS or region (1)	Total number of existing bovine		Officially free herds		Infected herds		Routine tuberculin testing		Number of tuberculin tests carried out before the introduction into the herds [Annex A(I)(2)(c) 3°indent(1) of Directive 64/432/EEC]	Number of animals with suspicious lesions of tuberculosis examined and submitted to histopathological and bacteriological examinations	Number of animals detected positive in bacteriological examination
	Herds	Animals	Number of herds	%	Number of herds	%	Interval between routine tuberculin tests (2)	Number of animals tested			
AT	69,586	1,976,527	69,586	100	0(*)	0	(a) & (g)	7,865	29	16	0
BE	34,540	2,682,370	34,539	99.997	1*	0.003	(a)**	234,996	395,000	225	19
BG	103,383	586,434	103,382	99.999	1*	0.001	(b)	537,426	4,097	78	36
CH	41,095	1,591,233	41,095	100	0	0	(a)	0	0	2	0
CY	324	56,374	273	84.25	o	o	(b) & (c)	4,0642	o	o	o
CZ	19,658	1,324,350	19,658	100	0	0	(g)(*)	6,338	6,338	5	0
DE	175,330	12,776,773	175,325	99.997	5*	0.003	(a) or/and (b)	44,365	127	88	24
DK	20,384	1,623,400	20,384	100	0	0	(g)	4.084*	0	1	0
EE	4,716	238,684	4,716	100	0	0	(d)	44,571	1,401	0	0
ES*											
FI*	14,935	914,053	14,935	100	0	0	(a)	639*	0	3	0
FR	232,592	19,005,674	232,337	99.9	173*	0.07	(a) 60 départs (b) 4 départs (c) 7 départs (d) 10 départs (e) 2 départs (f) 13 départs	733,476	121,933	163	36**
UK(E,W,NI											
UK(SC)	13,323(a)	1,741,130(b)	13,322(a)	99.99	5(c)	0.04	(f)	228,640(d)	5,685(e)	10(f)	9(g)
UK(IoM)	280	32,803	280	100	0	0	(e)	6,684	0	1	0
GR	31,381	708,397	14,295*	45.55*	176**	0.56**	(b)	181,003	0	0	0
HU*	16,608	75,6721	16,599	99.95	1*	0,006	(b)	668,729	86,452	564**	4
IE*											
IT*	48,467	2,590,982	48,466	99.998	1	0.002	*	26597	35384	15	5
LT	86,207	669,190	86,207	100	0	0	(b)	580,861	41,318	5	0
LU	1,525	187,066	1,525	100	0	0	(a)	0	0	0	0
LV	33,998	380,612	33,998	100	0	0	(a)	1,501	5,984	0	0
MT*	125	13,912	125	100	0	0	(b)	23,593	0	0	0
NL	51,119	3,912,112	51,119	99.99	4	0.01	(a)	0	0	9	5
NO	16,400	856,000	16,400	100	0	0	(a)	1	0	0	0
PL	646,016	6,068,806	646,003	99.998	13	0.002	(g)	1,174,019	203	148	60
PT*											
RO*	751,595	2,220,939	751,534	99.99	61	0.01	(b)	2,039,581	0	377	122
SE*	20,503	1,511,846	20,503	100	0	0	(g)	242*	0	69**	0
SI	32,225	457,634	35,225	100	0	0	(d)	148,956	0	21	0
SK	8,635	463,574	8,635	100	0	0	(f)	71,680	0	0	0

Table continued overleaf

Table 6 (continued). Data on bovine tuberculosis

MS or region [1]	Total number of existing bovine		Officially free herds		Infected herds		Routine tuberculin testing		Number of tuberculin tests carried out before the introduction into the herds [Annex A(I)(2)(c) 3°indent(1) of Directive 64/432/EEC]	Number of animals with suspicious lesions of tuberculosis examined and submitted to histopathological and bacteriological examinations	Number of animals detected positive in bacteriological examination
	Herds	Animals	Number of herds	%	Number of herds	%	Interval between routine tuberculin tests [2]	Number of animals tested			

[1] Detailed regional information is required, unless the officially free status has been granted to the whole territory of the Member State.
[2] a) No routine tests, b) Tests once a year, c) Tests each 2 years, d) Tests each 3 years, e) Tests each 3 years concerning 24 months aged animals, f)Tests each 4 years, g) Others (please give details)

Additional information:

AT: *3 herds positive for *M. caprae*. Measures applied like in case *M. bovis*.
BE: *All bacteriological positive animals belonged to the tuberculosis breakdown herd of end 2010; **intensive testing by tracing-back and tracing-on in case of an infected herd or follow up testing of infected herd .
BG: *1 herd in Sredets, Burgas region. Follow-up and investigation of all cattle which entered the herd over the past five years were carried out. There are no agents in the herd of origin of animals. Epidemiological studies continue. Burgas region is free from tuberculosis in 1995
CZ: * Single tuberculin test on:
all imported females (except for slaughter) older than 6 weeks of age from third countries.
all removed females (except for slaughter) older than 6 weeks of age and breeding bulls older than 6 weeks of age from Member States which have not the officially TB free status.
all breeding bulls
DE:* 2 herds in Bavaria, 3 herds in Lower Saxony.
DK:* Bulls at semen collection centres are subjects to serological test for bovine brucellosis before entry to the centres and once a year. Furthermore, some bovine animals are tested before export.
ES: 2008/940/EC
FI: * intradermal tuberculin tests were done on bulls standing at the semen collection centres or new bulls introduced to the centres.
FR: *officially free herds (mentioned in the relevant column) include 123 herds suspended on 31/12/2011 and suspension was removed between 1/1/2012 and 1/2/2012; **Moreover the % of officially free herds was superior or equal to 99.9% from July to November 2011, on 1 November 2011 it was 99.9% (232027/232258).
UK (England, Wales & Northern Ireland): 2008/940/EC
UK (Scotland):
(a) (Number of herds as at 31 December 2010
(b) Bovine animal number sourced from Cattle Tracing System
(c) Number of infected herds (OTF status withdrawn) of indigenous origin.
(d) All tuberculin skin tests and interferon-gamma blood tests done on individual animals.
(e) Number of cattle required to receive a negative pre-movement test before leaving England / Wales and arrival in Scotland (does not include imported cattle)
(f) Carcases investigated after disclosure of suspect TB lesions at routine slaughter of cattle from OTF herds (ie test reactors excluded)
(g) Cattle carcases with suspect TB lesions at routine slaughter from which *Mycobacterium.bovis* was isolated. Excludes tuberculin and gamma-interferon test reactors
UK(IoM): Isle of Man
GR: *data not complete for the regions: Athina, Kavala, Kefallinia, Lesvos, Peiraias, Rethymno; ** data not complete for most of the regions
HU: *beside the officially free herds and the infected herd in Somogy county there were 8 herds where the officially free status was suspended (in Baranya, Borsod-Abaúj-Zemplén, Heves, Komárom-Esztergom, Nógrád and Somogy counties). ** It means the number of animals sent to slaughter for diagnostic reasons. Samples from these animals were sent to NRL for histopathological and bacteriological examinations. 9 cases of *M. bovis* in wild boars in Somogy county.
IE: 2008/940/EC
IT: *[Abruzzo (Pescara) - (c), Bolzano - (a), Emilia Romagna - (c), Friuli Venezia Giulia - (a), Lazio – (c), Lombardia - (c), Marche (Ascoli Piceno, Fermo) - (c), Piemonte (Novara, Verbano-Cusio-Ossola, Vercelli) - (c), Sardegna (Cagliari, Medio-Campidano, Ogliastra, Olbia-Tempio, Oristano) - (c), Toscana - (f); Trento (b), Veneto (d)]; other regions: 2008/940/EC.
PT: 2008/940/EC
SE: *235 tests at semen collection centres have been performed in accordance with Council Directive 88/407/EEC. Test has been performed in 6 animals in connection with import of water buffalos (*bubalus bubalus*) and one bovine animal has been examined due to lesion at slaughter (or *post mortem* examination). ** animals submitted to histopathological and bacteriological examinations were from one bovine animal and 44 swine, 14 deer and 10 animals o other species (elks, caprine etc…).

Source: European Commission, 2012. Bovine and swine diseases 2011 Annual report. Available online: http://ec.europa.eu/food/animal/liveanimals/bovine/docs/final_report_2011_en.pdf

CONCLUSIONS AND RECOMMENDATIONS

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

Conclusions

- In this report harmonised epidemiological indicators (HEIs) are proposed for food-borne biological hazards related to bovine animals and meat thereof in the context of the Scientific Opinion on public health hazards to be covered by inspection of meat from bovine animals (EFSA BIOHAZ Panel, 2013). These hazards include *Cysticercus* (*Taenia saginata*) and mycobacteria, which are already covered by meat inspection of bovine animals, as well *Salmonella* and pathogenic verocytotoxin-producing *Escherichia coli* (VTEC), which were identified by the BIOHAZ Scientific Opinion. 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 hazards, such as audits of farms, that correlates with a human health risk caused by the hazard.

