

SCIENTIFIC REPORT OF EFSA

Technical specifications on harmonised epidemiological indicators for biological hazards to be covered by meat inspection of poultry¹

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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 poultry and meat thereof and that can be addressed within meat inspection. These hazards include *Salmonella*, *Campylobacter* and extended-spectrum/AmpC beta-lactamase producing *Escherichia coli* as well as generic *E. coli* as an indicator for process hygiene. An epidemiological indicator is defined as the prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard that correlates to human health risk caused by the hazard. The indicators can be used by the European Commission and the Member States to consider when adaptations in meat inspection methods may be relevant and to carry out risk analysis to support such decisions. It is foreseen that the indicators will be used in the integrated food safety assurance system for poultry meat outlined in the EFSA Scientific Opinion, particularly to help categorise farms/flock and slaughterhouses according to the risk related to the hazards and process hygiene as well as setting appropriate targets. 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/flock level. It is recommended that risk managers should define the harmonised requirements for the controlled housing 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 light of new information and the data generated by their implementation.

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KEY WORDS

Meat inspection, biological hazard, epidemiological indicators, poultry

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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, HEIs) for specific public health hazards in food and animals to be used by risk managers when they consider that the current methods of meat inspection do not adequately address the relevant risks. It is related to the mandate from the Commission for a Scientific Opinion on the public health hazards to be covered by inspection of meat. The second Opinion and this report under this mandate concern the meat inspection of poultry and they were published in June 2012.

In this report, harmonised epidemiological indicators are proposed for food-borne biological hazards to public health that are related to poultry and meat thereof and that can be addressed within meat inspection. These hazards include *Salmonella*, *Campylobacter* and *Escherichia coli* producing extended-spectrum and/or AmpC beta-lactamase (ESBL/AmpC). In addition, an HEI is proposed for generic *E. coli* as an indicator for process hygiene. An epidemiological indicator is understood to mean the prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard (such as audits or evaluation of process hygiene) that correlates to a human health risk caused by the hazard. The epidemiological indicators can be used by the European Commission and the Member States to consider when adaptations to meat inspection methods may be relevant, and to enable the Member States to carry out risk analysis to support any such decisions. It is foreseen that the epidemiological indicators will be used in the integrated food safety assurance system for poultry meat outlined in the Scientific Opinion on the public health hazards to be covered by inspection of meat from poultry, particularly to help to categorise farms/flocks and slaughterhouses according to the risks related to particular hazards or level of process hygiene. The indicators may also be used in setting appropriate targets foreseen by the Opinion.

The risk managers should decide on the most appropriate use of the epidemiological indicators at the European Union and national levels. Depending on the purpose and the epidemiological situation of the country, the indicators may be applied at national, regional, slaughterhouse or farm/flock level. The indicators can be used alone or in combination. They may be applied to classify the countries, regions, farms or slaughterhouses according to the infection or colonisation status related to the hazards. Some indicators may also be used to evaluate the measures taken in the slaughterhouses to control a specific hazard or to assess process hygiene. The accumulated historical data from implementation of the epidemiological indicators will in particular be useful for categorisation of farms and slaughterhouses and may be applied to justify reduction in the sampling frequencies for the indicators.

Most of the epidemiological indicators are proposed for poultry populations or poultry carcasses at the farm or slaughterhouse level. Some indicators include auditing of the farms for controlled housing conditions or the provision of food chain information with respect to the use of partial depopulation (thinning) of the flocks or the use of antimicrobials during rearing.

Comparable data from the European Union Member States were available for only some of the proposed epidemiological indicators. This was the case with some of the indicators relating to *Salmonella* and *Campylobacter*.

For each epidemiological indicator addressed, the key elements of minimum monitoring or inspection requirements are defined. This includes the animal population to be targeted, the stage of the food chain at which the sampling should take place, sampling strategy, type and details of the specimen to be taken, diagnostic or analytical method to be used, and a case definition. A general description is provided on how to choose the sampling strategy for the different types of indicators.

In the case of ESBL-/AmpC-producing *E. coli* it is accepted that there is a need for more research to clarify the factors that place poultry at risk of colonisation as well as most appropriate analyses methods. In addition, more information would be welcome regarding the use of quantitative data on

bacterial counts to assess slaughterhouses and regarding the sensitivity of boot swabs for sampling of *Campylobacter* at poultry farms. The Member States are invited to support research and studies on these subjects.

It is recommended that the European Commission and the Member States define the harmonised requirements for controlled housing 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 and these data may be used to update the indicators, when appropriate. It is recommended that the Member States report the data generated from implementation of these indicators in accordance with and using the framework prescribed in Directive 2003/99/EC. The proposed indicators should be reviewed regularly in 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, when appropriate.

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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/EC⁴ 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, 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 854/2004⁵ 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, 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 882/2004⁶, Regulation (EC) No 2160/2003⁷, Regulation (EC) No 852/2004⁸, Regulation (EC) No 853/2004⁹, 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, 30.4.0224, 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 on the hygiene of foodstuffs. OJ L 139, 30.4.0224, p. 55–205.

TECHNICAL SPECIFICATIONS

1. Introduction

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

A recent scientific Opinion from the EFSA's Panel on Biological Hazards (BIOHAZ) (EFSA, 2012a) estimates that 10.6 % and 2.6 % of salmonellosis cases in humans at European Union (EU) level are attributable to broilers and turkeys, respectively. An earlier BIOHAZ Opinion on *Campylobacter* (EFSA, 2010a) concluded that poultry is a major source, if not the largest single source, of human *Campylobacter* infection. In the same Opinion, it is assessed that handling, preparation and consumption of broiler meat may account for 20 % to 30 % of human cases of campylobacteriosis in EU, while 50 % to 80 % may be attributed to the chicken reservoir as a whole.

According to the European Union (EU) Summary Report on zoonoses and food-borne outbreaks in 2010 (EFSA and ECDC, 2012a), 6 % and 0.1 % of the reported food-borne outbreaks with information on food vehicle were caused by broiler meat and turkey meat, respectively. Of the food-borne outbreaks caused by broiler meat, 42.9 % were caused by *Salmonella*, 40.5 % by *Campylobacter*, 4.8 % by staphylococcal enterotoxins, 2.4 % by *Clostridium* spp. and 2.4 % by norovirus, while the only food-borne outbreak reported to be due to the consumption of turkey meat was caused by *Salmonella*. The relevant hazards related to poultry meat vary among the Member States in accordance with the epidemiological situation and food consumption habits.

Meat inspection generally offers an opportunity to control some food-borne hazards; however, most of the biological hazards related to poultry are not specifically addressed by the current meat inspection system in place in the EU.

It is possible to use the data on prevalence and incidence of the biological hazards in animals, meat and humans as one aspect of the criteria when determining and ranking the human health importance of the hazards to be covered by meat inspection. These epidemiological criteria or indicators may be used by the risk managers when considering adaptations of current meat inspection methods for poultry. In the case of poultry, relevant prevalence data that may be used when designing the epidemiological indicators have been collected from the EU Member States within the framework of the annual reporting on the monitoring of zoonoses in accordance with Directive 2003/99/EC. Also, the EU-wide baseline surveys on *Salmonella* in laying hens, flocks of broilers and turkeys as well on broiler carcasses provide for fully harmonised datasets from the Member States (EFSA, 2007a, d, 2008a, 2010b). 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/EC¹⁰ on setting up a network for the epidemiological surveillance and control of communicable diseases in the EU.

The Scientific Opinion of the EFSA (later referred to as the EFSA Scientific Opinion) on the public health hazards to be covered by inspection of meat from poultry (EFSA, 2012b) outlines an integrated food safety assurance system for poultry meat. It is foreseen that the harmonised epidemiological indicators will be used as part of this system. Therefore, this report should be read in parallel with that Opinion.

As the EU Regulations do not include different inspection requirements for the different poultry species, and because only limited or no data are available for "minor" poultry species, all poultry species are considered together in this report. The general description of risk factors, available data

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, 03.10.1998, p. 1–7.

and epidemiological indicators focuses on the main species (broilers/hens and turkeys), but any important differences concerning other species were considered when necessary.

This report applies only to rearing and production of poultry that is sent to slaughterhouses as defined in Regulation (EC) No 853/2004. It does not apply to the direct supply, by the producer, of small quantities of poultry to the final consumer or to local retail establishments directly supplying the final consumer.

2. Definitions

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

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

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

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

Controlled housing conditions - a type of animal husbandry in which poultry are kept at all times and for their whole life under conditions controlled by the food business operator with regard to feeding, housing and biosecurity of the holding (examples of proposed requirements for controlled housing conditions can be found in Appendix 1). The controlled housing condition requirements are in some cases not applicable to free-range production of poultry.

Flock - all poultry of the same health status kept on the same premises or in the same enclosure and constituting a single epidemiological unit; in the case of housed poultry, this includes all birds sharing the same airspace (Regulation (EC) No 2160/2003).

Free range poultry - poultry which has been allowed access to the outside.

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

Partial depopulation (thinning) - removal of a portion of the flock, during the production cycle, before the house is finally depopulated.

Poultry - fowl, turkeys, guinea fowl, ducks, geese, quails, pigeons, pheasants and partridges reared or kept in captivity for breeding, the production of meat or eggs for consumption, or for restocking supplies of game (Directive 90/539/EEC)¹¹.

Poultry meat - edible parts of the animal species mentioned above, including blood (Regulation (EC) No 853/2004).

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

Slaughterhouse - 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

¹¹ Council Directive 90/539/EEC of 15 October 1990 on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs. OJ L 303, 31.10.1990, p. 6–28.

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.

Slaughter batch - a group (or batch) of birds which have been raised in the same flock and which are delivered and slaughtered on one single day.

3. Approach applied to select the epidemiological indicators

3.1. Harmonised epidemiological indicators

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

The purpose of the harmonised epidemiological indicators proposed in this report is to enable the European Commission and the Member States to consider whether adaptations to meat inspection methods may be made at the Member State level and to enable the Member States to carry out a risk analysis (or components thereof) to support decisions on any such adaptations of meat inspection methods. The hazards addressed in this report were those identified in the complementary EFSA Scientific Opinion (EFSA, 2012b) as the most relevant in the context of meat inspection of poultry. The epidemiological indicators provide information to be used in the integrated food safety assurance system outlined in the EFSA Scientific Opinion. This applies particularly in the process of classification of the farms/flocks and slaughterhouses according to risk related to a particular hazard as well as the setting of related targets. The indicators, either alone or in combination, may be used by risk managers at the national, regional, slaughterhouse or farm/flock level depending on the purpose.

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

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

In this report the following approach is applied to select the harmonised epidemiological indicators (the first Terms of Reference (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 poultry as the source of human infections is discussed for each hazard.
- For each hazard, the main poultry food chain and the risk and risk-reducing factors along the chain, as well as the meat inspection and other risk mitigation strategies, are presented. This description includes an identification of possible epidemiological indicators.

- The possible epidemiological indicators are evaluated against selected criteria (i.e. their quality, appropriateness, data availability and feasibility) using a scoring system. The epidemiological indicators that received the highest scores are selected.

Following the selection of the harmonised epidemiological indicators, the available data from the annual reporting in accordance with the Directive 2003/99/EC, as well as from the EU-wide baseline surveys, were reviewed for comparable data from the Member States. This comparable data are presented in Chapter 8 (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 at which the sampling should take place, type and details of the specimen to be taken, diagnostic or analytical method to be used and a case definition. A general description is provided on how to choose the sampling strategy for each case.

In addition, a generic epidemiological indicator is proposed to assess the process hygiene during the slaughtering of poultry. Two case studies on the use of the proposed epidemiological indicators are presented in a scientific report submitted to EFSA (Cameron, 2012).

3.2. The biological hazards addressed

The first ToR of the mandate for technical assistance from the Commission asks for the harmonised epidemiological indicators to be defined for specific hazards already covered by current meat inspection (such as trichinellosis, tuberculosis, cysticercosis, etc.). In the case of meat inspection of poultry, there were no such hazards.

In addition, according to the first ToR, the epidemiological indicators for possible additional hazards identified in a Scientific Opinion on the hazards to be covered by inspection of meat from poultry (EFSA, 2012b), which can be used to consider adaptations of meat inspection methodology, should be addressed as well. The EFSA Scientific Opinion identifies *Salmonella*, *Campylobacter* and extended-spectrum and/or AmpC beta-lactamase (ESBL/AmpC)-producing *Escherichia coli* as such hazards. Furthermore, an epidemiological indicator is suggested for process hygiene during the slaughter in line with the above-mentioned EFSA Scientific Opinion.

4. Epidemiological indicators for the biological hazards

4.1. *Salmonella*

4.1.1. Introduction

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 subspecies, and most *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.

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

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

In animals, subclinical infections are common. The organism may easily spread between animals in a herd or flock without detection and animals may become intermittent or persistent carriers. Fever and diarrhoea are less common in pigs than in cattle, sheep and horses; goats and poultry usually show no signs of infection (EFSA and ECDC, 2012a).

4.1.2. Current situation and trends in the EU

In 2010, salmonellosis was the second most commonly reported zoonotic disease in humans in the EU (EFSA and ECDC, 2012a). A total of 99 020 confirmed cases of human salmonellosis were reported, and the number of cases decreased by 8.8 % compared with 2009, continuing the statistically significant decreasing trend in the EU for the sixth consecutive year. The continuous decrease in the numbers of salmonellosis cases in humans is likely to be mainly related to the successful *Salmonella* control programmes in poultry populations, particularly in laying hens. In foodstuffs, *Salmonella* was most often detected in fresh broiler and turkey meat, on average at levels of 4.8 % and 9.0 %, respectively (EFSA and ECDC, 2012a).

In 2010, a total of 0.7 % of breeding flocks of *Gallus gallus* in the EU were positive for the five *Salmonella* target serovars (*S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, *S. Virchow* and *S. Hadar*) during the production period, and altogether 2.0 % of breeding flocks in EU were positive for *Salmonella* spp. Among laying hen and broiler flocks, 5.9 % and 4.1 %, respectively, were positive for *Salmonella* spp., while the EU prevalence of the two target serovars (*S. Enteritidis* and *S. Typhimurium*) in laying hen and broiler flocks was 1.9 % and 0.4 %, respectively. In 2010, 0.3 % of

adult turkey breeding flocks were positive for the two target serovars (*S. Enteritidis* and *S. Typhimurium*), while overall 6.9 % of turkey breeding flocks were found positive for *Salmonella* spp. Among turkey fattening flocks before slaughter, the EU prevalence of the two target serovars (*S. Enteritidis* and *S. Typhimurium*) was 0.5 %, while overall 12.1 % of fattening turkey flocks were positive for *Salmonella* spp. *Salmonella* findings were also reported from other poultry species, pigs and cattle (EFSA and ECDC, 2012a).

4.1.3. Poultry meat as a source of infection for humans

The BIOHAZ Panel has carried out or reviewed several source attribution studies on *Salmonella* in its opinions. The most recent Opinion is from 2012 (EFSA, 2012a), in which the panel concludes that, based on the results of the source attribution model developed by Hald et al. (2012), in total 2.6 % of all human salmonellosis cases in the EU in 2010 were attributable to turkeys and 10.6 % to broilers. For the other *Salmonella* food animal reservoirs, it was estimated that 17.0 %, and 56.8 % of the estimated numbers of human salmonellosis cases could be attributed to laying hens (eggs) and pigs, respectively. It should be noted that compared with earlier EU-level source attribution studies (Pires et al., 2011; Vose et al., 2011), the model used in the Opinion attributed a relatively higher proportion of human salmonellosis cases to the pig reservoir and smaller one to laying hen reservoir.

4.1.4. Risk and protective factors

Risk factors related to *Salmonella* infection of broilers have been summarised in the Scientific Opinion of the BIOHAZ Panel on a quantitative estimation of the public health impact of setting a new target for the reduction of *Salmonella* in broilers (EFSA, 2011b). The risk factors related to *Salmonella* infection of broilers include the use of infected breeding flocks (Skov et al., 1999; Maijala et al., 2005) as well as the specific hatchery from which the animals originate (Angen et al., 1996; Chriel et al., 1999). Pseudovertical transmission is associated with contamination of the hatchery environment or equipment in which eggs are processed and hatched or chicks held and handled. It is likely that the primary source of hatchery contamination is contaminated eggs, trays or trolleys originating from the breeding flocks (EFSA, 2011b). Other on-farm risk factors include the presence of infection in previous flocks (Angen et al., 1996; Rose et al., 2000), poor biosecurity and deficiencies in cleaning and disinfection (Henken et al., 1992; Rose et al., 1999, 2000; Gradel and Rattenborg, 2003; Elgroud et al., 2009), quality of staff (Namata et al., 2009), presence of rodents at the farm (Rose et al., 2000) and use of medication of the birds during the rearing period (Chriel et al., 1999; EFSA, 2007b). Free-range production often appears at reduced risk, but this may be partly associated with the higher age of birds at sampling (EFSA, 2007b; Snow et al., 2008). There is also evidence of a seasonal effect on the prevalence of *Salmonella* infections of flocks (Angen et al., 1996; EFSA, 2007b; Van der Fels-Klerx et al., 2008). The use of *Salmonella*-contaminated feed is another risk factor for *Salmonella* infection of a flock (Williams, 1981; Jones et al., 1991; Henken et al., 1992; Angen et al., 1996).

Transportation of birds to the slaughterhouse causes stress, resulting in increased *Salmonella* excretion rates and exterior carriers (Rigby and Pettit, 1980; Mulder, 1995). Washing and disinfection of transport crates is not always adequate to remove *Salmonella* contamination (Rigby et al., 1980; Corry et al., 2002; Olsen et al., 2003).