- The epidemiological indicators proposed in this report will provide relevant information to risk managers (i.e. the European Commission (EC) and the Member States (MSs)), to enable them to consider whether adaptations to meat inspection methods may be relevant and to enable the MSs to carry out a risk analysis to support such decisions. The epidemiological indicators could be also used in the future to help categorise countries, regions, slaughterhouses, or potentially farms or herds/flocks, according to risk related to a particular hazard as well as to set appropriate specific hazard-based targets (hazard prevalence and/or concentration) in/on bovine carcases and, when appropriate, in bovine farms/herds. Thus, the indicators could facilitate the implementation of risk-based meat inspection.

- The risk managers should decide on the most appropriate use of the epidemiological indicators. Depending on the purpose and the epidemiological situation of the country, the indicators may be applied at national, regional, slaughterhouse or farm/herd level and they can be used alone or in different combinations. For *Salmonella* and pathogenic VTEC, the proposed HEIs include microbiology-based indicators, which will give specific information on *Salmonella* and VTEC infection or contamination in the animal, hide or carcase as well as HEIs based on audits at farm or transport conditions and visual inspection of bovine hide, which will a give more general assessment of microbiological risk and when used in combination with microbiological HEIs will support assessment and knowledge of *Salmonella*/VTEC risk. The epidemiological indicators may be used in the classification of the countries, regions, farms or slaughterhouses according to the infection, colonisation or contamination status related to the hazards. In addition, some indicators may be used to evaluate the measures taken in the slaughterhouses to control a specific hazard.

- The epidemiological indicators for *Salmonella* and pathogenic VTEC can be used in the classification of slaughter batches according to the infection status of the herd at farm level. In addition, other indicators have been proposed to evaluate the measures taken in the slaughterhouses to control the hazard or to guarantee process hygiene.

- The epidemiological indicators for *T. saginata* (*Cysticercus*) can be used in the classification of slaughter animals according to the infection status related to the hazard at farm level.

- In cases of rare biological hazards in bovine animal production, epidemiological indicators are suggested to enable surveillance for possible emergence of such hazards. This is the case for mycobacteria.

- The data accumulated from the implementation of the HEIs will provide for historical information over time of the infection, colonisation or contamination status of the animals, farms and slaughterhouses. This information will be useful for the categorisation of farms and slaughterhouse and areas regarding their status. Where there is a history of negative test results, the information can also be used to reduce the testing frequency applied for HEIs.

- The epidemiological indicators suggested for bovine animals address risks at region, at farm and at slaughterhouse level using a variety of methods. The proposed HEIs are summarised in Table 7.

Recommendations

- It is recommended that the EC and the MSs define the harmonised requirements for the controlled husbandry conditions at farms related to the specific hazards. The EC and the MSs should define the detailed rules for the content of this food chain information.

- Regular use of the proposed indicators will provide knowledge on risk factors at the different stages of the food chain and add certainty to current sparse evidence. In addition, the proposed epidemiological indicators can generate data that will provide information on the epidemiological situation in the EU. 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 HEIs proposed by this report should be reviewed regularly in the light of new information and the data generated from monitoring of them.

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

Conclusions

- Comparable data from the EU MSs were available only for mycobacteria, 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 where the sampling should take place, type and details of the specimen to be taken, diagnostic or analytical method to be used, and a case definition.

Recommendations

- It is recommended that monitoring at any stage is designed to be epidemiologically sound with clearly stated objectives and acceptable levels of uncertainty.

- It is recommended that the EC and the MSs organise training to ensure harmonised implementation of the monitoring and inspection requirements for the HEIs.

Table 7: Proposed harmonised epidemiological indicators for bovine animals

Indicators (animal/ food category/other)	Food chain stage	Analytical /diagnostic method	Specimen
Salmonella			
HEI 1: Practices which increase the risk of introducing *Salmonella* into the farm (purchase policy, mixing with other herds, access to pasture, access to surface water)	Farm	Auditing	Not applicable
HEI 2: On-farm practices and conditions	Farm	Auditing	Not applicable
HEI 3: *Salmonella* status of the group(s) of bovine animals containing animals to be slaughtered within one month	Farm	Microbiology	Pooled faeces
HEI 4: Transport and lairage conditions	Transport and lairage	Auditing	Not applicable
HEI 5: Visual inspection of hide conditions of animals at lairage (clean animal scoring system)	Slaughterhouse	Visual inspection	Not applicable
HEI 6: *Salmonella* on incoming animals (after bleeding and before dehiding)	Slaughterhouse	Microbiology (detection and serotyping)	Hide swabs
HEI 7: *Salmonella* in incoming animals (evisceration stage)	Slaughterhouse	Microbiology (detection and serotyping)	Lymph nodes
HEI 8: *Salmonella* on carcases pre-chilling	Slaughterhouse	Microbiology (detection and serotyping)	Carcase swabs
HEI 9: *Salmonella* on carcases post-chilling	Slaughterhouse	Microbiology (detection and serotyping)	Carcase swabs
Pathogenic VTEC			
HEI 1. Practices which increase the risk of introducing pathogenic VTEC into the farm (purchase policy, mixing with other herds, access to pasture, access to surface water)	Farm	Auditing	Not applicable
HEI 2. On-farm practices and conditions	Farm	Auditing	Not applicable
HEI 3. Pathogenic VTEC status of the group(s) of bovine animals containing animals to be slaughtered within one month	Farm	Microbiology	Pooled faeces or floor samples
HEI 4. Transport and lairage conditions	Transport and lairage	Auditing	Not applicable
HEI 5. Visual inspection of hide conditions of animals at lairage (clean animal scoring system)	Slaughterhouse	Visual inspection	Not applicable
HEI 6. Pathogenic VTEC on incoming animals (after bleeding and before dehiding)	Slaughterhouse	Microbiology	Hide swabs
HEI 7. Pathogenic VTEC on carcases pre-chilling	Slaughterhouse	Microbiology	Carcase swabs
HEI 8. Pathogenic VTEC on carcases post-chilling	Slaughterhouse	Microbiology	Carcase swabs

Tables continued overleaf.

Table 7 (continued): Proposed harmonised epidemiological indicators for bovine animals

Indicators (animal/ food category/other)	Food chain stage	Analytical /diagnostic method	Specimen
Cysticercus			
HEI 1. Audit of farming practices	Farm	Auditing	Not applicable
HEI 2. Prevalence of *T. saginata* cysticerci-positive slaughter animals (excluding white veal calves)	Slaughterhouse	Serology. At individual level. Direct method to detect circulating parasite antigens	Blood
HEI 3. *T. saginata* cysticerci in suspected lesions from all types of farms (excluding white veal calves)	Slaughterhouse	Visual meat inspection and PCR for confirmation of *Taenia* DNA in the lesion	Suspect lesion (meat)
Mycobacteria			
HEI 1. Official status of bovine herd as regards bovine tuberculosis (OTF status)	Farm	Food chain information	Not applicable
HEI 2. Human pathogenic mycobacteria in bovines at slaughter (identification of tuberculosis-like lesions through visual *post mortem* inspection and microbiology of suspect lesions)	Slaughterhouse	Visual meat inspection and microbiology	Suspected lesions

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Appendices

Appendix A. Proposed requirements for controlled husbandry conditions on farms

Table 8. Proposed requirements for controlled husbandry conditions on farms by pathogen

Measures	*Salmonella*	Pathogenic VTEC	*Cysticercus*	Mycobacteria	
				M. bovis	NTM
Practices which increase the risk of introducing pathogens into the farm					
Purchase policy	√	√	√	√	√
Contact with other animals/ herds	√	√		√	√
Contact with wildlife (including avifauna)				√	√
Access to pasture	√	√	√		
Access to surface water	√	√	√		
Feeding fresh grass			√		
On-farm practices and conditions contributing to transmission of pathogens					
Animal density	√	√		√	
Ventilation	√	√		√	
Bedding	√	√			
Slurry	√	√			
Storage conditions of feed	√				
Age mixing	√	√			
Waste management	√	√			

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

Salmonella

I. Identification of potential epidemiological indicators

Table 9: Potential epidemiological indicators for *Salmonella* in bovine animals

	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Farm (including contribution from wildlife)			
Risk Factor 1 Practices which increase the risk of introducing *Salmonella* into the farm	Data on purchase practices readily available through national identification and registration system. Data on risky farming practices readily available.	Data on purchase practices readily available. Data on risky farming practices available from audits of farms.	Purchase policy Contact with other animals / herds Access to pasture Access to surface water by auditing
Risk Factor 2 Buy-in from *Salmonella*-positive supply farms	Some data on prevalence of *Salmonella* status of supply farms.	It is possible to gather data on prevalence of *Salmonella* status of supply farms.	*Salmonella* status of supply farms
Risk Factor 3 On-farm practices and conditions contributing to transmission of *Salmonella* (i.e. animal density, bedding, slurry, storage conditions of feed, age mixing, waste management, including biosecurity measures)	Data available from research Some data on *Salmonella* in bovines are available.	Data available from audits of farms It is possible to obtain such data.	Auditing of farm practices and conditions *Salmonella* status of farms Microbiology
Risk Factor 4 Shedding of *Salmonella* by bovines to be slaughtered within one month			*Salmonella* status of the group(s) of bovine animals, containing animals to be slaughtered within one month Microbiology
Risk factor 5 Feed (possibly *Salmonella*-positive)	Some data available from the industry and literature on commercial feed Home-produced feed – few data	It is possible to obtain such data. There is no systematic monitoring at present in most MSs.	*Salmonella* prevalence in feed or occurrence in feed mill

Table continued overleaf.