Incoming birds infected with *Salmonella* are an important risk factor for *Salmonella* contamination of poultry carcasses in the slaughterhouse (Rasschaert et al., 2008). The slaughter of *Salmonella*-positive poultry flocks/batches may result in the contamination not only of carcasses but also of the slaughter line (Corry et al., 2002; Olsen et al., 2003). *Salmonella* can spread through faecal contamination and the slaughterhouse equipment can remain contaminated with *Salmonella* even after cleaning and disinfection (Heyndrickx et al., 2002; Rasschaert et al., 2007, 2008). Slaughterhouse technology can influence greatly direct and indirect cross-contamination between slaughtered birds, and variability in slaughterhouse equipment contributes to differences in the final microbial load of the carcass (EFSA, 2012b). Chilling usually reduces *Salmonella* contamination (James et al., 2006; Huezo et al., 2007); however, it may also lead to cross-contamination between carcasses. The capacity of the slaughterhouse and type of chilling used for the carcasses have been found to be significantly

associated with the risk of *Salmonella*-contaminated broiler carcasses (EFSA, 2011c). The Opinion from EFSA on the public health hazards to be covered by inspection of meat from poultry (EFSA, 2012b) concluded that each slaughterhouse can be viewed as unique, owing to differences in poultry species slaughtered logistics, processing practices, plant layout, equipment design and performance, standardised and documented procedures, personnel motivation and management, and other factors. These variations individually and in combination lead to between-slaughterhouse differences in risk reduction capacities and, consequently, in the microbiological status of the final carcass.

Cross-contamination with *Salmonella* from other poultry carcasses can also take place during cutting and processing (Carraminana et al., 1997; Uyttendaele et al., 1999). Products of poultry with skin have been found to have higher *Salmonella* prevalence than products without skin (Uyttendaele et al., 1999; Anonymous, 2010).

4.1.5. Proposed harmonised epidemiological indicators (HEIs)

The epidemiological indicators selected for *Salmonella* in poultry are shown in Table 1 and Figure 1.

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

Indicators (animal/food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
HEI 1 <i>Salmonella</i> in breeding parent flocks ^(a)	Farm	Microbiology (detection and serotyping)	Pooled faeces (e.g. boot swabs) possibly combined with dust samples
HEI 2 <i>Salmonella</i> in poultry flocks prior to slaughter ^(a)	Farm	Microbiology (detection and serotyping)	Pooled faeces (e.g. boot swabs)
HEI 3 Controlled housing conditions at farm for laying hens and fattening flocks (including biosecurity)	Farm	Auditing	Not applicable
HEI 4 <i>Salmonella</i> in birds - carcasses after slaughter process and chilling	Slaughterhouse	Microbiology (detection and serotyping)	Neck and breast skin

(a) In accordance with the *Salmonella* control programmes laid down by EU Regulations for breeding flocks of *Gallus gallus*,¹² laying hens,¹³ broilers¹⁴ and turkeys.¹⁵

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 2.

¹² Commission Regulation (EC) No 200/2010 of 10 March 2010 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of *Salmonella* serotypes in adult breeding flocks of *Gallus gallus*. OJ L 61, 11.3.2010, p. 1–9.

¹³ Commission Regulation (EC) No 517/2011 of 25 May 2011 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of certain *Salmonella* serotypes in laying hens of *Gallus gallus* and amending Regulation (EC) No 2160/2003 and Commission Regulation (EU) No 200/2010. OJ L 138, 26.5.2011, p. 45–51.

¹⁴ Commission Regulation (EC) No 646/2007 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of certain *Salmonella enteritidis* and *Salmonella typhimurium* in broilers and repealing Regulation (EC) No 1091/2005. OJ L 151, 13.6.2007, p. 21–25.

¹⁵ Commission Regulation (EC) No 584/2008 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of certain *Salmonella enteritidis* and *Salmonella typhimurium* in turkeys. OJ L 162, 21.6.2008, p. 3–8.

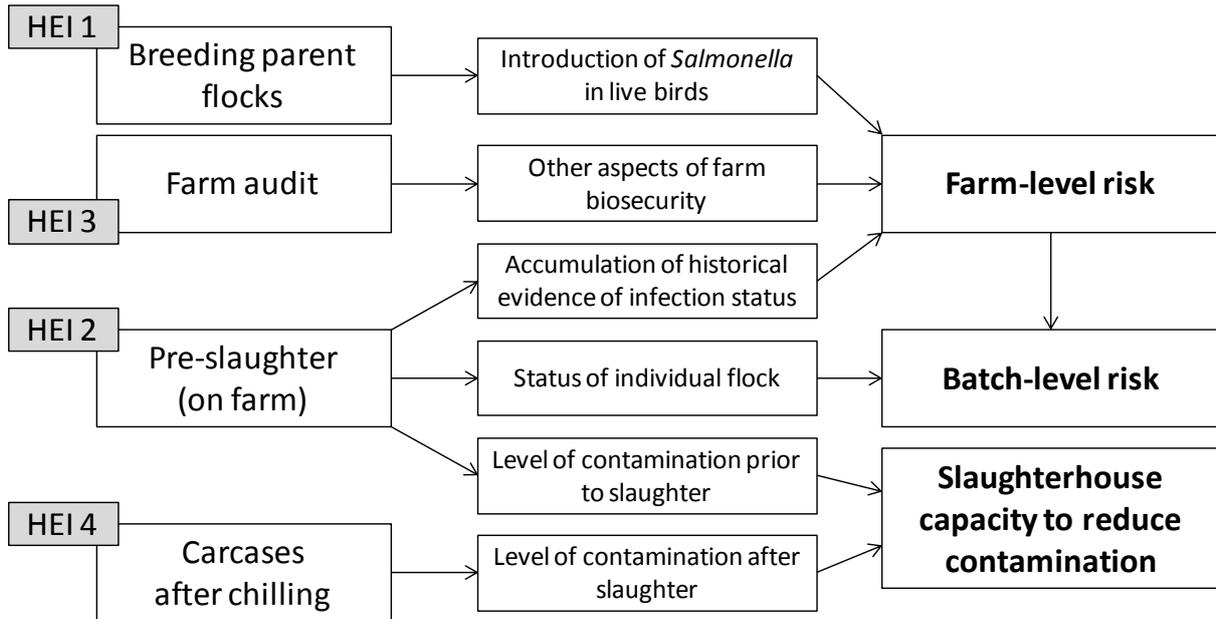


Figure 1: Schematic diagram illustrating the harmonised epidemiological indicators for *Salmonella* in poultry

Microbiological testing of pooled faeces and skin is the proposed analytical method for HEIs related to sampling of poultry or their carcasses for *Salmonella* infection or contamination. Microbiological analysis and typing of *Salmonella* spp. will provide data on specific new zoonotic serovars such as monophasic variants of *S. Typhimurium* and new emerging serovars. Particular *Salmonella* clones of special public health significance (e.g. clones with high virulence or resistance towards antimicrobials deemed critically important for treatment of human infections, but not necessarily related to particular serovars) may also be identified. The HEIs apply to all serovars of *Salmonella*, even though specific serovars can be targeted, when appropriate. Most of the proposed HEIs utilise the testing of poultry flocks or carcasses already foreseen by existing EU legislation on *Salmonella* controls in poultry flocks and on microbiological criteria for food¹⁶. In addition, auditing of farms for control housing conditions is applied in the proposed HEIs.

HEI 1 evaluates the risk of introducing *Salmonella* into poultry flocks from infected breeding flocks. Data from the mandatory *Salmonella* control programmes of breeding flocks of *Gallus gallus* and turkeys will provide information for this indicator.

HEI 2 provides information on the occurrence of the *Salmonella* and the specific serovars present in the poultry farms providing birds for slaughter. The indicator also gives information on the *Salmonella* infection status of the incoming slaughter batch to the slaughterhouse, since the transport and lairage conditions do not have a significant impact on this status. For this HEI the flocks should be tested before the whole flock or a part of the flock (thinning) is submitted to the slaughterhouse. Data from the mandatory *Salmonella* control programmes in broilers and fattening turkeys will provide information for this indicator.

Regular sampling of birds from the same farm as foreseen in HEI 1 and HEI 2 will enable the *Salmonella* status of the farm to be trended over time and thus collection of historical information regarding the farm.

¹⁶ Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, 1-26 as amended by Commission Regulation (EC) No 1441/2007 of 5 December amending regulation (EC) No 2073/2005 on microbiological criteria for foodstuff: OJ L 322, 07.12.2007, p. 12–29.

In the case of *Salmonella*, is not proposed to sample caeca after evisceration (as a HEI) because epidemiological studies have shown that *Salmonella*-positive flocks lead to *Salmonella*-positive carcasses at the slaughterhouse. *Salmonella* prevalence within a flock tends to decrease at the end of rearing and thus the probability of finding positive caeca at the slaughterhouse would be lower. This lower prevalence would therefore require a large number of samples to be taken to detect *Salmonella* and as such the proposed HEI 2 is regarded as a more sensitive indicator for incoming positive birds to the slaughterhouse.

HEI 3 classifies farms by using audit techniques which address the type and quality of housing, site and house biosecurity and overall management practices. The risk managers should define the detailed controlled housing conditions to be applied for *Salmonella* in poultry farms. An example of possible requirements is presented in Appendix 1.

HEI 4 provides information on the standard of process hygiene achieved after slaughter and chilling. It is an indicator of the *Salmonella* status of the carcasses after the entire slaughter process (including chilling) has been completed. The prevalence found at this point in the process reflects the *Salmonella* contamination level entering the food chain from the slaughterhouse. The data derived from monitoring of HEI 4 can be used to set *Salmonella* targets for slaughterhouses as referred to in the EFSA Scientific Opinion (EFSA, 2012b).

By combining the results (especially regarding the obtained serovars) from HEI 2 it is possible to assess the ability of the slaughter process to influence *Salmonella* contamination of the carcasses. The combined use of HEI 4 and HEI 2 also allows comparison of the *Salmonella* serovars/strains present. The EFSA Scientific Opinion (EFSA, 2012) recommends that this information is used for monitoring the effect on *Salmonella* reduction of the cleaning and disinfection process performed after the slaughter activities. If there is no association between the findings and if the same *Salmonella* strains are found on the carcasses over a period of time, the possibility of “house strain” contamination should be investigated.

The historical data from the implementation of HEI 4 provide information on the performance of the slaughterhouse as regards process hygiene and *Salmonella* control. The EFSA Scientific Opinion concludes that collection and analysis of such data over time would enable continuous monitoring of the abattoirs’ performance and thereby act as an indicator of the efficiency of the technology- and hygiene-based processes in reducing the final microbial load of the carcasses. Such analyses could indicate whether the abattoirs are improving or whether they might be failing to maintain previously high standards. An assessment of historical data could also be used for adjusting the sampling frequency of the main hazards in order to focus control efforts where the process hygiene does not ensure satisfactory sanitary conditions.

The proposed HEIs give different types of information on the risk of *Salmonella* infection in poultry or contamination of the carcasses and risk managers should choose the HEIs to be applied and then also interpret the available information in the appropriate way. The indicators may be used alone or in different combinations. A case study to illustrate the use of the proposed HEIs for *Salmonella* is presented in a scientific report submitted to EFSA (Cameron, 2012).

4.1.6. Harmonised monitoring requirements

Animal population

At farm:

- Breeding parent flocks.
- All flocks providing birds for slaughter.

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

At slaughterhouse:

- Slaughter batches of poultry.

Stage of the food chain

- Farm for breeding and fattening flocks.
- Farm for laying hens.
- Slaughterhouse for poultry slaughter batches.

Sampling

HEI 1 Breeding parent flocks

- Objective: classify the flock as positive or negative for *Salmonella* spp. (or specified serovars)
- Target population: the flock (all flocks requiring risk categorisation should be included) at farm level (no hatchery sampling included)
- Epidemiological unit: the flock
- Sampling strategy: census, all flocks providing chicks. The sampling unit is the flock.
- Survey interval:
 - According to Regulations (EC) No 584/2008 and 200/2010, every 2 weeks during the laying period for *Gallus gallus* and turkeys. If adequate (negative) historical testing has been performed, and audits have demonstrated a low risk of introduction of infection, this interval may be decreased (based on the principles described in Annex III (4), pages 101–103, of the report on HEIs for meat inspection of swine (EFSA, 2011e)). A similar sampling scheme can be used for the other poultry species.

HEI 2 Poultry flocks

- Objective: classify the flock as positive or negative for *Salmonella* spp. (or specified serovars).
- Target population: the flocks submitting birds for slaughter (all flocks requiring risk categorisation should be included).
- Epidemiological unit: the flock.
- Sampling strategy: all flocks are to be sampled before they send birds for slaughter. The sampling unit is the flock.
- Survey interval:

- In the case of broilers and fattening turkeys, according to Regulation (EC) No 646/2007 and Regulation (EC) No 584/2008, a maximum of 3 weeks prior to slaughter. In the case of spent hens to be sent for slaughter, faecal samples should be collected in accordance with Regulation (EC) No 517/2011 a maximum of 3 weeks prior to slaughter. If adequate (negative) historical testing has been performed, and audits have demonstrated a low risk of introduction of disease, this interval may be decreased such that it is not necessary to test every batch from low-risk farms (based on the principles described in Annex III(4), pages 101–103, of the report on HEIs for meat inspection of swine (EFSA, 2011e)). A similar sampling scheme can be used for the other poultry species.

HEI 3 Farm audits for controlled housing conditions

- Objective: estimate the likelihood of introduction of *Salmonella* infection into farms.
- Target population: all poultry farms.
- Epidemiological unit: the farm.
- Sampling strategy: each farm requiring risk classification to be audited.
- Audit interval: audit of farms repeated at a frequency (to be determined by risk managers) adequate to characterise the risk of introduction of *Salmonella* spp.

HEI 4 Carcase after chilling

- Objective: estimate the prevalence of poultry carcasses contaminated with *Salmonella* spp. (or specified serovars) after processing in order to assess the capacity of the slaughterhouse to prevent cross-contamination and the prevalence of *Salmonella*-contaminated carcasses entering the food chain.
- Target population: the slaughter population.
- Epidemiological unit: the slaughter batch.
- Sampling strategy:
 - Representative sample (random or systematic). As an example, Regulation (EC) No 2073/2005 on microbiological criteria lays down a sampling scheme for *Salmonella* process hygiene criterion in poultry carcasses, according to which 15 carcasses are sampled every week.
- Sample size:
 - Adequate to assess the difference in prevalence of *Salmonella* before and after processing (calculated as described in Annex 3 of the report on HEIs for meat inspection of swine (EFSA, 2011e)). This should include samples from a representative number of batches. Additional guidance on the sample size selection is given in Chapter 7 of this report.
- Survey interval:
 - Initial survey
 - Repeated at a frequency (to be determined by risk managers) adequate to characterise the slaughterhouse risk (required particularly when procedures in the slaughterhouse change).

Type and details of sample

- Pooled faeces at farm:

- For breeding parent flocks (HEI 1), boot/sock swabs possibly combined with dust samples in accordance with Regulation No 200/2010/EC.
- For other poultry flocks (HEI 2), boot/sock swabs in accordance with existing Regulations (No 517/2011/EC, No 584/2008/EC and No 646/2007/EC).
- Neck and breast skin from carcasses after chilling at slaughterhouse (HEI 4). As an example, Regulation (EC) No 2073/2005 on microbiological criteria lays down rules for taking samples from poultry carcasses for *Salmonella* process hygiene criterion, according to which 15 carcasses are sampled during the sampling session and five pools of three neck skins are analysed. Another published sampling scheme is in the baseline survey protocol for *Salmonella* in broiler carcasses (Decision 2007/516/EC),¹⁷ according to which a 25-g specimen of neck skin and other skin of the carcass is analysed for *Salmonella*.
- Questionnaire-based audit of farm procedures including specific conditions for *Salmonella* (HEI 3).

Diagnostic/analytical methods

Microbiological sampling: ISO 6579/A1:2007 (ISO, 2007);¹⁸ detection and serotyping (White–Kaufmann–Le Minor scheme).

Case definition

- Finding of *Salmonella* spp. in a sample.
- Farms found not complying with the controlled housing conditions.

Many of the proposed HEIs for *Salmonella* are set to the same stage of the food chain and target populations as the current EU *Salmonella* reduction targets and microbiological criteria. In many cases, the same types of samples are suggested to be taken. The relationship between the HEIs and the *Salmonella* targets and criteria are summarised in Table 2.

¹⁷ Commission Decision 2007/516/EC of 19 July 2007 concerning a financial contribution from the Community towards a survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and on the prevalence of *Campylobacter* spp. and *Salmonella* spp. in broiler carcasses to be carried out in Member States. OJ L 190, 21.7.2007, p. 25-37.

¹⁸ ISO 6579:2002/Amd 1 2007. Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.

Table 2: Relationships between proposed HEIs for *Salmonella* in poultry and current EU *Salmonella* reduction targets and microbiological criteria for poultry and meat thereof

Population	Stage of the food chain	Proposed HEI	EU reduction target	Microbiological criterion	Sample	EU legislation
Breeding flocks of poultry	Farm	<i>Salmonella</i> spp. and specific serovars			Boot swabs and dust	
Breeding flocks of <i>Gallus gallus</i> and turkeys	Farm		<i>S. Enteritidis</i> * <i>S. Typhimurium</i> * <i>S. Virchow</i> <i>S. Hadar</i> <i>S. infantis</i>		Boot swabs and dust	Regulation No 200/2010/EC (<i>Gallus gallus</i>), Regulation 584/2008/EC (turkeys)
Poultry flocks prior to slaughter	Farm	<i>Salmonella</i> spp. and specific serovars			Boot swabs or pooled faeces	
Flock of broilers and fattening turkeys	Farm		<i>S. Enteritidis</i> <i>S. Typhimurium</i>		Boot swabs	Regulation No 646/2007/EC (broilers), Regulation No 584/2008/EC (turkeys)
Flocks of laying hens	Farm		<i>S. Enteritidis</i> <i>S. Typhimurium</i>		Pooled faeces	Regulation No 517/2011/EC
Poultry carcasses after chilling	Slaughterhouse	<i>Salmonella</i> spp. and specific serovars			Neck and breast skin	
Poultry carcasses after chilling	Slaughterhouse			<i>Salmonella</i> spp. (process hygiene criterion)	Neck skin	Regulation No 2073/2005/EC as amended
Fresh poultry meat (derived from <i>Gallus gallus</i> and turkeys)	Products on the market			<i>S. Typhimurium</i> <i>S. Enteritidis</i> (food safety criterion)	Skin and/or surface muscle slice	Regulation No 2073/2005/EC as amended

* The target for breeding flocks of turkeys covers only *S. Enteritidis* and *S. Typhimurium*

4.2. *Campylobacter*

4.2.1. Introduction

Campylobacteriosis in humans is caused by thermotolerant *Campylobacter* spp. The infective dose of these bacteria is generally low. The species most commonly associated with human infection are *C. jejuni* followed by *C. coli* and *C. lari*, but other *Campylobacter* species are also known to cause human infection.