Table 9 (continued): Potential epidemiological indicators for *Salmonella* in bovine animals

	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Transport to slaughterhouse			
Risk factor 1 Loading and transport - cross-contamination, cleanliness of transport vehicle	Data available from research and studies on impact of transport on *Salmonella* prevalence	It is possible to obtain such data. Data are not readily available.	*Salmonella* contamination of transport vehicles Microbiology Audit of transport conditions: - animal density, mixing of animals from different origins - sanitary conditions of vehicle - measurement of duration of transport
Slaughterhouse			
Lairage Risk Factor 1 Cross-contamination (mixing of animals from different origins), cleanliness of lairage	Data available from research and studies on impact of lairage on *Salmonella* prevalence in bovine animals	It is possible to obtain such data	*Salmonella* contamination of lairage Microbiology Audit of lairage conditions: - mixing of animals from different origins - sanitary conditions of lairage (cleanliness) - measurement of duration of lairage - re-use of pens without cleaning between
Risk Factor 2 Cleanliness of hide of animals	Data available	Data available	Visual inspection of hide conditions of animals (clean cattle policy)

Table continued overleaf.

Table 9 (continued): Potential epidemiological indicators for *Salmonella* in bovine animals

	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Slaughterline			
Risk factor 3 Hide contamination after bleeding and before dehiding	Data available from literature	It is possible to obtain such data	*Salmonella* on incoming animals (after bleeding and before dehiding) Microbiology
Risk factor 4 Carcase dressing techniques and cross-contamination of carcases	Data available from literature Pre-chilling data available from slaughterhouses (Regulation 2073/2005: process hygiene criteria)	It is possible to obtain such data	*Salmonella* on the carcase: - after dehiding and pre-chilling - post-chilling Microbiology
Processing of meat and products thereof			
Risk factor 1 Cross-contamination during processing	Data available from literature and from national surveillance/monitoring	It is possible to obtain such data	Detection of *Salmonella* on fresh meat and on other meat products
Retail			
Risk factor 1 Temperature abuses	Data should be available from HACCP programmes	A temperature above 12 °C is considered high risk for *Salmonella* growth	Detection of *Salmonella* on fresh meat Temperature of the chilling rooms
Risk factor 2 Cross-contaminations at retail	Some prevalence data available from literature and national surveillance/monitoring	It is possible to obtain such data	
Consumer			
Risk factor 1 Handling in the kitchen and cross-contamination	Limited data available	Difficult to obtain	
Risk factor 2 Undercooking of bovine meat	Limited data available	Difficult to obtain	
Risk factor 3 Temperature abuses	Limited data available A study exists in France indicating the percentage of domestic refrigerators having temperature above 8 °C and above 12 °C	Difficult to obtain	

II. Evaluation of suggested indicators

Table 10: Evaluation of suggested indicators for *Salmonella* in bovine animals

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**
Practices which increase the risk of introducing *Salmonella* into the farm (purchase policy, mixing with other herds, access to pasture, access to surface water)	Farm	Auditing	Not applicable	2	1	1	2	1.45
Salmonella status of supply farms	Farm	Microbiology	Pooled faeces	1	1	1	1	1
On-farm practices and conditions	Farm	Auditing	Not applicable	2	1	1	2	1.45
Salmonella status of the farm	Farm	Microbiology	Pooled faeces	1	1	1	1	1
Salmonella status of the group(s) of bovine animals containing animals to be slaughtered within one month	Farm	Microbiology	Pooled faeces	2	2	1	1	1.7
Salmonella presence in feed or occurrence in feed mill	Farm /feed mill	Microbiology	Feed	2	1	0	1	1.15
Salmonella contamination of transport vehicles and lairage	Transport and lairage	Microbiology	Environmental swabs	1	1	0	1	0.85
Transport and lairage conditions	Transport and lairage	Auditing	Not applicable	2	1	2	2	1.6
Visual inspection of hide conditions of animals at lairage (clean animal scoring system)	Slaughterhouse	Visual inspection	Not applicable	2	1	2	2	1.6
Salmonella on incoming animals (after bleeding and before dehiding)	Slaughterhouse	Microbiology	Hide swabs	2	2	2	1	1.85

Table continued overleaf.