The incubation period in humans averages from 2 to 5 days. Patients may experience mild to severe symptoms, with common clinical symptoms including watery, sometimes bloody, diarrhoea, abdominal pain, fever, headache and nausea. Usually infections are self-limiting and last only a few days. Infrequently, extraintestinal infections or post-infection complications such as reactive arthritis and neurological disorders occur. *C. jejuni* has become the most recognised antecedent cause of Guillain–Barré syndrome, a polio-like form of paralysis that can result in respiratory and severe neurological dysfunction and even death.

Thermotolerant *Campylobacter* spp. are widespread in nature. The principal reservoirs are the alimentary tracts of wild and domesticated birds and mammals. They are prevalent in food animals such as poultry, cattle, pigs and sheep; in pets, including cats and dogs; in wild birds; and in environmental water sources. Animals, however, rarely succumb to disease caused by these organisms.

The bacteria can readily contaminate various foodstuffs, including meat, raw milk and dairy products, and less frequently fish and fishery products, mussels and fresh vegetables. Among sporadic human cases, contact with live poultry, consumption of poultry meat, drinking water from untreated water sources, and contact with pets and other animals have been identified as the major sources of infection. Cross-contamination during food-preparation in the home has also been described as an important transmission route. Raw milk and contaminated drinking water have been causes of larger outbreaks (EFSA and ECDC, 2012a).

4.2.2. Current situation and trends in the EU

Campylobacter was the most commonly reported gastrointestinal bacterial pathogen in humans in EU from 2005 to 2010. A total of 212 064 confirmed cases of human campylobacteriosis were reported in EU in 2010 and the number of cases increased by 6.9 % in 2010 compared with 2009. The EU notification rate of confirmed cases of campylobacteriosis has shown a significant increasing trend in the past 5 years, being more evident since 2008 (EFSA and ECDC, 2012a).

In foodstuffs, *Campylobacter* was most often reported in broiler meat and products thereof. In 2010, the prevalence of *Campylobacter* in fresh broiler meat in the EU was 31.2 %, varying between reporting Member States from 3.1 % to 67.5 %. In the case of fresh turkey meat, 29.5 % of units were found positive for *Campylobacter* (EFSA and ECDC, 2012a). In samples of fresh pig meat and fresh bovine meat at retail, *Campylobacter* was detected less frequently, at levels of 0.6 % and 0.4 %, respectively. In other foodstuffs *Campylobacter* was detected only occasionally, including some findings from ready-to-eat meat products of broiler and turkey meat origin, milk, cheese and vegetables (EFSA and ECDC, 2012a). In animals, the majority of data on *Campylobacter* were from investigations of broilers. In 2010, the proportion of *Campylobacter*-positive broiler flocks at reporting Member State level was 18.2 % ranging from 0 % to 92.9 %. For pigs and cattle, fewer Member States provided data; however, the prevalence in reporting Member States was generally high to very high for pig herds (34.5 % to 59.9 %) and low to very high for cattle herds (1.7 % to 67.0 %) (EFSA and ECDC, 2012a).

An EU-wide baseline survey on *Campylobacter* in broiler batches and on broiler carcasses was carried out in 2008 (EFSA, 2010b). At EU level, the prevalence of *Campylobacter*-colonised broiler batches among the 26 participating Member States was 71.2 % and that of *Campylobacter*-contaminated

broiler carcasses was 75.8 %. The country-specific *Campylobacter* prevalence varied widely among the participating Member States.

4.2.3. Poultry meat as a source of infection for humans

The BIOHAZ Panel's Opinion on *Campylobacter* in animals and foodstuffs (EFSA, 2005) indicated that poultry meat products appear to be a major source of campylobacteriosis, through cross-contamination to ready-to-eat (RTE) foods and through direct hand-to-mouth transfer, during food preparation, and to a lesser extent from the consumption of undercooked poultry meat. Contaminated meat acts as a vehicle of *Campylobacter*, especially those present in meat juices, which can easily contaminate kitchen equipment such as cutting boards, plates and knives, and thereby other foods (e.g. salads) that might be eaten without further bactericidal treatment (EFSA, 2010a).

The BIOHAZ Panel's Opinion on quantification of the risk of human campylobacteriosis posed by broiler meat in the EU (EFSA, 2010a) concluded that handling, preparation and consumption of broiler meat accounts for 20 % to 30 % of human cases of campylobacteriosis, while 50 % to 80 % may be attributed to the chicken reservoir as a whole. In the same Opinion, it was indicated that source attribution analysis based on investigations of outbreaks with a known source attributed 29 % of human campylobacteriosis outbreaks to chicken (EFSA, 2010a).

4.2.4. Risk and protective factors

The scientific Opinion of the BIOHAZ Panel *Campylobacter* in broiler meat production and control options and performance objectives and/or targets at different stages of the food chain (EFSA, 2011a) concluded that some risk factors associated with *Campylobacter* infection in broilers are frequently implicated regardless of the country investigated or the robustness of the study design. These primary factors include season, increasing bird age, thinning (partial depopulation), the presence of flocks of various ages on the farm, the farming of multiple animal species, the use of extensive rearing at any stage and poor biosecurity. In particular, the biosecurity measures aiming primarily to control the entry of *Campylobacter* into the house are relevant as it has been found that, once *Campylobacter* enters the broiler house and infects the first birds, spread is very rapid and virtually all birds are colonised within one week (EFSA, 2011a). This Opinion also concluded that colonisation with *Campylobacter* of flocks with outdoor access is very likely to occur. Other risk factors are more intermittently implicated in the Opinion. These include the use of non-potable drinking water, lack of farmer awareness regarding the importance of biosecurity, the presence of insects or vermin and the use of antibiotics. These secondary risk factors may be related more to specific management practices or even geographical location. Additional factors that may increase the risk of *Campylobacter* infection in broilers include the presence of workers and other farm visitors and the use of *Campylobacter*-contaminated drinking water. The Opinion furthermore concluded that biosecurity measures are considered essential to prevent flock colonisation with *Campylobacter* and that colonisation of flocks with outdoor access is very likely to occur.

Several studies have indicated that applied hygienic practices and barriers as well as the type of the ventilation system can be associated with the risk of *Campylobacter* infection of broiler flocks (Van de Giessen et al., 1998; Evans and Sayers, 2000; Barrios et al., 2006; Hald et al., 2007; McDowell et al., 2008; Hansson et al., 2010). The analyses of the EU-wide baseline survey on *Campylobacter* in broiler batches and carcasses indicated that the previous thinning of the flock, the age of the broilers and the quarter of sampling (3-month period) were associated with *Campylobacter*-colonised broiler batches (EFSA, 2011c).

The crates used to transport live birds from the farm to the slaughterhouse as well as the personnel carrying out the catching and placing the birds in the crates are known sources of *Campylobacter* contamination of the birds and of colonisation of the remaining flock at the farm in case of partial depopulation (Slader et al., 2002; Allen et al., 2008). The stress on birds during transport to the slaughterhouse and lairage is likely to increase faecal shedding of *Campylobacter* and the external contamination of the birds with *Campylobacter* (Stern et al., 1995; Whyte et al., 2001).

Contamination of carcasses with *Campylobacter* takes place during slaughter by faecal contamination, exterior contamination of the birds (skin and feathers) or cross-contamination from other carcasses or equipment within the slaughterhouses (EFSA, 2011a). *Campylobacter* contamination of carcasses can occur throughout the entire slaughter process, including chilling (Berndtson et al., 1996). However, a number of studies have found that air chilling results in a decrease in *Campylobacter* levels on the carcass (Sanchez et al., 2002; Allen et al., 2007; Berrang et al., 2007; Hinton et al., 2004; Huezo et al., 2007; Boysen and Rosenquist 2009). When birds of varying size are processed using automated evisceration equipment, rupture of viscera and subsequent faecal contamination of other carcasses may occur (Rosenquist et al., 2006; EFSA, 2011a). In addition, the hygienic measures prevailing in a given slaughterhouse are likely to have a major impact on the final numbers of *Campylobacter* on the carcasses (EFSA, 2011a).

In the EU-wide baseline survey on *Campylobacter* in broiler batches and carcasses (EFSA, 2011a), the factors associated with *Campylobacter* contamination of broiler carcasses included the *Campylobacter* colonisation status of the batch and the type of carcass chilling. The survey findings also indicate that some slaughterhouses are more capable than others of preventing/reducing *Campylobacter* contamination and of controlling the contamination and/or the *Campylobacter* counts on carcasses.

Skinless poultry meat products have been found to have lower *Campylobacter* counts than the corresponding meat products with skin (Uyttendaele et al., 1999; Davis and Conner, 2000, 2007; Sampers et al., 2008).

The number (counts) of *Campylobacter* on the poultry carcasses is an important risk factor for public health. The Opinion from the BIOHAZ Panel (EFSA, 2011a) concluded, based on quantitative risk assessment, that reducing the numbers of *Campylobacter* on broiler carcasses by 1 log₁₀ unit would reduce the public health risk caused by broiler meat by between 50 % and 90 %. Reducing counts by more than 2 log₁₀ units would reduce the public health risk caused by broiler meat by more than 90 %.

4.2.5. Proposed harmonised epidemiological indicators (HEIs)

The epidemiological indicators selected for *Campylobacter* in poultry are shown in Table 3 and Figure 2.

Table 3: Harmonised epidemiological indicators for *Campylobacter* in poultry

Indicators (animal/food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
HEI 1 <i>Campylobacter</i> in poultry flocks prior to slaughter ^(a)	Farm	Microbiology - real-time PCR	Caecal droppings
HEI 2 Controlled housing conditions at farm for poultry flocks (including biosecurity)	Farm	Auditing	Not applicable
HEI 3 Use of partial depopulation in the flock	Farm	Food chain information	Not applicable
HEI 4 <i>Campylobacter</i> in birds - incoming to slaughter process (evisceration stage)	Slaughterhouse	Microbiology - enumeration	Caecal content
HEI 5 <i>Campylobacter</i> in birds - carcasses after slaughter process and chilling	Slaughterhouse	Microbiology - enumeration	Neck and breast skin

(a) Sampling of caecal droppings should be carried out 2–3 days prior slaughter.

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 2.

Microbiological testing of caecal droppings, caecal content and skin is the proposed analytical method for the HEIs related to sampling of poultry or their carcasses for *Campylobacter* colonisation or contamination. In addition, auditing of farms for control housing conditions is applied in the proposed HEIs.

In the absence of a more accurate sampling method, caecal droppings are suggested as samples at the farm level. This matrix is proposed instead of caecal content sampling or boot swabs in order to avoid sacrificing the animals and because of the lack of data in the published literature regarding the sensitivity of boot swabs for *Campylobacter* detection. Caecal droppings allow better *Campylobacter* spp. survival and provide more accurate results than boot swabs, but they have some practical limitations because the droppings are difficult to spot in the poultry house and they are shed at specific times of the day. Boot swabs might be the most suitable sampling method as they could also be used as the sample type for other bacteria, making sampling more cost-effective, but some experimental work is needed to define the optimal number of samples by flock to achieve an acceptable level of sensitivity allowing the detection of *Campylobacter*-positive flocks.

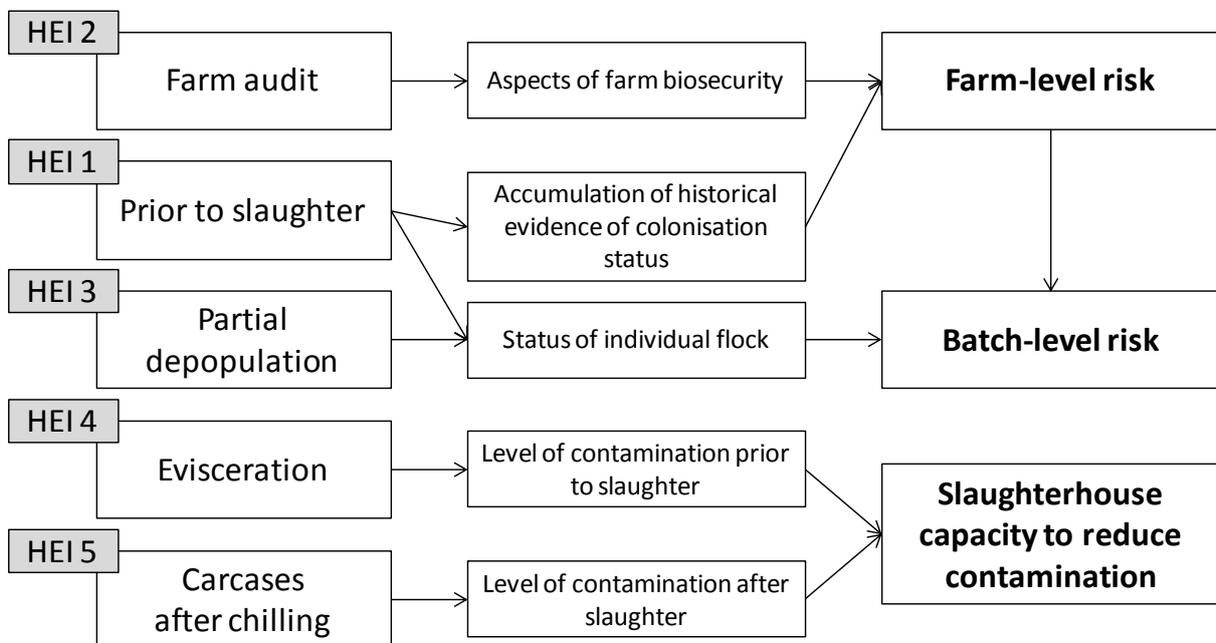


Figure 2: Schematic diagram illustrating the purpose of each of the harmonised epidemiological indicators for *Campylobacter* in poultry

The main factors determining the risk of carcasses contamination with *Campylobacter* spp. are the type of production system used, the practice of partial depopulation (thinning) and the level of slaughter hygiene in the abattoir.

HEI 1 provides information on the occurrence of *Campylobacter* in the poultry farms providing birds for slaughter by categorising the tested flocks as either colonised or not colonised with *Campylobacter*. For this HEI the flocks should be tested each time birds are delivered to the slaughterhouse and the sampling should be repeated if partial depopulation (thinning) was applied, unless the first sample was already positive. The timing of farm sampling is crucial, as a flock may become infected with *Campylobacter* rapidly. Therefore, the interval between testing and slaughter should be as short as possible while still providing adequate time for the results to be available (from a rapid polymerase chain reaction (PCR) method) before slaughter (e.g. sampling 2–3 days prior to

slaughter). Monitoring of trends in the *Campylobacter* status of the farm will be enabled by regular sampling of birds from the same farm and historical information derived from this sampling can be used to update the risk status of the farm. According to the EFSA Scientific Opinion, in most countries, it can be assumed that slaughter batches from flocks with outdoor access or flocks that have been thinned more than 3 days previously are likely to be positive for *Campylobacter* and could be directly allocated to a higher risk category. In summer, this would apply even to countries with an overall lower flock prevalence. Thus, the risk managers or food business operators may decide to consider such flocks automatically *Campylobacter* positive without testing.

HEI 2 classifies farms on the basis of controlled housing conditions by applying auditing techniques. The risk managers should define the detailed controlled housing conditions to be applied for *Campylobacter* in poultry farms. Examples of possible requirements are presented in Appendix 1.

HEI 3 provides information on whether partial depopulation (thinning) was carried out in the flock sent to slaughter. This information must be included in the food chain information provided by the farm to the slaughterhouse for each slaughter batch.

HEI 4 provides for quantitative information on numbers of *Campylobacter* bacteria (counts) in caecal contents of poultry at slaughter. This information will reflect the status of the incoming slaughter batch with respect to not only initial on-farm colonisation but also the impact of transport and lairage conditions on *Campylobacter* colonisation of the birds.

HEI 5 provides information on the overall level of carcass contamination after the slaughter and chilling processes. In combination with HEIs 1 and 4 it can be used to assess the ability of the slaughter process to influence *Campylobacter* contamination of the carcasses, particularly the numbers of *Campylobacter*. If a problem in the process hygiene in the slaughterhouse is observed, the risk managers or the food business operator could further explore at which process step the contamination occurs, for example in the context of hazard analysis and critical control points (HACCP). HEI 5 is also an indicator of the *Campylobacter* status of the carcasses after the entire slaughter process (including chilling) has been completed. The microbial counts found at this point in the process reflect the *Campylobacter* contamination level entering the food chain from the slaughterhouse. The data derived from monitoring of HEI 5 can be used to set *Campylobacter* targets for slaughterhouses as referred to in the EFSA Scientific Opinion (EFSA, 2012b).

The historical data from the implementation of HEI 5 provide information on the performance of the slaughterhouse as regards process hygiene and control of *Campylobacter* contamination. The EFSA Scientific Opinion concludes that collection and analysis of such data over time would enable continuous monitoring of the abattoirs' performance and thereby act as an indicator of the efficiency of the technology- and hygiene-based processes in reducing the final microbial load of the carcasses. Such analyses could indicate whether the abattoirs are improving or whether they might be failing to maintain previously high standards. An assessment of historical data could also be used for adjusting the sampling frequency of the main hazards in order to focus control efforts where the process hygiene does not ensure satisfactory sanitary conditions.

The proposed HEIs give different types of information on the risk of *Campylobacter* colonisation in poultry or contamination of the carcasses, and risk managers should choose the HEIs to be applied and then also interpret the available information in the appropriate way. The indicators may be used alone or in different combinations. A case study to illustrate the use of the proposed HEIs for *Campylobacter* is presented in a scientific report submitted to EFSA (Cameron, 2012).