Table 10 (continued): Evaluation of suggested indicators for *Salmonella* in bovine animals

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**
Salmonella in incoming animals (evisceration stage)	Slaughterhouse	Microbiology	Lymph nodes	2	1	1	1	1.3
Salmonella on carcases pre-chilling	Slaughterhouse	Microbiology	Carcase swabs	2	2	2	1	1.85
Salmonella on carcases post-chilling	Slaughterhouse	Microbiology	Carcase swabs	2	2	2	1	1.85

(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 and how much would the sampling/testing cost or are the data already available (no additional sampling/testing needed)?
(e): 0 = bad, 1 = moderate, 2 = good

Pathogenic verocytotoxin-producing *Escherichia coli*

I. Identification of potential epidemiological indicators

Table 11: Risk factors and potential epidemiological indicators for pathogenic VTEC in bovine animals

	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Farm (including contribution from wildlife)			
Risk Factor 1 Practices which increase the risk of introducing pathogenic VTEC into the farm	Data available through national identification and registration system	Data available	Purchase policy Contact with other animals / herds Access to pasture Access to surface water by auditing
Risk Factor 2 Buy-in from pathogenic VTEC positive supply farms	Some data on prevalence of pathogenic VTEC status of supply farms.	It is possible to gather data on prevalence of pathogenic VTEC status of supply farms.	Pathogenic VTEC status of supply farms
Risk Factor 3 On farm practices and conditions contributing to transmission of pathogenic VTEC (i.e. animal density, bedding, slurry, storage conditions of feed, age mixing, waste management including biosecurity measures)	Data available from research Data on pathogenic VTEC in bovines to be slaughtered as well as on carriers can be obtained.	Data available from audits of farms It is possible to obtain such data. There is no monitoring at present	Auditing of farm practices and conditions Pathogenic VTEC status of the farm Microbiology
Risk Factor 4 Shedding of pathogenic VTEC by bovines to be slaughtered within one month			Pathogenic VTEC status of the group(s) of bovine animals, containing animals to be slaughtered within one month Microbiology

Table continued overleaf.

Table 11 (continued): Risk factors and potential epidemiological indicators for pathogenic VTEC in bovine animals

	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Transport to slaughterhouse			
Risk factor 1 Loading and transport - cross-contamination, cleanliness of transport vehicle	Data available from research and studies on impact of transport on pathogenic VTEC prevalence	It is possible to obtain such data	Pathogenic VTEC contamination of transport vehicles Microbiology Audit of transport conditions: - animal density, mixing of animals from different origins - sanitary conditions of vehicle - measurement of duration of transport
Slaughterhouse			
Lairage			
Risk Factor 1 Cross-contamination (mixing of animals from different origins), cleanliness of lairage	Data available from research and studies on impact of lairage on pathogenic VTEC prevalence in bovine animals	It is possible to obtain such data	Pathogenic VTEC contamination at lairage Microbiology Audit of lairage conditions: - mixing of animals from different origins - sanitary conditions of lairage (cleanliness) - measurement of duration of lairage - re-use of pens without cleaning between
Risk Factor 2 Cleanliness of hide of animals	Data available	Data available	Visual inspection of hide conditions of animals (clean animal scoring policy)

Table continued overleaf.

Table 11 (continued): Risk factors and potential epidemiological indicators for pathogenic VTEC in bovine animals

	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Slaughterline			
Risk factor 3 Hide contamination after bleeding and before dehiding	Data available from literature	It is possible to obtain such data from the slaughterhouse	Pathogenic VTEC on incoming animals (after bleeding and before dehiding) Microbiology
Risk factor 4 Carcase dressing techniques and cross-contamination of carcases	Data available from literature	It is possible to obtain such data	Pathogenic VTEC on the carcase: - after dehiding and pre-chilling - post-chilling Microbiology
Processing of meat and products thereof			
Risk factor 1 Cross-contamination during processing	Data available from literature and from national surveillance/monitoring	It is possible to obtain such data	Detection of pathogenic VTEC on fresh meat products and other meat products
Retail			
Risk factor 1 Temperature abuses	Data should be available from HACCP programmes	A temperature above 12 °C is considered high risk for VTEC growth	Detection of pathogenic VTEC on fresh meat products Temperature of the chilling rooms
Risk factor 2 Cross-contaminations at retail	Some prevalence data available from literature and national surveillance/monitoring	It is possible to obtain such data	
Consumer			
Risk factor 1 Handling in the kitchen and cross-contamination	Limited data available	Difficult to obtain	
Risk factor 2 Undercooking of bovine meat	Limited data available	Difficult to obtain	
Risk factor 3 Temperature abuses	Limited data available A study exists in France indicating the percentage of domestic refrigerators having temperature above 8 °C and above 12 °C	Difficult to obtain	

II. Evaluation of suggested indicators

Table 12: Evaluation of suggested indicators for pathogenic VTEC in bovine animals

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**
Practices which increase the risk of introducing pathogenic VTEC into the farm (purchase policy, mixing with other herds, access to pasture, access to surface water)	Farm	Auditing	Not applicable	2	1	1	2	1.45
Pathogenic VTEC status of supply farms	Farm	Microbiology	Pooled faeces	1	1	1	1	1
On-farm practices and conditions	Farm	Auditing	Not applicable	2	1	1	2	1.45
Pathogenic VTEC status of the farm	Farm	Microbiology	Pooled faeces	1	1	1	1	1
Pathogenic VTEC status of the group(s) of bovine animals containing animals to be slaughtered within one month	Farm	Microbiology	Pooled faeces or floor samples	2	2	1	1	1.7
Pathogenic VTEC contamination of transport vehicles and lairage	Transport and lairage	Microbiology	Environmental swabs	1	1	0	1	0.85
Transport and lairage conditions	Transport and lairage	Auditing	Not applicable	2	1	2	2	1.6

Table continued overleaf.

Table 12 (continued): Evaluation of suggested indicators for pathogenic VTEC in bovine animals

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**
Visual inspection of hide conditions of animals at lairage (clean animal scoring system)	Slaughterhouse	Visual inspection	Not applicable	2	1	2	2	1.6
Pathogenic VTEC on incoming animals (after bleeding and before dehiding)	Slaughterhouse	Microbiology	Hide swabs	2	2	2	1	1.85
Pathogenic VTEC on carcases pre-chilling	Slaughterhouse	Microbiology	Carcase swabs	2	2	2	1	1.85
Pathogenic VTEC on carcases post-chilling	Slaughterhouse	Microbiology	Carcase swabs	2	2	2	1	1.85

(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 and how much would the sampling/testing cost or are the data already available (no additional sampling/testing needed)?
(e): 0 = bad, 1 = moderate, 2 = good

***Cysticercus* (*Taenia saginata*)**

I. Identification of potential epidemiological indicators

Table 13: Risk factors and potential epidemiological indicators for *Cysticercus* (*Taenia saginata*) in bovine animals

	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Farm (including contribution from wildlife)			
Risk factor 1 Farming practices, such as - access to pastures, feeding fresh grass, age (*grazing of bovines in areas where human activities take place, e.g. open defecation, fertilisation of pastures with waste from septic tanks or slurry from sewage plants or effluents from sewage treatment plants, contaminated water sources, grazing areas contaminated after flooding*).	Data available	It is possible to obtain such data	Prevalence of *Cysticercus*-positive slaughter animals based on serology Audit of farming practices
Risk factor 2 Presence of human carriers on the farm	Data available	No data available	Audit (eating habits) Prevalence of *Cysticercus*-positive slaughter animals
Transport to slaughterhouse			
Risk factor 1	-	-	-

Table continued overleaf.

Table 13 (Continued): Risk factors and potential epidemiological indicators for *Cysticercus* (*Taenia saginata*) in bovine animals

	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Slaughterhouse			
Risk factor 1 Inspection procedure			Audit of inspection procedure (visual inspection of carcase surfaces) Suspected lesions of *T. saginata* cysticerci from all types of farms
Processing of meat and products thereof			
Risk factor 1	-	-	-
Retail			
Risk factor 1			
Consumer			
Risk factor 1 Eating raw or undercooked meat	-		Consumer behaviour

II. Evaluation of suggested indicators

Table 14: Evaluation of suggested indicators for *Cysticercus* (*Taenia saginata*) in bovine animals

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**
Audit of farming practices	Farm	Auditing	Not applicable	2	1	2	2	1.6
Prevalence of *T. saginata* cysticerci-positive slaughter animals (excluding white veal calves)	Slaughterhouse	Serology (antigen based test)	Blood	1	2	1	2	1.55
Prevalence of *T. saginata* cysticerci-positive slaughter animals (excluding white veal calves)	Slaughterhouse	Serology (antibody based test)	Blood	1	1	1	2	1.15
T. saginata cysticerci in suspected lesions from all types of farms (excluding white veal calves)	Slaughterhouse	PCR	Suspect lesions	2	2	1	2	1.85
Audit of inspection procedure	Slaughterhouse	Auditing	Not applicable	1	1	1	2	1.15

(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 and how much would the sampling/testing cost or are the data already available (no additional sampling/testing needed)?
(e): 0 = bad, 1 = moderate, 2 = good

Mycobacteria

I. Identification of potential epidemiological indicators

Table 15: Risk factors and potential epidemiological indicators for mycobacteria in bovine animals