4.2.6. Harmonised monitoring requirements

Animal population

- At farm: all poultry flocks providing birds for slaughter.
- At slaughterhouse: slaughter batches of poultry.

Farms are subjected to an audit regarding the production system standards to define the biosecurity and controlled housing conditions and the application of thinning of the flock is to be included in the food chain information following the birds from farm to slaughterhouse.

Stage of the food chain

- Farm for poultry flocks.
- Farm for controlled housing conditions and partial depopulation of flocks.
- Slaughterhouses for slaughter batches of poultry.

Sampling

HEI 1 On-farm sampling

- Objective: classify the flock as positive or negative for *Campylobacter*.
- Epidemiological unit: the flock.
- Target population: the flock (all flocks requiring risk categorisation should be included).
- Sampling strategy:
 - The sampling unit is the flock. Each flock is sampled prior to sending birds to slaughter. The interval between testing and slaughter should be as short as possible (2–3 days) while providing adequate time for the results from rapid testing to be available before slaughter. In the case of prior partial depopulation, sampling of the flock should be repeated unless the first results were already positive.
- Survey interval
 - If adequate (negative) historical testing has been performed, and audits have demonstrated a low risk of introduction of disease, the census sampling may be decreased such that it is not necessary to test every batch from low-risk farms (based on the principles described in Annex 3 of the report on HEIs for meat inspection of swine (EFSA, 2011e)).

HEI 2 Audit of the farm for controlled housing conditions

- Objectives:
 - Characterise the farm as meeting the requirements for controlled housing conditions.
 - Classify the *Campylobacter* risk level of the farm.
- Target population: all farms producing poultry for slaughter.
- Epidemiological unit: the farm.
- Sampling strategy: each farm requiring risk classification to be audited.
- Audit interval: audit of farms repeated at a frequency (to be determined by risk managers) adequate to characterise the risk *Campylobacter*.

HEI 3 Use of partial depopulation (thinning)

- Target population: all poultry slaughter batches sent to slaughterhouse.
- Epidemiological unit: the slaughter batch.
- Sampling strategy: census; all slaughter batches of poultry are to be accompanied by the information if prior partial depopulation of the flock of origin has taken place.

HEI 4 Carcasses at evisceration

- Objective: estimate the prevalence of birds colonised and the *Campylobacter* counts in their caecal content at the slaughterhouse (before slaughter process).
- Sampling unit: the individual bird at slaughter
- Target population: all slaughter batches of poultry
- Epidemiological unit: the slaughter batch
- Sampling strategy:
 - Representative sample (random or systematic).
 - Microbiological testing of caecal content (enumeration)
- Sample size:
 - Adequate to assess the difference in prevalence and mean log count of *Campylobacter* before and after processing. Sample size for prevalence should be calculated as described in Annex 3 of the report on HEIs for meat inspection of swine (EFSA, 2011e). Sample size for comparison of means should be calculated as illustrated in Chapter 7 of the current report.
- 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 5 Carcase contamination after the slaughter and chilling

- Objective: estimate the prevalence and concentration of *Campylobacter* on carcasses at the slaughterhouse (after processing and chilling), to assess the capacity of the slaughterhouse to limit cross-contamination and to inform of the level of *Campylobacter* contamination of the carcasses leaving the slaughterhouse.
- Sampling unit: the individual bird.
- Target population: the slaughter population.
- Epidemiological unit: the slaughter batch.

Sampling strategy:

- Representative sample (random or systematic).
- Microbiological testing of neck and/or breast skin samples (enumeration)
- Sample size:
 - Adequate to assess the difference in prevalence and extent of *Campylobacter* colonisation/contamination before and after processing (calculated as described in Annex 3 of the report on HEIs for meat inspection of swine (EFSA, 2011e)).
- Survey interval:

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

Type and details of sample

- Pooled caecal droppings collected from litter in the poultry house within 2–3 days before slaughter. Caecal droppings look different for normal faecal material: they are more watery and have a brown colour (see pictures in Appendix 3). In total, at least 15 such droppings have to be collected from all over the poultry house.
- Caecal contents samples at the slaughterhouse, directly after evisceration: collection of one caecum/carcass. The number of birds to be sampled can be defined using the sample size calculations presented in Chapter 7. At the laboratory the necessary amount of caecal content is aseptically collected for analysis. An example of caecal sampling for *Campylobacter* is given in the EU baseline survey protocol set down in Commission Decision 2007/516/EC.
- Neck and/or breast skin samples at the slaughterhouse (e.g. as foreseen in Commission Decision 2007/516/EC).
- Questionnaire-based audit of farm procedures including specific conditions for *Campylobacter*.
- Food chain information concerning the thinning of the flock.

Diagnostic/analytical methods

- Caecal droppings: real-time PCR.
- Caecal content: enumeration according to ISO method 10272-2:2006(E), “Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of *Campylobacter* spp. Part 2: Enumeration method”¹⁹.
- Skin: enumeration of *Campylobacter* according to ISO method 10272-2:2006(E).

Case definition

- Finding and counts of *Campylobacter* in a sample.
- Farms found not complying with the controlled housing conditions.
- Flocks previously partially depopulated.

¹⁹ ISO/TS 10272-2:2006_Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of *Campylobacter* spp.—Part 2: Colony-count technique.

4.3. Extended-spectrum and/or AmpC beta-lactamase (ESBL/AmpC)-producing bacteria

4.3.1. Introduction

According to the Opinion from the BIOHAZ Panel on the public health risks of bacterial strains producing ESBL/AmpC in food and food-producing animals (EFSA, 2011d), extended-spectrum β -lactamases (ESBLs) have been defined as plasmid-encoded enzymes found in the *Enterobacteriaceae*, frequently in *Escherichia coli* and *Klebsiella pneumoniae*, that confer resistance to a variety of β -lactam antibiotics, including penicillins, second-, third- and fourth-generation cephalosporins and monobactams (e.g. aztreonam), but usually not the carbapenems or the cephamycins (e.g. ceftiofur). In contrast, AmpC β -lactamases are intrinsic cephalosporinases found on the chromosomal DNA of many Gram-negative bacteria that confer resistance to penicillins, second- and third-generation cephalosporins including β -lactam/inhibitor combinations, and cefamycins (ceftiofur), but usually not to fourth-generation cephalosporins (cefepime, ceftazidime) and carbapenems; a growing number of these AmpC enzymes are now plasmid borne (EFSA, 2011d).

ESBL-/AmpC-producing organisms are frequently co- or multiresistant and exhibit resistance to other antimicrobial classes, such as fluoroquinolones, aminoglycosides and trimethoprim-sulphamethoxazole. The broad resistance profile in ESBL-producing bacteria is important in human infections and is of public health concern (Pitout and Laupland, 2008; Rodriguez-Bano et al., 2010). The multiresistant nature of bacteria that produce ESBLs can affect the selection and timely administration of appropriate antimicrobials for community-acquired and healthcare-associated infections, since many first-line antimicrobials are no longer active against them. Furthermore, infections with such resistant organisms are associated with poorer patient outcomes, increased morbidity and mortality, increased length of stay and increased costs (Ibrahim et al., 2000; Lautenbach et al., 2001; Cosgrove et al., 2003; Anderson et al., 2006; Schwaber and Carmeli, 2007; Ben-Ami et al., 2009; Roberts et al., 2009).

Although person-to-person spread is recognised as the main method of spread of ESBL-/AmpC-producing *E. coli* both in hospitals and in the community, the primary reservoirs of such organisms are contentious (EFSA, 2011d). ESBL-/AmpC-producing organisms have been detected in a variety of food-producing animals, mainly poultry and cattle (but also swine, horse, rabbit, ostrich, wild boars), and food of animal origin (Blanc et al., 2006; Vo et al., 2007; Carattoli, 2008; Poeta et al., 2009; Rodriguez et al., 2009; Carneiro et al., 2010; Cortes et al., 2010; Dierikx et al., 2010; Escudero et al., 2010; Hunter et al., 2010) in many European countries. From these hosts, the species more commonly identified have been *E. coli* and non-typhoidal salmonellae. Among *E. coli*, the clonal lineages phylogroup B2 (*E. coli* O25:H4-ST131) and phylogroup D (*E. coli* O25a-ST648 and *E. coli* ST69 and ST393) are being increasingly detected among both humans and animals (Cortes et al., 2010; Vincent et al., 2010; Mora et al., 2011). The most common *Salmonella* serovars producing ESBLs are *S.* Typhimurium, Newport and Heidelberg, but such enzymes have also been detected in an expanding number of other serovars (Gonzalez-Sanz et al., 2009).

The potential contribution of food-producing animals or foods to public health risks by ESBL-/AmpC-producing bacteria is related to specific plasmid-mediated ESBL/AmpC genes encoded by a number of organisms. Although there are a large number of genes which encode ESBL/AmpC enzymes, not all are equally prevalent among human and animal bacteria. The predominant ESBL families encountered are CTX-M, TEM and SHV, while the predominant AmpC family is CMY (EFSA, 2011d).

The identification of ESBL-/AmpC-producing organisms is performed by determination of susceptibility to cefotaxime, ceftazidime and ceftiofur. ESBL producers are resistant to cefotaxime, variably resistant to ceftazidime and susceptible to ceftiofur. AmpC producers are susceptible to cefepime and resistant to cefotaxime, ceftiofur and ceftiofur (EFSA, 2011d).

4.3.2. Current situation and trends in the EU

EFSA's technical specifications for monitoring of antimicrobial resistance in food-producing animals (EFSA, 2007c, 2008b) state that cefotaxime is a good indicator of what are currently the most common and important ESBL/AmpCs in humans in Europe and can therefore be used as an indicator for ESBL/AmpC resistance.

Resistance to cefotaxime has been reported in non-typhoidal *Salmonella* isolates from human cases in recent years. In 2010, 1 % of a total of 24 251 *Salmonella* isolates from 17 Member States were reported to be resistance to cefotaxime, with prevalence of resistance varying from 0.1 % to 4.4 % across the Member States (EFSA and ECDC, 2012b).

Among *Salmonella* isolates from food in 2010, the overall percentage of resistance to third-generation cephalosporins in isolates from broiler meat in the reporting Member States was at the level of 4 % (for both cefotaxime and ceftazidime) (EFSA and ECDC, 2012b). In turkey meat, the overall percentage of resistance to both cefotaxime and ceftazidime in *Salmonella* isolates was approximately 1 % (data mainly from one Member State). In pig meat, the overall level of resistance to cefotaxime and ceftazidime in *Salmonella* isolates in all reporting Member States was 0.2 % and 0 %, respectively.

In animals, in 2010, a low level of resistance to cefotaxime of 1 % and to ceftazidime of 2 % was reported in *Salmonella* isolates from fowl (*Gallus gallus*) in the reporting Member States. Among *Salmonella* isolates from pigs, the overall level of resistance in all reporting Member States was 0.8 % for cefotaxime and 1 % for ceftazidime. Only one country reported cefotaxime or ceftazidime resistance in *Salmonella* isolates from cattle, which was found at a very low level of 0.5 %. Among indicator *E. coli* isolates tested from *Gallus gallus* in the reporting Member States group, the observed resistance to cefotaxime was 5 % and to ceftazidime was 7 %. In indicator *E. coli* isolates from pigs, the overall occurrence of resistance for all reporting Member States was 1 % to cefotaxime and 2 % to ceftazidime, and those for indicator *E. coli* isolates from cattle were 3 % and 4 %, respectively (EFSA and ECDC, 2012b).

4.3.3. Poultry meat as a source of infection for humans

According to the BIOHAZ Opinion on ESBL/AmpC (EFSA 2011d), in recent years the presence of ESBL-/AmpC-producing *Salmonella* and *E. coli* in animals and food has been increasingly reported both in Europe and globally. These enzymes have been described in bacteria from all major food-producing animals; however, poultry and products thereof are the ones most frequently reported to be contaminated with ESBL-/AmpC-producing bacteria. With regard to the possibility of bacteria that produce ESBL/AmpC being transmitted to humans, there are reports that provide circumstantial evidence that ESBL-producing *E. coli* can be associated with its transmission from food to humans (Lavilla et al., 2008), and studies whose findings suggest transmission of *E. coli* that produce ESBL from poultry to humans (Leverstein-van Hall et al., 2011), but also evidence (Fey et al., 2000; Zansky et al., 2002) of direct association of transmission of *Salmonella* resistant to third-generation cephalosporins during an outbreak in humans (EFSA, 2011d).

The BIOHAZ Opinion on ESBL/AmpC states that identification of common clones of ESBL-/AmpC-producing *E. coli* isolates in humans and food-producing animals and foods provide indirect evidence of transmission. Moreover, resistance genes may be transferred from food-borne commensal bacteria to non-food-borne human pathogens. This has been shown to be particularly applicable to ESBLs (Mesa et al., 2006; Lavilla et al., 2008). Recent findings indicate transmission of ESBL genes, plasmids and clones from poultry to humans is most likely to occur through the food chain (EFSA, 2011d). In a recent study from the Netherlands, the results are suggestive of transmission of ESBL genes, plasmids and clones from poultry to humans, most probably through the food chain (Leverstein-van Hall et al., 2011). Whereas, Dutil et al. (2010) reported on observed temporal links between the use of ceftiofur in chickens followed by the occurrence of resistant AmpC gene-carrying *S. enterica* subsp. *enterica* serovar Heidelberg and *E. coli* strains in chickens and humans in Canada.

4.3.4. Risk and protective factors

Information on risk and protective factors for the occurrence of bacterial strains producing ESBL/AmpC is limited. Risk factors contributing to the occurrence, emergence and spread of ESBL- and/or AmpC-producing bacteria have been summarised in the Opinion of the BIOHAZ Panel (EFSA, 2011d). This Opinion, however, notes that the establishment of such risk factors is particularly complicated by the lack of data or inaccurate data.

At the level of farm management, ESBL- and AmpC-producing bacteria may enter and proliferate in a farm through the stocking of new animals, exposure to contaminated air, through water or feed, insect or rodent vectors, human-to-animal and animal-to-animal transmission (EFSA, 2011d). In a study on Belgian broiler farms, risk factors associated with the occurrence of ESBL- and AmpC-producing *E. coli* included, besides antimicrobial use, the cleanliness of the environment (with a cleaner environment being a risk factor for appearance of such isolates), the lack of acidification of drinking water, the application of more than three feed changes during the production cycle, the breed and the litter material that is used (Smet et al., 2008; Persoons et al., 2010a). Persoons et al. (2010b) showed that antimicrobial resistance of *E. coli* from cloacal swabs of broilers persisted over consecutive production rounds. Use of antimicrobials is also an important risk factor and, importantly, this is not restricted to use of cephalosporins, but also applies to generic antimicrobial use (EFSA, 2011d; Dheilly et al., 2012). Risk factors for the presence of multi-drug resistance in *E. coli* in layers could include the housing of hens in raised-floor systems or the presence on the farm of animals (cattle, pigs ...) in which cephalosporins are used (Van Hoorebeke et al., 2011). Moreover, chemicals used in animal production, such as antiseptics, disinfectants and metals, could play a role in the appearance of such resistant isolates (Hasman and Aarestrup, 2002; Aarestrup and Hasman, 2004; Cavaco et al., 2010; EFSA, 2011d).

Trade and movement of animals is another contributing factor, with the pyramidal structure of broiler farming offering opportunity for spread of the microorganisms from higher to lower levels, if these are present in animals in the former. The BIOHAZ Opinion (EFSA, 2011d) noted that an extensive trade of animals occurs in EU Member States with few countries leading the production and export, and only a small number of companies producing pure line grandparent stock. How widespread ESBL-producing bacteria are in food-producing animals in the breeding/rearing/fattening sectors is generally unknown, although a few reports suggest that ESBL/AmpC-producing bacteria are not uncommon at the top of some production pyramids (breeding) (EFSA, 2011d). Indeed, vertical transmission is probable as ESBL- and/or AmpC-producing *E. coli* strains have been detected in day-old grandparent chicks, in day-old parents and in day-old broiler chicks (Dierikx et al., 2011) and thus carriage of ESBL- and/or AmpC-producing *E. coli* in breeding flocks should be considered a risk factor. Moreover, spread of ESBL-producing bacteria can happen through contamination during slaughter or during food handling, while contamination of fresh food of plant origin may also play a role in the spread and transmission of ESBLs (EFSA, 2011d).

4.3.5. Proposed harmonised epidemiological indicators (HEIs)

The epidemiological indicators in Table 4 and Figure 3 are selected for ESBL-/AmpC-producing bacteria in poultry. The HEIs are proposed for ESBL/AmpC-harbouring commensal *E. coli* bacteria. This is because these bacteria are more prevalent in poultry than *Salmonella* or other Enterobacteriaceae and, furthermore, they are easy to detect compared with ESBL/AmpC-positive *Salmonella* or other Enterobacteriaceae.

Table 4: Harmonised epidemiological indicators for ESBL/AmpC in commensal *Escherichia coli* in poultry

Indicators (animal/food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
HEI 1 ESBL-/AmpC-producing <i>E. coli</i> in elite, grandparent and parent breeding flocks producing chicks for meat production lines	Farm	Microbiology, enumeration, molecular methods for characterisation on a subsample	Pooled faeces (boot swabs)
HEI 2 ESBL-/AmpC-producing <i>E. coli</i> in incoming 1-day-old chicks for fattening purposes	Farm	Microbiology, detection with enrichment, molecular methods for characterisation on a subsample	Paper used in transport boxes
HEI 3 ESBL-/AmpC-producing <i>E. coli</i> in poultry flocks prior to slaughter	Farm	Microbiology, enumeration, molecular methods for characterisation on a subsample	Pooled faeces (boot swabs)
HEI 4 Controlled housing conditions	Farm	Auditing	Not applicable
HEI 5 Use of antimicrobials during the whole life time of the flock (including <i>in ovo</i> , hatching, rearing, laying, all types of flocks)	Hatchery/farm	Food chain information (from hatchery to farm, from farm to slaughterhouse)	Not applicable
HEI 6 ESBL-/AmpC-producing <i>E. coli</i> in birds - carcasses after slaughter process and chilling	Slaughterhouse	Microbiology, enumeration, molecular methods for characterisation on a subsample	Neck (and breast) skin

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

Microbiological testing of pooled faeces, transport box papers and carcasses skin is the proposed analytical method for those HEIs related to sampling of poultry or their carcasses for the presence of ESBL-/AmpC-producing *E. coli*. Microbiological analysis will provide data on the presence and numbers of these bacteria in the faeces/transport papers and carcasses. Bacteriological and molecular analysis on a subsample of the isolates will provide data on the mechanisms of resistance and their genetic support.

HEI 1 provides information on the risk of introducing chicks/poults that are colonised with ESBL-/AmpC-producing *E. coli* into poultry houses when originating from ESBL-/AmpC-producing *E. coli*-colonised breeding flocks, as vertical transmission of resistant *E. coli* from breeding flocks to their progeny is very probable, and birds originating from ESBL-/AmpC-producing *E. coli*-contaminated breeding flocks are at a higher risk of being colonised with ESBL-/AmpC-producing *E. coli*.

HEI 2 provides information on the risk of introducing chicks/poults colonised with ESBL-/AmpC-producing *E. coli* originating either from ESBL-/AmpC-producing *E. coli*-contaminated breeding flocks or from colonised hatchery into poultry houses. Detection of ESBL-/AmpC-producing *E. coli* contamination from the faeces present in the transport box papers will enable the detection of introduction of ESBL-/AmpC-producing *E. coli*-colonised chicks.

HEI 3 provides information on the presence of ESBL-/AmpC-producing *E. coli* in the poultry farms providing birds for slaughter. For this HEI the flocks should be tested each time the birds are submitted to the slaughterhouse.

Repeated sampling of birds from the same farm as foreseen in HEIs 1–3 will provide information on ESBL-/AmpC-producing *E. coli* status of the farm and also assist in the monitoring of trends of the ESBL-/AmpC-producing *E. coli* contamination of the farm.

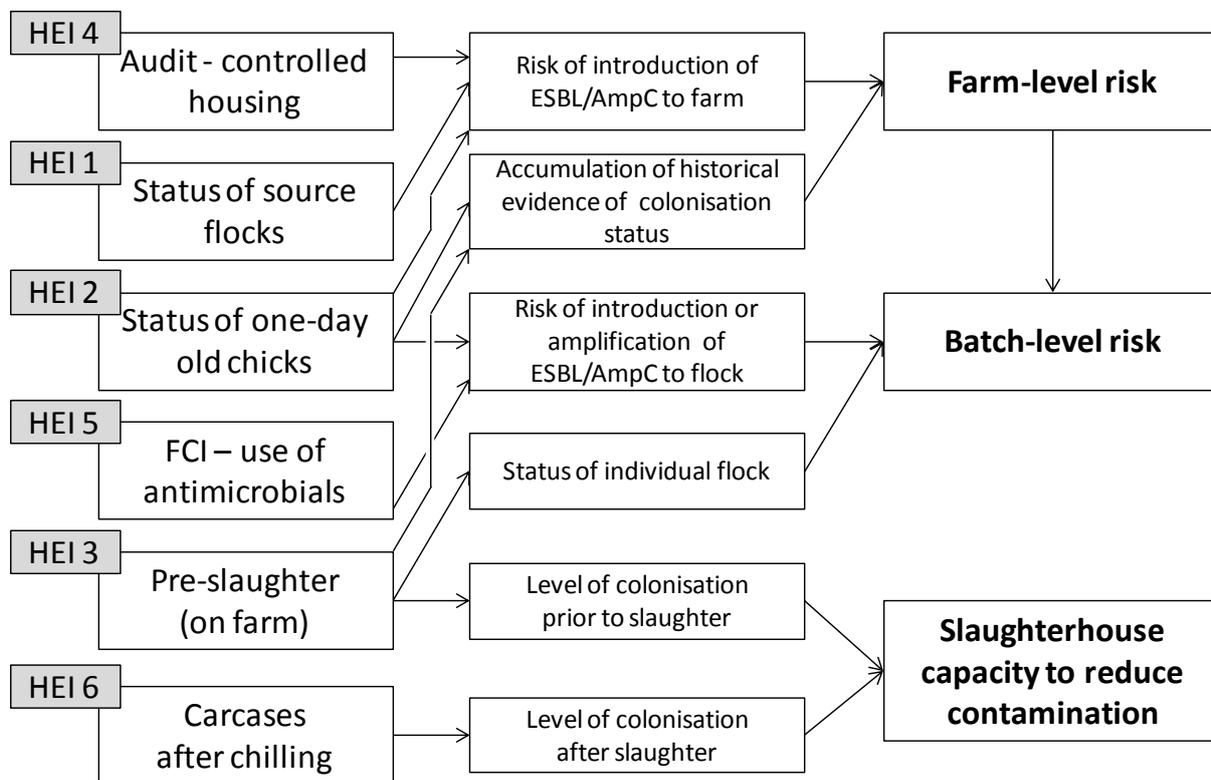


Figure 3: Schematic diagram illustrating the purpose of each of the harmonised epidemiological indicators for ESBL/AmpC *E. coli* in poultry

HEI 4 classifies farms on the basis of controlled housing conditions, including biosecurity and management practices in farms, by applying auditing techniques. Risk managers should define the detailed controlled housing conditions to be applied for ESBL-/AmpC-producing *E. coli* in poultry farms. Examples of possible requirements are presented in Appendix 1.

HEI 5 provides information on whether the flock sent to the slaughterhouse has received antimicrobials (any antimicrobial substance, ideally specifying the substance used) during its whole lifetime, including *in ovo* and in hatchery administration. This information should be included in the food chain information provided by the primary producer to the slaughterhouse and also in documentation (relates to breeding farm and hatchery) accompanying day-old chicks to the farms rearing fattening birds. Antimicrobial use in the hatchery may amplify the ESBL-/AmpC-producing *E. coli* bacterial population, raising the risk of presence of ESBL/AmpC *E. coli* on chicks/poults.

HEI 6 provides information on the overall level of ESBL/AmpC *E. coli* carcass colonisation after the slaughter and chilling process. In combination with HEI 3, it can be used to assess the ability of the slaughter process to influence ESBL-/AmpC-producing *E. coli* contamination of the carcasses. The microbial counts found at this point in the process reflect the ESBL-/AmpC-producing *E. coli* colonisation level entering the food chain from the slaughterhouse. The data derived from monitoring of HEI 6 can be used to set ESBL-/AmpC-producing *E. coli* targets for slaughterhouses as referred to

in the EFSA Scientific Opinion (EFSA, 2012). The historical data from the implementation of HEI 6 provide information on the status of the slaughterhouse as regards ESBL-/AmpC-producing *E. coli* contamination and its ability to control the contamination.

The proposed HEIs give different types of information on the risk of ESBL-/AmpC-producing *E. coli* colonisation in poultry or contamination of the carcasses and risk managers should choose the HEIs to be applied and then also interpret the available information in the appropriate way. The indicators may be used alone or in different combinations.

4.3.6. Harmonised monitoring requirements

Animal population

- At farm:
 - breeding flocks
 - consignments of 1-day-old chicks
 - poultry flocks providing birds for slaughter
- At slaughterhouse: slaughter batches of poultry.

Farms are subjected to an audit regarding the production system standards to define the safety, biosecurity and controlled housing conditions. Use of antimicrobials at farms and hatcheries should be included in the food chain information.

Stage of the food chain

- Farm for poultry flocks.
- Farm for controlled housing conditions and use of antimicrobials.
- Slaughterhouses for slaughter batches of poultry.

Sampling

HEI 1 Breeding flocks (elite, grandparent and parent flocks) producing chicks for meat production

- Objective: classify the flock as ESBL-/AmpC-producing *E. coli* positive or negative.
- Target population: the flock (all flocks requiring risk categorisation should be included) at farm level or by hatchery sampling.
- Epidemiological unit: the flock.
- Sampling strategy: all flocks should be sampled in the beginning of the production (laying) period. If adequate (negative) historical testing has been performed, and audits have demonstrated a low risk of introduction of colonisation, this interval may be decreased (based on the principles described in Annex 3 of the report on HEIs for meat inspection of swine (EFSA, 2011e)).

HEI 2 One-day-old chicks at farm

- Objective: classify the flock or consignments as colonised or uncolonised with ESBL-/AmpC-producing *E. coli*.
- Target population: delivered 1-day-old chicks.
- Epidemiological unit: consignment of chicks.

- Sampling strategy: all consignments should be sampled. If adequate (negative) historical testing has been performed, and audits have demonstrated a low risk of introduction of colonisation, this interval may be decreased (based on the principles described in Annex III(4), pages 101–103, of the report on HEIs for meat inspection of swine (EFSA, 2011e)).

HEI 3 Fattening poultry flocks at farm

- Objective: classify the flock as ESBL-/AmpC-producing *E. coli* positive or negative.
- Target population: all flocks submitting birds to be slaughtered.
- Epidemiological unit: the flock.
- Sampling strategy: all flocks should be sampled. If adequate (negative) historical testing has been performed, and audits have demonstrated a low risk of introduction of colonisation, this interval may be decreased (based on the principles described in Annex 3 of the report on HEIs for meat inspection of swine (EFSA, 2011e)).

HEI 4 Farm audits - controlled housing conditions

- Objective: estimate the likelihood of introduction of ESBL-/AmpC-producing *E. coli* infection into farms by the environment.
- Target population: all farms.
- Epidemiological unit: the farm.
- Sampling strategy: each farm requiring risk-classification to be audited.
- Audit interval: audit of farms repeated at a frequency (to be determined by risk managers) adequate to characterise the risk of ESBL-/AmpC-producing *E. coli*.

HEI 5 Food chain information on use of antimicrobials

- Objective: estimate the likelihood of introduction of ESBL-/AmpC-producing *E. coli* infection into farms due to the use of antimicrobials.
- Target population: all poultry slaughter batches sent to slaughterhouse and all consignments of chicks sent to fattening farms.
- Epidemiological unit: the slaughter batch or consignment of chicks.
- Sampling strategy: census; all slaughter batches of poultry and all consignments of chicks are to be accompanied by the information on use of antimicrobials during its/their lifetime, including also use of antimicrobials at hatchery and farm, specifying the substance used.

HEI 6 Carcass contamination after the slaughter and chilling

- Objective: estimate the prevalence of contaminated carcasses and enumerate ESBL-/AmpC-producing *E. coli* at the slaughterhouse (after processing and chilling), and to assess the capacity of the slaughterhouse to limit the contamination.
- Target population: the slaughter population.
- Epidemiological unit: the slaughter batch.
- Sampling strategy:
 - Representative sample (random or systematic).
 - Microbiological testing of neck and/or breast skin samples.
- Sample size:

- Adequate to assess the difference in prevalence and extent of ESBL-/AmpC-producing *E. coli* colonisation/contamination before and after processing (calculated as described in Annex 3 of report on HEIs for meat inspection of swine (EFSA, 2011e)).
- Survey interval:
 - Initial survey.
 - Repeated at a frequency (to be determined by risk managers) adequate to characterise the slaughterhouse risk (required particularly when procedures in the slaughterhouse change).

Type and details of sample

- Parts of transport box paper at farm for day-old chicks (HEI 2).
- Pooled faeces samples at farm (HEI 1 and 3): faeces or boot swabs collected for example in accordance with the sampling schemes for *Salmonella* included in Regulations No 646/2007, 584/2008 and 200/2010.
- Caecal contents samples at the slaughterhouse, directly after evisceration:
 - Collection of one caecum/carcass and at the laboratory the necessary amount of the caecum content is aseptically collected for analysis. An example of caecal sample collection is given in the EU baseline survey on broiler carcasses laid down by Commission Decision 2007/516/EC.
- Neck and/or breast skin samples at the slaughterhouse (e.g. as foreseen in the EU baseline survey on broiler carcasses laid down by Commission Decision 2007/516/EC).
- Questionnaire-based audit of farm procedures including specific conditions for ESBL-/AmpC-producing *E. coli*
- Food chain information concerning the use of antimicrobials during rearing of the flock including at the hatcheries

Diagnostic/analytical methods

In line with the scientific report of EFSA on technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food (EFSA, 2012c), it is not possible to provide comprehensive and exhaustive recommendations on the analytical methods to be used for ESBL-/AmpC-producing *E. coli* to cover all scenarios, and the scheme outlined is intended to cover what are considered to be the current issues of importance. This following recommendation will need periodic revision and updating, particularly according to epidemiological situations and results that could be obtained during a suggested EU baseline survey on ESBL-mediated resistance or investigations of the relative merits of the different methods.

Use of TBX (tryptone bile X-glucuronide) or another chromogenic medium specific for *E. coli* is recommended to avoid identification of the colonies belonging to other species within Enterobacteriaceae or even other bacterial species able to grow on less specific media, such as MacConkey medium. As stated in the EFSA scientific report (EFSA, 2012c), enrichment with or without cephalosporin may influence bacterial conjugation and exchange of resistance plasmids between bacteria, but it will increase the sensitivity of the method. Thus, enrichment is necessary for samples supposed to contain low numbers of ESBL-/AmpC-producing *E. coli*, such as papers from transport boxes for day-old chicks. For other samples, such as pooled faeces or skin samples, it is considered that at least in some Member States the numbers of ESBL-/AmpC-producing *E. coli* may be high enough to allow their direct enumeration on cefotaxime- or ceftriaxone-supplemented TBX or another chromogenic medium specific for *E. coli* according to ISO 16649-2 method (or equivalent

method). Cefotaxime (1 mg/L) (2 mg/L) is used as recommended by the EFSA Opinion (EFSA, 2011d). Further discussion on the analytical methods to be used for ESBL-/AmpC-producing *E. coli* can be found in the scientific report mentioned above (EFSA, 2012c).

Case definition

- Finding and counts of ESBL-/AmpC-producing *E. coli* in a sample.
- Farms found not complying with the controlled housing conditions.
- Flocks found to be treated with antimicrobials.

5. Generic harmonised epidemiological indicators

5.1. Use of process hygiene criteria in the slaughterhouse

Salmonella, *Campylobacter* as well as *E. coli* and *Salmonella* harbouring ESBL/AmpC are carried in the gastrointestinal tract and/or on the feathers of birds presented for slaughter, and carcasses become contaminated as a result of direct or indirect contamination that is highly dependent on the slaughterhouse technology. Although technical aspects of individual steps of poultry slaughter line may vary considerably between slaughterhouses, the type and generally the order in which these steps are carried out are less variable.

The Opinion (EFSA, 2012b) states that each slaughterhouse can be viewed as unique, owing to differences in poultry slaughtered, logistics, processing practices, plant layout, equipment design and performance, standardised and documented procedures, personnel motivation and management, and other factors. These variations, individually and their combinations, lead to between-slaughterhouse differences in risk reduction capabilities and, consequently, in the microbiological status of the final carcase.

Currently, EU legislation requires food business operators, as part of their food safety management systems, to identify and remove visible contamination (gastrointestinal spillages, faecal or other contamination) during slaughter. It is accepted that visible inspection does not always identify all faecal contamination, and thus process hygiene is evaluated by sampling poultry carcasses (neck skin) for *Salmonella* in accordance with Regulation No 2073/2005. This sampling involves only a small proportion of the slaughter batches in most slaughterhouses but, even so, a more targeted testing could be used to more accurately identify the need for hygiene improvements and to validate the changes made. Regulation No 2073/2005 also lays down process hygiene criteria for *Salmonella* and Enterobacteriaceae for carcasses of cattle, sheep, goat, horses and pigs.

A few studies have reported the variability of poultry slaughterhouses in respect of the microbiological status of carcasses. A relationship was reported between slaughterhouse operational hygiene inspection scores and *Campylobacter* contamination in broiler carcasses (Habib et al., 2012). The EU baseline survey on *Campylobacter* on broilers and their carcasses demonstrated that some slaughterhouses were more capable than others in preventing or reducing *Campylobacter* contamination and the *Campylobacter* counts on the carcasses (EFSA, 2011a). Consequently, a risk categorisation of slaughterhouses is possible, based on the assessment of individual hygiene process performance. This requires a standardised methodology and criteria for assessment of process hygiene. Until 2006 such a scoring system was implemented in slaughterhouses across Great Britain using the hygiene assessment system (HAS), in order to identify and encourage improvements (Pinillos et al., 2008).

5.2. Using generic indicators for microbiological hazards and process hygiene

The use of generic indicators for the biological hazards and process hygiene at slaughterhouse should be considered where

- there are no suitable epidemiological indicators for biological hazards for which the faecal contamination or contamination of carcasses by contaminated slaughter line are relevant transmission routes; or
- risk managers judge the use of a generic indicator to be preferable to alternative methods, e.g. for *Salmonella* or *Campylobacter*, for example because of the low prevalence of the pathogens; or
- there is a need to address new and emerging biological hazards for which faecal contamination or cross-contamination of carcasses are relevant transmission routes.

5.3. Use of *E. coli* as generic indicator of faecal contamination and process hygiene

Overall process hygiene is influenced by two major factors: the adequacy of slaughter and dressing techniques and the effectiveness of plant sanitation programmes. Risk for the consumer linked to the poor slaughterhouse evisceration practices can be assessed by the presence of pathogenic bacteria after chilling. The presence of pathogens on carcasses after chilling is influenced by many factors including, for example, the variation in bird size within a slaughter batch, the poor alignment of automated evisceration equipment to cater for bird size, the accidental rupture of the digestive tract and the spillage of gut contents. By using a generic indicator in a slaughter line, it is possible to evaluate the risk of faecal contamination of the carcasses with biological hazards originating from the gut contents and the risk of cross-contamination between the carcasses for these hazards during slaughtering. The risk of contamination of the carcasses with biological hazards from contaminated slaughter line surfaces or equipment can also be assessed by the indicator.