	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 Practices which increase the risk of introducing mycobacteria into the farm	Data on purchase practices readily available through national identification and registration system. Data on risky farming practices readily available. Few literature data available, few technical reports (e.g. France)	Data on purchase practices readily available. Data on risky farming practices available from audits of farms. It is possible to gather data	Purchase policy by auditing Contact other animals / herds Contact with wildlife including avifauna Prevalence of serological responses (in serum or meat juice) to *Mycobacterium* spp. in slaughter animals
Risk Factor 2 On-farm practices and conditions contributing to transmission of *M. bovis* (i.e. animal density, ventilation)	Data partially available	Data available from audits of farms It is possible to obtain such data. There is no monitoring at present	Official status of bovine herd as regards bovine tuberculosis *M. bovis* (OTF status) On-farm practices and conditions Auditing of on-farm structures and procedures for biosecurity
Risk Factor 3 Feed (possibly mycobacteria-positive)	Limited data available	It is possible to obtain such data. There is no monitoring at present.	Presence of mycobacteria in feed or occurrence in feed mill
Transport to slaughterhouse			
Risk factor 1			

Table continued overleaf.

Table 15 (continued): Risk factors and potential epidemiological indicators for mycobacteria in bovine animals

	Availability of prevalence data	Data availability to divide population into groups between which the risk varies	Suggested epidemiological indicator (HEI)
Slaughterhouse			
Risk factor 1 Detection of infected bovine animals in the slaughterhouse through visual inspection	Data available from current *post mortem* inspection data		Human pathogenic mycobacteria in bovine animals at slaughter (identification of tuberculosis-like lesions through visual inspection and microbiology of suspect lesions)
Risk factor 2 Removal of infected organs/ carcases after the detection through visual inspection and their disposal as the appropriate animal by-product category	Data available from current *post mortem* inspection data		Identification of tuberculosis-like lesions in bovines at slaughter through visual *post mortem* inspection
Processing of meat and products thereof			
Risk factor 1			
Retail			
Risk factor 1			
Consumer			
Risk factor 1			

II. Evaluation of suggested indicators

Table 16: Evaluation of suggested indicators for mycobacteria in bovine animals

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**
Practices which increase the risk of introducing mycobacteria into the farm	Farm	Auditing	Not applicable	1	0	1	2	0.75
On-farm practices and conditions Auditing of on-farm structures and procedures for biosecurity	Farm	FCI and auditing (e.g. animal density)	Not applicable	2	0	1	2	1.05
Prevalence of serological responses (in serum or meat juice) to *Mycobacterium* spp. in slaughter animals	Farm/ slaughterhouse	Serology	Blood/ meat juice	1	0	1	1	0.6
Presence of mycobacteria in feed or occurrence in feed mill	Farm	Microbiology	Feed	1	0	1	1	0.6
Official status of bovine herd as regards bovine tuberculosis (OTF status)	Farm	Information on food chain	Food chain information	2	0	2	2	1.2
Human pathogenic mycobacteria in bovines at slaughter (identification of tuberculosis like lesions through visual *post mortem* inspection and microbiology of suspect lesions)	Slaughterhouse	Visual meat inspection and microbiology[f]	Suspected lesions	1	1	1	2	1.15

(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 and how much would the sampling/testing cost or are the data already available (no additional sampling/testing needed)?
(e): 0 = bad, 1 = moderate, 2 = good
(f): Detection of the human pathogenic mycobacteria from lesions detected through visual inspection

ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
BIOHAZ	Biological Hazards
BSE	bovine spongiform encephalopathy
CFU	Colony-forming unit
CVO	Chief Veterinary Officer
DNA	Deoxyribonucleic acid
EAEC	enteroaggregative *E. coli*
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ESBL	extended-spectrum β-lactamase
EU	European Union
EUSR	European Union Summary Report
FCI	Food chain information
GHP	Good Hygiene Practices
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Points
HEI	Harmonised Epidemiological Indicator
HUS	Haemolytic-Uraemic Syndrome
ISO	International Organization for Standardization
MS(s)	Member State(s)
MAA	*Mycobacterium avium* subsp. *avium*
MAC	*Mycobacterium avium* complex
MAH	*Mycobacterium avium* subsp. *hominisuis*
MAP	*Mycobacterium avium* subsp. *paratuberculosis*
MIRU-VNTR	Mycobacterial interspersed repetitive unit-variable number tandem repeat
MTC	*Mycobacterium tuberculosis* complex
NTM	Non-tuberculous mycobacteria
OIE	World Organisation for Animal Health
OTF	Officially Tuberculosis Free
PCR	Polymerase Chain Reaction
RAJ	Recto-anal junction
RFLP	Restriction Fragment Length Polymorphism
TESSy	The European Surveillance System

ABBREVIATIONS

ToR	Term of Reference
TVC	Total viable count
VT	verocytotoxin
VTEC	verocytotoxin-producing *Escherichia coli*