When choosing the correct indicator organism to assess process hygiene, the following must be considered:

- The organism must be distributed over a wide range of values in numbers of bacteria present.

It must be capable of being used to assess the degree of faecal and cross contamination of the carcasses;

- It must be capable of being used to assess plant sanitation.
- It must identify the at-risk steps associated with carcass dressing.
- It must be capable of indicating the trends in general process hygiene over time;
- It must be identified following an easy and satisfactory analytical method.

Among the possible indicators that may be considered, it is suggested that *E. coli* is one of the most relevant. Its prevalence in the gut is 100 %, and thus enumeration of *E. coli* on the carcass is a very good indicator of faecal contamination during processing. Enteric commensal bacteria carrying chromosomal and transferable antimicrobial resistance genes, such as generic *E. coli*, can occasionally also infect humans and are good indicators of occurrence of antimicrobial resistance and therefore are identified as constituting a relevant public health risk.

Enterobacteriaceae are also used as a hygiene indicator for food including fresh meat and, for example, EU Regulation No 2073/2005 includes an Enterobacteriaceae process hygiene criterion for carcasses of cattle, sheep, goat, horses and pigs. However, *E. coli* is often considered a more specific indicator for faecal contamination (Craven et al., 2003; Baylis et al., 2006). This is because certain psychrotrophic strains of Enterobacteriaceae may multiply in meat and the Enterobacteriaceae also includes bacteria that are not always of faecal origin. By comparison, *E. coli* is generally of faecal origin, and growth of this organism under refrigeration conditions remains minimal (Baylis et al., 2006).

E. coli is a normal inhabitant of the intestinal tract of birds and warm-blooded mammals, and is commonly used as an indicator of faecal contamination and hygienic food handling and processing. Thus, there is a general recognition in the scientific literature that indicator microorganisms are much better suited for use in process hygiene assessment than pathogenic microorganisms (Bolton et al., 2000; Koutsoumanis and Sofos, 2004; Blagojevic et al., 2011). This is due mainly to the varying frequency at which pathogens occur in animals/on carcasses. Additionally, pathogens are often more difficult to count/quantify and require more laborious handling in better-equipped laboratories.

Berrang et al. (2004) evaluated the impact on intestinal content contamination of the broiler carcass on the counts of *Campylobacter*, *E. coli* and coliforms and total count. The application of approximately 10 mg of caecal contents did not result in significantly higher numbers of total aerobic bacteria but did cause a significant increase in the numbers of *E. coli*, coliforms and *Campylobacter*. In case of application of 5 mg of caecal contents, a significant increase was observed only in *Campylobacter* counts. The numbers of *E. coli*, coliforms and total aerobic bacteria did not increase. When only about

2 mg of caecal contents was applied to broiler carcass halves, no significant increase in any of the bacteria enumerated was noted.

A useful reference for the design of a process control plan was outlined by Griffith (1996), who reported that the setting of a specific number of *E. coli* could be used to define two distinct groups of establishments, those with higher and those with lower means. Such a value also suggested a possible tolerance above the mean for the purpose of process control. It was also suggested that it may be possible to develop control plans that define acceptable frequencies for small, medium and large deviations above the process mean.

The potential use of *E. coli* numbers as a measure of slaughter process control has been recognised by regulatory agencies and food business operators. The United States Department of Agriculture's (USDA) hazard analysis and critical control point rule (USDA, 1996) specifies two criteria for evaluating process control: establishments are to maintain less than 100 cfu of *E. coli* per mL in 80 % of poultry carcass rinses and never exceed 1 000 cfu/mL. Surveys have been performed to define precise *E. coli* performance criteria for poultry (Ghafir, 2008), to monitor microbial reduction during slaughter processing (Gill, 2006), and to validate interventions to reduce microbial numbers on poultry (Stopforth et al., 2007).

Measuring *E. coli* at the end of the slaughter line or after chilling could be a means to verify the efficiency of microbial process controls that are designed to ensure sanitary conditions on carcasses. Using *E. coli* as an indicator organism to assess process hygiene at the slaughterhouse level can form the basis for risk classification of the slaughterhouses. However, it must be recognised that the scientific literature contains only a limited amount of data on the quantitative levels of *E. coli* on poultry carcasses from slaughterhouses in the EU and on the usefulness of a process hygiene criteria based on *E. coli* counts.

Pathogen testing is valuable for the purposes of consumer exposure assessment and pathogen reduction programmes and for such purposes testing for *E. coli* cannot substitute for testing for pathogens.

5.3.1. Proposed harmonised epidemiological indicators

The epidemiological indicators selected for faecal contamination of poultry carcasses are shown in Table 5 and Figure 4.

Table 5: Harmonised generic epidemiological indicators for faecal contamination of poultry

Indicators(animal/food category/other)	Food chain stage	Analytical/diagnostic method	Specimen
HEI 1 Generic <i>E. coli</i> in birds – carcasses after slaughter process and chilling	Slaughterhouse	Microbiology - <i>E. coli</i> - enumeration	Neck skin/breast skin

Generic *E. coli* is suggested as an indicator for faecal contamination of the carcasses during the slaughter process.

HEI 1 provides information on the overall level of carcass contamination after the slaughter and chilling processes. The microbial counts found at this point reflect the extent of the faecal contamination taking place during the slaughter process. In addition, in combination with HEI 5 for ESBL-/AmpC-producing *E. coli* HEI1 can be used to assess the proportion of the ESBL-/AmpC-producing *E. coli* on the carcasses and entering the food chain.

Collection and analysis of data from the implementation of HEI 1 would enable a continuous monitoring of the abattoir's performances over time and thereby be an indicator of the efficiency of the technology and hygiene-based process to reduce the final microbial load of the carcasses. Such analyses could indicate whether the abattoirs are improving or whether they might be failing to maintain previously high standards. An assessment of historical data could also be used for adjusting the sampling frequency of the main hazards in order to focus control efforts where the process hygiene does not ensure satisfactory sanitary conditions.

It is also possible to use Enterobacteriaceae as a more general process hygiene indicator.

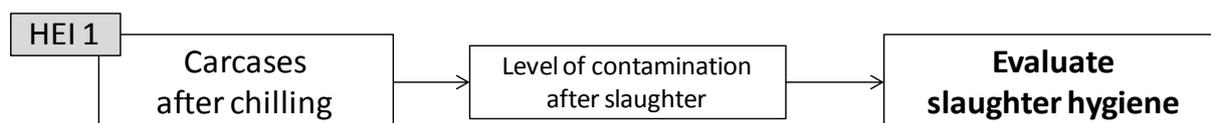


Figure 4: Schematic diagram illustrating the purpose of the harmonised epidemiological indicator for generic *E. coli* in poultry

5.3.2. Harmonised monitoring requirements

Animal population

- Slaughtered poultry.

Stage of the food chain

- The slaughterhouse.

Sampling

HEI 1 Carcass contamination after slaughter and chilling

- Objective: estimate the prevalence of poultry carcasses contaminated with generic *E. coli* after processing in order to assess the capacity of the slaughterhouse to prevent contamination.
- Target population: Carcasses at the slaughterhouse.
- Epidemiological unit: the slaughterhouse.
- Sampling strategy:
 - Representative sample (random or systematic).
- Sample size: calculate to enable assessment of whether the prevalence of indicator is above a threshold defined by the risk manager. Additional guidance on the sample size selection is given in Chapter 7 of this report.
- Survey interval:
 - Periodic survey as required.

Type and details of sample

Neck skin and/or breast skin at the end of slaughter line. An example of sampling details is given in Commission Decision 2007/516/EC concerning a financial contribution to the baseline survey of *Campylobacter* spp. and *Salmonella* spp. in broilers and broiler carcasses.

Diagnostic/analytical methods

- ISO method 16649-2.

Case definition

- Number of *E. coli* bacteria in a sample above an agreed limit.

6. Combined sampling and audits for the epidemiological indicators

HEIs including sampling or audits at farm and at slaughterhouse have been proposed for *Salmonella*, *Campylobacter*, ESBL-/AmpC-producing *E. coli* and generic *E. coli* 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 6.

Table 6: Proposed combined sampling for the epidemiological indicators in poultry

Animal/food category	Hazard (related HEI)	Food chain stage	Type of sample	Combined sampling
Breeding flocks	<i>Salmonella</i> (HEI 1) ESBL-/AmpC-producing <i>E. coli</i> (HEI 1)	Farm	Pooled faeces samples	Same sample or same sampling session
Poultry farms	<i>Salmonella</i> (HEI 3) <i>Campylobacter</i> (HEI 2) ESBL-/AmpC-producing <i>E. coli</i> (HEI 4)	Farm	Audit	Same audit session
Poultry flocks 3 weeks prior to slaughter	<i>Salmonella</i> (HEI 2) ESBL-/AmpC-producing <i>E. coli</i> (HEI 3)	Farm	Pooled faeces (boot swabs)	Same sample or same sampling session
Bird carcasses after slaughter process	<i>Salmonella</i> (HEI 4) <i>Campylobacter</i> (HEI 5) ESBL-/AmpC-producing <i>E. coli</i> (HEI 6) Generic <i>E. coli</i> (HEI 1)	Slaughterhouse (after chilling)	Neck/breast skin	Same sample or same sampling session

6.1. On-farm sampling

On-farm sampling of breeding poultry flocks will provide information on the risk of introducing *Salmonella* and ESBL-/AmpC-producing *E. coli* in poultry farms from infected/colonised breeding flocks. Specifically, microbiological testing of pooled faecal samples has been recommended for HEI 1 of both *Salmonella* and ESBL-/AmpC-producing *E. coli*. The same pooled faecal samples (collected during the same sampling session and sent to the same microbiology laboratory) could therefore be used for culture detection of *Salmonella* and ESBL-/AmpC-producing *E. coli* for testing both hazards.

On-farm sampling of poultry flocks prior to slaughter including collection of pooled faecal samples (boot swabs) a maximum of 3 weeks before slaughter has been recommended for *Salmonella* (HEI 2) and ESBL-/AmpC-producing *E. coli* (HEI 3), while samples of caecal droppings collected 2–3 days prior to slaughter have been proposed for *Campylobacter* (HEI 1). Out of these, the sampling for *Salmonella* and ESBL-/AmpC-producing *E. coli* can be combined since the timeline and type of sample is similar. The same pooled faecal samples could be sent to the same microbiology laboratory and used for culture detection of *Salmonella* and ESBL-/AmpC-producing *E. coli* for testing both hazards.

Furthermore, on-farm audits for controlled housing conditions are proposed for *Salmonella*, *Campylobacter* and ESBL-/AmpC-producing *E. coli*, and these audits, possibly using a combined questionnaire, may be carried out during same audit visit.

6.1.1. Slaughterhouse sampling

HEIs at the end of the slaughter line (after slaughter and chilling) have been proposed for *Salmonella*, *Campylobacter*, ESBL-/AmpC-producing *E. coli* and generic *E. coli* using neck/breast skin samples. It is possible to use the same sampling sessions and even same samples for these different micro-organisms. For example, the sampling details and specimen instructions given in Commission Decision 2007/516/EC foresee the same neck skin sample being used for both *Campylobacter* and *Salmonella*. As the sample is intended for microbial examination of *Salmonella*, *Campylobacter*, ESBL-/AmpC-producing *E. coli* and generic *E. coli*, a unique sample can be collected for the different tests.

6.1.2. Handling of samples in the laboratory

A sample taken by a combined sampling session can be used for the microbial examination of different parameters.

For boot swab samples, a fixed volume of the pre-enrichment for the isolation of *Salmonella* (buffered peptone water, BPW) can be added. After homogenisation, a small volume (maximum 5 mL) can be taken for the enumeration of the ESBL-/AmpC-producing *E. coli*.

For all other sample types (faeces, neck and breast skin) a 26-g sample can be taken. After homogenisation in 234 mL of BPW, 10 mL of homogenate has to be withdrawn and can be used for the enumeration of other bacteria, such as *Campylobacter*, ESBL/AmpC *E. coli* and generic *E. coli*. The rest of the homogenate is used for *Salmonella* testing. This procedure was used in the EU-wide baseline survey on *Salmonella* and *Campylobacter* on broiler carcasses (Commission Decision 2007/516/EC).

7. Sampling strategies to be used when estimating epidemiological indicators

The sampling strategy or plan describes the methodology used for selecting the sample from the population (EFSA, 2006). The strategy should be aligned to the objectives of the surveillance (representative or risk based), the population of interest, as well as the constraints of the environment in which sampling is to be done. This section and Table 7 provides a number of examples of different sampling strategies that may be used in the collection of data for HEIs in poultry.

Details on methods for calculating appropriate sample sizes are described in Annex 3 of the report on HEIs for meat inspection of swine (EFSA, 2011e).

Table 7: Examples of sampling strategies for different sampling locations and populations of interest

Sampling location	Possible units of interest	Example sampling strategies
On-farm	Flock	Systematic transects
Slaughterhouse	Slaughter batch	Systematic

7.1. On-farm sampling

On-farm sampling is used in several of the HEIs to assess the farm, flock or batch status, including:

- *Salmonella*
 - Boot swabs/pooled faeces.
- *Campylobacter*
 - Caecal droppings.
- ESBL/AmpC *E.coli*
 - Boot swabs/pooled faeces.

In all these cases, pooled analysis is usually used to make a single assessment of farm/flock status (either positive/negative or, in some cases, a quantitative assessment based on the number of colony-forming units cultured). The sampling approach thus aims to collect a limited number of separate samples (between two and five boot swabs, for example), which aim to present the status of the entire flock. In most cases, the objective is to assess the presence or absence of the agent in question. Representative sampling of the flock may be used for this purpose, but risk-based sampling (collecting samples in such a way as to increase the probability of detecting disease if it is present) will provide a greater sensitivity for the test. Risk-based sampling can be justified only if appropriate risk factors for stratifying the population are available.

The methodology for pooled faeces sampling and boot swab sampling is described in the specific EU Regulations as regards the EU targets for the reduction of the prevalences of *Salmonella* in poultry flocks (Regulation (EC) No 200/2010, (EC) 517/2011, (EC) 646/2007, (EC) 584/2008). These Regulations specify:

- For faeces
 - A number of sites at random (breeding and laying hens flocks).
- For boot swabs
 - “The samples shall be taken while walking through the house using a route that will produce representative samples for all parts of the house or the respective sector. This

shall include littered and slatted areas provided that slats are safe to walk on. All separate pens within a house shall be included in the sampling.”

While risk-based sampling may have theoretical advantages, it is unlikely that reliable information on risk factors within a single flock will be available to guarantee that one part of the flock or house will have a higher probability than another of having the agent present. The objective should therefore, in practice, be to obtain a representative sample.

In this sampling approach, the infection/colonisation is associated with birds, but sampling of faeces in the environment is used for practical reasons. To obtain a formal representative sample, representative spatial sampling techniques could be used (e.g. the selection of random coordinates identifying different locations in the house). Again, in practice, this approach is likely to be too time-consuming for routine application.

A more practical approach to spatial sampling is to use systematic transects, which aim to represent every part of the house. The exact location of the transects needs to be determined according to the layout of the specific house, but, as indicated in the Regulation, all pens should be included. Some thought should be put into the path chosen to avoid obvious bias. For example:

- If there are parts of the house which are routinely avoided by the poultry and therefore have few droppings, more heavily covered areas should be selected instead.
- If there are paths crossing the house that are regularly used by staff, these should be avoided.

In caged layer flocks, boot swabs cannot be used and transects are inappropriate. Instead, systematic sampling of cages should be used, with either collection of droppings or the use of a hand swab. A method to achieve this is described in the EU regulations on *Salmonella* reduction targets in poultry flocks.

7.2. Slaughterhouse sampling

Slaughterhouse sampling is used for the following HEIs:

- *Salmonella*
 - Neck and breast skin after chilling.
- *Campylobacter*
 - Caecal content at evisceration.
 - Neck and breast skin after chilling.
- ESBL-/AmpC-producing *E. coli*
 - Neck and breast skin after chilling.
- Generic *E. coli*
 - Neck and breast skin after chilling.

In contrast to on-farm sampling, individual birds (carcasses) are directly sampled in the slaughterhouse. The objective is not solely to determine the batch status, but also may be to estimate the prevalence and counts of the bacteria. Unbiased prevalence estimates require that sampling is representative, so risk-based approaches should not be used for slaughterhouse sampling.

For caecal contents at evisceration, systematic sampling (e.g. taking every 100th bird in the chain) is appropriate. The same approach may be used for neck and breast skin sampling after chilling, but the practical sampling procedure and method used to count the sampling interval will depend on how he chilled carcasses are kept.

As with all systematic sampling, the sampling interval should be calculated based on the required sample size and the size of the population being sampled. For example, to take a sample of 30 birds from a batch of 2 000, the sampling interval should be 2 000/30 or 67. Selecting the first bird at random (in this case, using a random number between 1 and 67) before then applying the sampling interval for subsequent birds ensures that sampling will meet the strict definition of random systematic sampling.

7.3. Calculation of sample sizes

7.3.1. General guidelines

Appropriate methods should be used to estimate sample sizes for the different HEIs. Three different approaches are required:

- Demonstration of freedom from infection/colonisation
 - An adequate sample size is required to achieve a target sensitivity (probability of detecting the agent in the population if it is present at a defined prevalence).
- Comparison of means
 - An adequate sample size is required to determine if the mean (in this case, the adjusted mean \log_{10} (count) of *Campylobacter*) as measured at one point (evisceration) is different to the mean at a second point (on skin after chilling). As sampling is occurring at two different points, two sample sizes are required—assumed to be the same if the variance is the same at both sampling points.
- Comparison of proportions (prevalence)
 - Comparison of a prevalence to a threshold
 - Only one sample size is required, adequate to determine if the population prevalence is above or below the threshold.
 - Comparison of two prevalences
 - Two sample sizes are required, to determine if there is a difference in prevalence (in this case, prior to slaughter and after slaughter).

7.3.2. Demonstrating freedom from infection or colonisation

A number of HEIs relate to assessing whether a batch, flock or farm is free from infection or colonisation (e.g. *Campylobacter* HEIs 1, 2 and 3 and *Salmonella* HEIs 1 and 2). This section describes the calculation of sample size required to demonstrate that a population (be it a batch, flock, farm or series of batches in a slaughterhouse) is free from infection or colonisation, based on the assumption of representative sampling. The probability of freedom from infection can be calculated based on the balance between the cumulative evidence from freedom from historical testing (measured in terms of flock- or batch-sensitivity), and the risk of introduction of infection.

In most cases, a flock-level test is applied (e.g. a boot swab or similar), which has a predetermined sample size. However, where individuals (e.g. birds) are being tested, the formula for calculation of the sample size when demonstrating freedom from infection is:

$$n = \ln(1 - SSe) / \ln(1 - P^* Se_a)$$

where SSe is the target surveillance (flock or batch) sensitivity, P^* is the design prevalence, or the hypothetical prevalence of infection that the surveillance is designed to be able to detect, and Se_a is the sensitivity of the individual test (e.g. bird test).

This formula assumes a large population size relative to the sample size, generally a valid assumption in the case of poultry flocks.

Commission Regulation No 200/2010, Annex section 2.2.2.1, provides indicative figures for the number of separate faecal samples to take for pooled faecal testing. These figures are based on the above formula but include the assumption that the individual animal test used is 100 % sensitivity. When this is not true, the recommended figures may fail to meet the target surveillance sensitivity of 95 %.

To illustrate this difference, Table 8 presents the sample size required to achieve a surveillance sensitivity of 95 % depending on the sensitivity of the test used. The effect of design prevalence is also shown.

Table 8: Sample size required when sampling caecal droppings, with a target surveillance sensitivity of 95 %, and using different design prevalence (P^*) values and different assumptions regarding the sensitivity of the individual test

P^*	Test sensitivity							
	40 %	48 %	60 %	70 %	75 %	80 %	90 %	95 %
1 %	748	623	498	427	398	373	332	314
2 %	373	311	249	213	199	186	165	157
5 %	149	124	99	85	79	74	66	62
10 %	74	61	49	42	39	36	32	31
20 %	36	30	24	20	19	18	16	15
50 %	14	11	9	7	7	6	6	5
75 %	9	7	6	5	4	4	3	3
100 %	6	5	4	3	3	2	2	1

The use of a fixed design prevalence is useful for planning sampling; however, it is also useful to assess what level of infection a given sample size should be able to detect with 95 % sensitivity. Table 9 shows the minimum detectable prevalence of infection for a range of test sensitivities and sample sizes.

Table 9: Minimum detectable prevalence of infection based on different sample sizes (N) and test sensitivities

N	Sensitivity							
	40 %	48 %	60 %	70 %	75 %	80 %	90 %	95 %
5	-	94 %	75 %	64 %	60 %	56 %	50 %	47 %
10	65 %	54 %	43 %	37 %	35 %	32 %	29 %	27 %
15	45 %	38 %	30 %	26 %	24 %	23 %	20 %	19 %
20	35 %	29 %	23 %	20 %	19 %	17 %	15 %	15 %
30	24 %	20 %	16 %	14 %	13 %	12 %	11 %	10 %
40	18 %	15 %	12 %	10 %	10 %	9.0 %	8.0 %	7.6 %
50	15 %	12 %	10 %	8.3 %	7.8 %	7.3 %	6.5 %	6.1 %
100	7.4 %	6.1 %	4.9 %	4.2 %	3.9 %	3.7 %	3.3 %	3.1 %
200	3.7 %	3.1 %	2.5 %	2.1 %	2.0 %	1.9 %	1.7 %	1.6 %
300	2.5 %	2.1 %	1.7 %	1.4 %	1.3 %	1.2 %	1.1 %	1.0 %
500	1.5 %	1.2 %	1.0 %	0.9 %	0.8 %	0.7 %	0.7 %	0.6 %

In practice, the target surveillance sensitivity depends on how many historical data are available. A long history of low sensitivity testing but a low risk of introduction of infection (good biosecurity) will provide a high probability of freedom, as will a single round of testing with very high flock sensitivity.

A Bayesian estimate can be made of the extra sensitivity required to achieve a target probability of freedom (the posterior), given a specified prior probability of freedom, using the formula:

$$Se = 1 - [(PF_{\text{prior}} \times (1 - PF_{\text{post}})) / (PF_{\text{post}} \times (1 - PF_{\text{prior}}))]$$

where Se is the required surveillance sensitivity, PF_{prior} is the prior probability of freedom (based on historical testing) and PF_{post} is the posterior probability of freedom (the target).

The two formulae presented here are derived from the formulae presented by Martin (2008).

7.3.3. Calculating sample sizes for evisceration caecal samples and skin swabs

For *Campylobacter*, HEI 4 and HEI 5 involve estimating the mean \log_{10} count in caecal and skin samples, respectively. The purpose is to compare counts before and after slaughter to assess the capacity of slaughterhouses to manage cross-contamination.

When comparing two means, sample size calculations aim to ensure that any observed differences are likely to be real rather than being due to chance. The factors influencing sample size are therefore:

- the magnitude of difference between the two means that one wants to be able to detect
- the confidence and power, describing the chances of either concluding there is a difference when there is not, or concluding there is not when there actually is
- the variance in the population.

This last factor plays an important role. If there is a great deal of variation in the population, an estimate of the mean using a small number of samples will be relatively imprecise. This is because, by chance, there is a risk that many of the samples could be either larger or smaller than the true mean. In contrast, when there is very little variability in the population, even with a small number of samples, each sample must be close to the true mean, so there is little chance of making an error.

The formula for sample size has been described by Fleiss et al. (1980) and is rather unwieldy. There are numerous free on-line calculators implementing this formula which are much more convenient for everyday use, for example, EpiTools (Sample size calculations → Compare two means) (Sergeant, 2009).

Unfortunately, this approach cannot be directly applied when comparing HEI 4 and HEI 5, as they are based on different samples and are expected to have different means. Instead, establishment of the normal relationship between the two measures is first required, allowing one to be adjusted to the same scale as the other. Sample size calculation should be made based on these adjusted figures.

Table 10 illustrates example sample sizes required for a number of different situations. While this may be used as a guide, it is better to use the on-line calculator cited above to determine the appropriate sample size for each particular situation. The sample sizes indicated are based on achieving 95 % confidence and 80 % power, based on a two-tailed test (implying that the alternative hypothesis is that the two values are different without assuming which is larger than the other). Variance is expressed in terms of the coefficient of variation (variance divided by the mean) and the difference to be detected is expressed as the percentage difference between the means ($\mu_1 - \mu_2/\mu_1$).

Table 10: Example sample sizes required to compare two means with confidence of 95 % and power of 80 %, based on varying coefficients of variation and varying percentage differences between the means to be compared

Ratio of means % difference	0.95 5 %	0.9 10 %	0.85 15 %	0.8 20 %	0.75 25 %	0.7 30 %	0.65 35 %	0.6 40 %	0.55 45 %	0.5 50 %
Coefficient of variation										
5 %	16	4	2	1	1	1	1	1	1	1
10 %	61	15	7	4	2	2	1	1	1	1
15 %	137	33	14	8	5	3	2	2	2	1
20 %	244	58	25	13	8	6	4	3	2	2
25 %	381	91	38	21	13	8	6	4	3	3
30 %	548	130	55	29	18	12	8	6	5	3
35 %	745	177	75	40	24	16	11	8	6	5
40 %	974	231	97	52	31	21	14	10	8	6
45 %	1,232	292	123	66	40	26	18	13	10	7
50 %	1,521	361	152	81	49	32	22	16	12	9
55 %	1,840	437	184	98	59	39	27	19	14	11
60 %	2,190	519	219	116	70	46	32	23	17	12
65 %	2,570	609	256	136	82	54	37	26	19	15
70 %	2,980	707	297	158	95	62	43	31	22	17
75 %	3,421	811	341	181	109	71	49	35	26	19
80 %	3,893	923	388	206	124	81	56	40	29	22
85 %	4,394	1042	438	233	140	91	63	45	33	25
90 %	4,926	1168	491	261	157	102	70	50	37	27
95 %	5,489	1301	547	291	175	114	78	56	41	31
100 %	6,082	1442	606	322	194	126	87	62	45	34

7.3.4. Calculating sample sizes for *Salmonella* skin samples

For *Salmonella* HEI 4, the objective is to assess the prevalence of carcasses after chilling that are positive for *Salmonella*. This may be compared either with the prevalence prior to slaughter or with some acceptable standard as defined by risk managers.

7.3.4.1. Evaluating prevalence against a standard threshold

Calculation of sample size for the number skin samples required is based on the estimate of a prevalence values. The aim is to be confident that the observed prevalence is truly below the threshold value. The principles of this calculation are described in detail in Annex 4 of the report on HEIs for meat inspection of swine (EFSA, 2011e). Briefly, the sample size calculation requires the following parameters:

- Estimated true proportion
 - This is the estimated prevalence. If uncertain, err towards 50 %.
- Confidence level
 - For consistency, this should always be set to a standard value of 0.95.
- Desired precision (\pm)

- This should be equal to the difference between the expected true proportion and the standard threshold. For instance, if it is expected that the prevalence is 2 %, and the threshold is 3 %, then the precision should be $3 \% - 2 \% = 1 \%$.
- Population size
 - This is the size of the population under study. If the batch prevalence is being estimated, the population size is equal to the number of birds in the batch.

Calculation of the sample size may be done using the on-line calculator cited above (Sample size calculations → Estimate a single proportion). Examples of sample sizes for different circumstances are shown in Table 11.

Table 11: Sample size required to compare a prevalence to a threshold value, based on different expected prevalence and threshold values (using a fixed confidence level of 95 %), assuming a large population (>10 000 birds)

Estimated prevalence	Precision required (\pm)																
	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1	0.12	0.14	0.16	0.18	0.2	0.3	0.4
0.01	195	49	22	13	8	6	4	4	3	2	2	1	1	1	1	1	1
0.05	931	233	104	59	38	26	19	15	12	10	7	5	4	3	3	2	1
0.1	1,764	441	196	111	71	49	36	28	22	18	13	9	7	6	5	2	2
0.15	2,499	625	278	157	100	70	51	40	31	25	18	13	10	8	7	3	2
0.2	3,136	784	349	196	126	88	64	49	39	32	22	16	13	10	8	4	2
0.25	3,675	919	409	230	147	103	75	58	46	37	26	19	15	12	10	5	3
0.3	4,116	1,029	458	258	165	115	84	65	51	42	29	21	17	13	11	5	3
0.35	4,459	1,115	496	279	179	124	91	70	56	45	31	23	18	14	12	5	3
0.4	4,704	1,176	523	294	189	131	96	74	59	48	33	24	19	15	12	6	3
0.45	4,851	1,213	539	304	195	135	99	76	60	49	34	25	19	15	13	6	4
0.5	4,900	1,225	545	307	196	137	100	77	61	49	35	25	20	16	13	6	4
0.55	4,851	1,213	539	304	195	135	99	76	60	49	34	25	19	15	13	6	4
0.6	4,704	1,176	523	294	189	131	96	74	59	48	33	24	19	15	12	6	3
0.65	4,459	1,115	496	279	179	124	91	70	56	45	31	23	18	14	12	5	3
0.7	4,116	1,029	458	258	165	115	84	65	51	42	29	21	17	13	11	5	3
0.75	3,675	919	409	230	147	103	75	58	46	37	26	19	15	12	10	5	3
0.8	3,136	784	349	196	126	88	64	49	39	32	22	16	13	10	8	4	2
0.85	2,499	625	278	157	100	70	51	40	31	25	18	13	10	8	7	3	2
0.9	1,764	441	196	111	71	49	36	28	22	18	13	9	7	6	5	2	2
0.95	931	233	104	59	38	26	19	15	12	10	7	5	4	3	3	2	1
0.99	195	49	22	13	8	6	4	4	3	2	2	1	1	1	1	1	1

7.3.4.2. Comparing two prevalence estimates

When comparing the batch prevalence prior to slaughter to the prevalence after slaughter, a slightly different calculation is required in order to take into account the uncertainty in both estimates. This calculation can be achieved using the same parameters, with the on-line calculator (Sample size calculations → Compare two proportions).

Table 12 illustrates sample sizes required to compare two proportions.

Table 12: Two-tailed sample size required for each group to detect a difference between the prevalence in two populations with 95 % confidence and 80 % power, based on the estimated proportion in each population

Population 1	Population 2											
	0.01	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0.95
0.99	5	5	6	7	9	12	15	21	30	50	121	333
0.95	5	6	7	8	11	14	19	27	43	88	474	
0.9	6	7	8	10	13	17	25	38	72	219		
0.8	7	8	10	13	19	28	45	91	313			
0.7	9	11	13	19	29	49	103	376				
0.6	12	14	17	28	49	107	408					
0.5	15	19	25	45	103	408						
0.4	21	27	38	91	376							
0.3	30	43	72	313								
0.2	50	88	219									
0.1	121	474										
0.05	333											

8. Comparable data on the harmonised epidemiological indicators

8.1. *Salmonella*

8.1.1. Comparable data on farm-level indicators

In the case of *Salmonella*, comparable data from the EU Member States (MSs) on the proposed farm-level indicators (HEI 1 and HEI 2 in Table 1) are available from the *Salmonella* control programmes in breeding flocks of *Gallus gallus*, broilers and breeding and fattening turkeys (presented in Tables 13–16).

Table 13: *Salmonella* in breeding flocks of *Gallus gallus* during the production period (all types of breeding flocks, flock-based data) in countries running control programmes in accordance with Regulation (EC) No 2160/2003, 2009–2010 (Source: Table SA16, ECDC and EFSA, 2012a)

Country	Breeding flocks (elite, grandparent and parent)												
	2010									2009			
	N	% positive									N	% positive	
		pos (all)	S target serovars ¹	<i>S. Enteritidis</i>	<i>S. Typhimurium</i>	<i>S. Infantis</i>	<i>S. Virchow</i>	<i>S. Hadar</i>	Other serovars, non-typeable, and unspecified	pos (all)		S target serovars ¹	
Austria ²	124	0	0	0	0	0	0	0	0	120	1.7	0.8	
Belgium ³	568	3.9	0.5	0.2	0.2	0	0	0.2	3.5	526	3.0	0	
Bulgaria	1,831	0.3	<0.1	0	0	<0.1	0	0	0.3	2,193	1.2	0.9	
Cyprus ⁴	44	0	0	0	0	0	0	0	0	55	1.8	1.8	
Czech Republic	586	1.9	1.4	1.0	0.3	0	0	0	0.5	620	1.5	1.0	
Denmark	227	2.2	2.2	0	1.3	0.9	0	0	0	249	1.6	1.2	
Estonia	4	0	0	0	0	0	0	0	0	3	0	0	
Finland	171	0.6	0	0	0	0	0	0	0.6	172	0	0	
France	1,669	1.6	0.5	0.3	0.2	0	<0.1	0	1.1	1,480	1.4	0.2	
Germany	927	0.6	0.3	0.2	0.1	0	0	0	0.3	1,041	1.9	0.9	
Greece	323	0.6	0.6	0	0	0	0	0.6	0	272	10.3	7.0	
Hungary	1,187	1.3	1.3	0.9	0.4	0	0	0	0	714	6.3	2.7	
Ireland	114	1.8	1.8	0	1.8	0	0	0	0	129	0	0	
Italy	956	3.5	0.4	0	0.1	0	0.1	0.2	3.0	512	6.6	1.6	
Latvia	31	0	0	0	0	0	0	0	0	25	0	0	
Lithuania	68	0	0	0	0	0	0	0	0	73	0	0	
Netherlands	925	0.6	0.6	0.5	0.1	0	0	0	0	850	0.6	0.5	
Poland	1,366	3.2	2.5	2.0	<0.1	0.2	0.2	0	0.7	1,056	3.5	2.7	
Portugal	246	0.8	0	0	0	0	0	0	0.8	219	4.1	0.5	
Romania	304	12.8	0.3	0	0	0.3	0	0	12.5	325	1.5	0.6	
Slovakia	49	0	0	0	0	0	0	0	0	129	3.1	2.3	
Slovenia	165	0	0	0	0	0	0	0	0	155	0	0	
Spain ²	1,385	3.8	0.7	0.4	0	0	<0.1	0.2	3.0	1,266	6.6	3.3	
Sweden	155	0	0	0	0	0	0	0	0	162	0	0	
United Kingdom	1,550	1.2	<0.1	0	<0.1	0	0	0	1.2	1,637	1.3	0.1	
EU Total	14,975	2.0	0.7	0.4	0.1	<0.1	<0.1	<0.1	1.3	13,983	2.7	1.2	
Norway	159	0	0	0	0	0	0	0	0	187	0	0	
Switzerland	75	0	0	0	0	0	0	0	0	93	0	0	

Note: Luxembourg and Malta do not have breeding flocks.

1. *S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, *S. Virchow*, *S. Hadar*.
2. Two serovars in one flock in 2009.
3. Two serovars in one flock in 2010.
4. One positive flock in 2009.
5. *S. Typhimurium* includes monophasic *S. Typhimurium*.

Table 14: *Salmonella* in broiler flocks of *Gallus gallus* before slaughter (flock-based data) in countries running control programmes, 2010 (Source: Table SA22, ECDC and EFSA, 2012a)

Country	2010						2009		
	N	% positive					N	% positive	
		pos (all)	<i>S. Enteritidis</i> and/or <i>S. Typhimurium</i>	<i>S. Enteritidis</i>	<i>S. Typhimurium</i>	Other serovars, non-typable, and unspecified		pos (all)	<i>S. Enteritidis</i> and <i>S. Typhimurium</i>
Austria	3,402	2.9	0.6	0.4	0.2	2.3	3,302	3.4	1.1
Belgium ¹	8,481	3.7	0.5	<0.1	0.5	3.3	8,049	3.1	0.5
Bulgaria ²	769	3.3	0.1	0	0.1	3.1	1,152	1.4	0.4
Cyprus	643	19.3	0	0	0	19.3	239	7.9	0
Czech Republic	5,591	6.5	3.9	3.9	<0.1	2.6	6,035	7.4	4.0
Denmark ⁵	3,773	1.1	0.3	<0.1	0.3	0.9	3,767	0.9	0.3
Estonia	434	0	0	0	0	0	414	0	0
Finland	3,070	0.2	0	0	0	0.2	2,972	0.4	0
France ^{4,5}	49,024	7.1	0.4	0.1	0.3	6.7	35,913	8.1	0.5
Germany	4,354	4.4	0.2	0.1	<0.1	4.2	4,339	7.0	0.4
Greece	8,319	0.3	<0.1	<0.1	<0.1	0.3	6,577	0.3	0
Hungary	6,515	13.7	0.3	0.2	0.1	13.4	4,491	32.4	0.4
Ireland	600	0.5	0	0	0	0.5	665	0	0
Italy	13,895	7.3	<0.1	<0.1	0	7.3	2,072	19.2	1.0
Latvia	593	1.2	1.2	1.2	0	0	566	7.1	5.3
Lithuania	198	0.5	0.5	0.5	0	0	218	2.3	2.3
Luxembourg	-	-	-	-	-	-	4	25	0
Malta	587	32.9	4.1	3.6	0.5	28.8	87	31.0	2.3
Netherlands	18,036	3.1	0.3	0.2	0.1	2.8	29,193	2.7	0.2
Poland	26,801	0.9	0.7	0.7	<0.1	0.2	20,665	3.2	1.7
Portugal	7,981	1.8	0.4	0.4	<0.1	1.3	654	5.4	1.8
Romania	6,040	6.4	<0.1	<0.1	0	6.3	3,160	4.8	0.1
Slovakia	2,801	1.6	1.6	1.3	0.3	0	544	14.0	7.7
Slovenia ¹	2,153	1.1	<0.1	0	<0.1	1.2	3,080	0.7	0
Spain	18,344	3.6	0.4	0.4	<0.1	3.2	13,620	6.7	1.6
Sweden	3,702	0.5	0.5	0	0.5	0	2,713	0.1	0
United Kingdom	33,611	1.6	<0.1	0	<0.1	1.5	27,780	1.3	0
EU Total	229,717	4.1	0.4	0.3	0.1	3.7	182,271	5.0	0.7
Norway	4,549	<0.1	0	0	0	<0.1	4,243	0	0
Switzerland	368	2.7	0.3	0.3	0	2.4	740	1.6	0.5

1. More than one serotype found in several samples.

2. For Bulgaria, sample unit is single animal.

3. For the United Kingdom, the number of existing flocks and number of flocks tested is derived from the number of samples submitted to private and Government veterinary laboratories.

4. *S. Typhimurium* includes monophasic *S. Typhimurium*.

5. The number tested is an underestimate as all flocks are tested but not all negative flocks are recorded.

6. More than one serotype found in two flocks in 2010.

Table 15: *Salmonella* in breeding flocks of turkeys during the production period (flock-based data), 2010 (Source: Table SA23, ECDC and EFSA, 2012a)

Country	N	% positive				
		pos (all)	<i>S. Enteritidis</i> and/or <i>S. Typhimurium</i>	<i>S. Enteritidis</i>	<i>S. Typhimurium</i>	Other serovars, non-typeable, and unspecified
Czech Republic	12	50.0	0	0	0	50.0
Finland	10	0	0	0	0	0
France ¹	785	4.3	0.5	0	0.5	3.8
Germany	141	0	0	0	0	0
Greece	4	0	0	0	0	0
Hungary	118	0	0	0	0	0
Ireland	14	0	0	0	0	0
Italy	177	26.6	0	0	0	26.6
Poland	66	13.6	0	0	0	13.6
Slovakia	21	0	0	0	0	0
Spain	17	52.9	5.9	0	5.9	47.1
Sweden	4	0	0	0	0	0
United Kingdom	249	2.8	0	0	0	2.8
Total (13 MSs)	1,618	6.9	0.3	0	0.3	6.6
Norway	15	0	0	0	0	0

1. *S. Typhimurium* result includes the reporting of monophasic variants.

Table 16: *Salmonella* in fattening flocks of turkeys before slaughter (flock-based data), 2010
(Source: Table SA24, EFSA and ECDC, 2012a)

Country	N	% positive				
		pos (all)	<i>S. Enteritidis</i> and/or <i>S. Typhimurium</i>	<i>S. Enteritidis</i>	<i>S. Typhimurium</i>	Other serovars, non-typeable, and unspecified
Austria ¹	355	8.5	0.3	0.3	0	8.2
Belgium	146	0.7	0	0	0	0.7
Czech Republic ¹	283	19.1	0.7	0.4	0.4	18.4
Denmark	24	4.2	0	0	0	4.2
Finland	348	0	0	0	0	0
France ^{2,3}	9,394	7.7	0.6	0.1	0.5	7.1
Germany ²	971	1.0	0.6	0.1	0.5	0.4
Greece	14	7.1	0	0	0	7.1
Hungary	2,997	29.9	0.2	<0.1	0.2	29.7
Ireland	103	1.0	0	0	0	1.0
Italy	2,468	17.7	0.2	0	0.2	17.6
Lithuania	6	16.7	0	0	0	16.7
Netherlands	229	2.6	0	0	0	2.6
Poland	3,434	5.2	0.7	0.3	0.4	4.5
Portugal	25	0	0	0	0	0
Romania	54	13.0	0	0	0	13.0
Slovakia	24	0	0	0	0	0
Slovenia	112	0.9	0	0	0	0.9
Spain	1,316	19.8	1.7	0	1.7	18.2
Sweden	155	0	0	0	0	0
United Kingdom	3,078	15.4	0.1	0	0.1	15.3
Total (21 MSs)	25,536	12.1	0.5	0.1	0.4	11.6
Norway	385	0	0	0	0	0
Switzerland	60	3.3	0	0	0	3.3

1. More than one serovar found in a sample.

2. *S. Typhimurium* result includes the reporting of monophasic variants.

3. The number tested is an underestimate as all flocks are tested but not all negative flocks are recorded.

8.1.2. Comparable data on slaughterhouse-level indicators

The 2008 EU-wide baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses (EFSA, 2010b) provides comparable data from the EU Member States (MSs) on *Salmonella*-contaminated broiler chilled carcasses at the end of the slaughter line (HEI 4; Table 1). This data is presented in Table 17.

Table 17: Prevalence of *Salmonella*-contaminated broiler carcasses, by country and in the EU*, 2008 (Source EFSA, 2010b)

Country	<i>Salmonella</i> spp.-contaminated broiler carcasses			<i>Salmonella</i> Enteritidis and Typhimurium-contaminated broiler carcasses			Other than <i>Salmonella</i> Enteritidis and Typhimurium-contaminated broiler carcasses		
	No of broiler batches	% prevalence ²	95 % CI ²	No of broiler batches	% prevalence ²	95 % CI ²	No of broiler batches	% prevalence ²	95 % CI ²
Austria	408	2.7	1.3 - 5.5	408	0.6	0.3 - 1.3	408	1.9	0.6 - 6.0
Belgium	380	18.7	10.2 - 31.9	380	3.2	1.0 - 10.0	380	11.9	6.1 - 21.9
Bulgaria	316	26.6	20.1 - 34.3	316	6.6	3.0 - 13.6	316	15.6	10.5 - 22.6
Cyprus	357	10.5	7.5 - 14.6	357	0	0 ¹ - 1.0 ¹	357	10.5	7.5 - 14.6
Czech Republic	422	4.9	2.4 - 9.9	422	0.9	0.4 - 2.1	422	3.8	1.5 - 9.4
Denmark	396	0	0 ¹ - 0.9 ¹	396	0	0 ¹ - 0.9 ¹	396	0	0 ¹ - 0.9 ¹
Estonia	102	0	0 ¹ - 3.6 ¹	102	0	0 ¹ - 3.6 ¹	102	0	0 ¹ - 3.6 ¹
Finland	369	0	0 ¹ - 1.0 ¹	369	0	0 ¹ - 1.0 ¹	369	0	0 ¹ - 1.0 ¹
France	422	7.4	3.8 - 13.7	422	0.2	0 - 1.7	422	6.7	3.4 - 13.1
Germany	432	14.5	6.8 - 28.4	432	2.7	0.4 - 16.5	432	9.0	4.5 - 17.2
Hungary	321	85.6	79.5 - 90.1	321	4.6	2.6 - 8.1	321	83.7	76.8 - 88.8
Ireland	394	11.2	3.4 - 31.4	394	0	0 ¹ - 0.9 ¹	394	11.2	3.4 - 31.4
Italy	393	17.4	12.1 - 24.3	393	0.3	0 - 1.8	393	13.4	9.3 - 19.0
Latvia	122	4.9	1.2 - 18.2	122	4.9	1.2 - 18.2	122	0	0 ¹ - 3.0 ¹
Lithuania	374	5.4	2.2 - 12.4	374	0.3	0 - 1.4	374	1.9	0.9 - 3.8
Luxembourg	13	0	0 ¹ - 24.7 ¹	13	0	0 ¹ - 24.7 ¹	13	0	0 ¹ - 24.7 ¹
Malta	367	19.3	12.2 - 29.2	367	0	0 ¹ - 1.0 ¹	367	13.0	6.4 - 24.4
Netherlands	429	10.1	6.2 - 16.1	429	0.2	0 - 1.5	429	9.4	5.9 - 14.6
Poland	419	25.4	20.9 - 30.5	419	9.6	7.0 - 12.9	419	16.0	12.2 - 20.7
Portugal	421	10.4	6.7 - 15.7	421	8.3	5.1 - 13.1	421	1.9	0.8 - 4.5
Romania	357	4.9	2.6 - 9.0	357	0.8	0.2 - 2.9	357	4.1	2.0 - 8.5
Slovakia	422	22.8	7.8 - 50.7	422	5.6	2.6 - 11.7	422	17.2	4.7 - 46.3
Slovenia	413	2.0	0.9 - 4.5	413	0.4	0.3 - 0.5	413	1.4	0.5 - 3.8
Spain	389	14.4	10.1 - 20.2	389	6.8	4.4 - 10.4	389	7.5	4.6 - 11.9
Sweden	410	0.3	0.1 - 1.3	410	0	0 ¹ - 0.9 ¹	410	0.3	0.1 - 1.3
United Kingdom	401	3.6	1.7 - 7.2	401	0	0 ¹ - 0.9 ¹	401	3.4	1.6 - 7.1
EU (26 MS)*	9,249	15.6	13.6 - 17.9	9,249	3.6	2.8 - 4.6	9,249	11.1	9.5 - 13.0
Norway	396	0	0 ¹ - 0.9 ¹	396	0	0 ¹ - 0.9 ¹	396	0	0 ¹ - 0.9 ¹
Switzerland	390	2.3	2.3 - 2.4	390	0.8	0.3 - 1.9	390	1.5	1.0 - 2.4

1. Exact binomial Confidence Interval (CI), the clustering of data is not taken into account.
2. Prevalence estimates and CIs at national as well as at EU level were obtained taking into account correlation among observations within the same slaughterhouse. In addition, at EU level, prevalence estimates and CIs were weighted for the national numbers of slaughtered broilers during 2008. *Greece did not participate in the baseline survey and two non-Member States, Norway and Switzerland, participated.

8.2. *Campylobacter*

The 2008 EU-wide baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses (EFSA, 2010b) provides comparable data from the Member States (MSs) on *Campylobacter*-colonised broiler batches (caecal content) presented in Table 18 at evisceration stage (HEI 4, Table 2) and on *Campylobacter*-contaminated broiler chilled carcasses presented in Table 19 at the end of the slaughter line (HEI 5, Table 2). No comparable data from the Member States are available on the proposed farm-level indicator (HEI 1, Table 2).

Table 18: Prevalence of *Campylobacter*-colonised broiler batches, by country and in the EU*, 2008 (Source: EFSA, 2010b)

Country	No of broiler batches	% prevalence ³	95 % CI ³
Austria	408	47.8 ⁴	41.5 ⁴ - 54.2 ⁴
Belgium	337	31.0	23.6 - 39.4
Bulgaria	275	29.6	21.9 - 38.6
Cyprus	375	30.6	25.7 - 36.0
Czech Republic	422	61.3	56.1 - 66.3
Denmark	396	19.0	15.9 - 22.6
Estonia	102	2.0 ¹	0.5 ¹ - 7.5 ¹
Finland	411	3.9	3.8 - 4.0
France	422	76.1	70.4 - 81.0
Germany	432	48.9	40.3 - 57.7
Hungary	321	50.1	44.5 - 55.7
Ireland	394	83.1	75.2 - 88.8
Italy	393	63.3	54.5 - 71.3
Latvia	122	41.0	17.0 - 70.2
Lithuania	374	41.5	40.7 - 42.2
Luxembourg	12	100	73.5 ² - 100 ²
Malta	367	96.8	95.0 - 98.0
Netherlands	429	24.4	20.3 - 29.0
Poland	419	78.9	74.1 - 83.0
Portugal	421	82.0	76.3 - 86.6
Romania	357	77.0	63.9 - 86.4
Slovakia	422	73.6	63.6 - 81.6
Slovenia	413	78.2	78.1 - 78.2
Spain	389	88.0	84.0 - 91.2
Sweden	410	13.2	8.0 - 21.0
United Kingdom	401	75.3	69.9 - 80.1
EU (26 MS)*	9,224	71.2	68.5 - 73.7
Norway	396	3.2	2.1 - 4.8
Switzerland	296	59.0	55.0 - 62.9

1. As one slaughterhouse contributed to the entire survey, point estimate and 95 % CI are based on logistic regression.

2. Exact binomial CI, the clustering of data is not taken into account.

3. Prevalence estimates and CIs at national as well as at EU level were obtained taking into account correlation among observations within the same slaughterhouse. In addition, at EU level, prevalence estimates and CIs were weighted for the national numbers of slaughtered broilers during 2008.

4. Results assuming independent covariance structure.

* Greece did not participate in the baseline survey and two non-Member States, Norway and Switzerland, participated.

Table 19: Prevalence of *Campylobacter*-contaminated broiler carcasses, based on the combined results of the detection and enumeration method, by country and in the EU*, 2008 (Source: EFSA, 2010b)

Country	N (No of broiler batches)	% prevalence ³	95 % CI ³
Austria	408	80.6	76.7 - 83.9
Belgium	380	52.7	44.8 - 60.5
Bulgaria	280	45.2	38.9 - 51.7
Cyprus	357	14.1	14.0 - 14.2
Czech Republic	422	68.6	65.5 - 71.5
Denmark	396	31.4	26.1 - 37.2
Estonia	102	4.9 ¹	2.1 ¹ - 11.2 ¹
Finland	369	5.5	5.4 - 5.5
France	422	88.7	84.3 - 91.9
Germany	432	60.8	53.6 - 67.7
Hungary	321	55.3	48.9 - 61.6
Ireland	394	98.3	98.0 - 98.5
Italy	393	49.6	39.5 - 59.7
Latvia	122	33.6	11.3 - 66.7
Lithuania	374	45.8	42.0 - 49.6
Luxembourg ²	13	100	75.3 ³ - 100 ³
Malta	367	94.3	93.6 - 95.0
Netherlands	429	37.6	31.8 - 43.7
Poland	419	80.4	75.8 - 84.3
Portugal	421	70.2	58.7 - 79.7
Romania	357	64.2	51.9 - 75.0
Slovakia	422	79.1	68.8 - 86.7
Slovenia	413	77.8	70.7 - 83.6
Spain	389	92.6	89.8 - 94.7
Sweden	410	14.6	8.4 - 24.2
United Kingdom	401	86.3	79.6 - 91.0
EU (26 MS)*	9,213	75.8	73.2 - 78.3
Norway	396	5.1	3.1 - 8.3
Switzerland	408	71.7	63.8 - 78.5

1. As one slaughterhouse contributed to the entire survey, point estimate and 95 % CI are based on logistic regression.
 2. Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples.
 3. Prevalence estimates and CIs at national as well as at EU level were obtained taking into account correlation among observations within the same slaughterhouse. In addition, at EU level, prevalence estimates and CIs were weighted for the national numbers of slaughtered broilers during 2008.
- * Greece did not participate in the baseline survey and two non-Member States, Norway and Switzerland, participated.

CONCLUSIONS AND RECOMMENDATIONS

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

Conclusions

- In this report harmonised epidemiological indicators (HEIs) are proposed for food-borne biological hazards related to poultry and meat thereof in the context of the Scientific Opinion on public health hazards to be covered by inspection of meat from poultry (EFSA, 2012a). These hazards include *Salmonella*, *Campylobacter* and ESBL-/AmpC-producing *E. coli* that were identified by the Scientific Opinion. In addition, HEIs are proposed for generic *E. coli* as an indicator for process hygiene as also foreseen in the Scientific Opinion. An epidemiological indicator is understood to mean the prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazards, such as audits of farms or evaluation of process hygiene, which correlates to a human health risk caused by the hazard.
- The epidemiological indicators proposed in this report will provide relevant information to the risk managers (i.e. the European Commission and the Member States), in order to consider whether adaptations in meat inspection methods may be relevant and to enable the Member States to carry out a risk analysis to support such decisions. It is also foreseen that the epidemiological indicators will be used in the integrated food safety assurance system for poultry meat outlined by the Scientific Opinion, in particular to help categorise the farms/flocks and slaughterhouses according to risk related to a particular hazard or level of process hygiene, as well as setting appropriate targets. Thus, the indicators can 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/flock level, and they can be used alone or in different combinations. 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 or to guarantee process hygiene.
- 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 farms and slaughterhouses. This information will be useful for the categorisation of farms and slaughterhouse 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.
- Most epidemiological indicators are suggested for poultry flocks at the farm or for poultry carcasses on the slaughter line using bacteriological testing methods. Some epidemiological indicators that are assessed by auditing apply for controlled housing conditions and some indicators refers to food chain information regarding use of partial depopulation (thinning) of the flocks or use of antimicrobials at farm or hatchery.
- The proposed harmonised epidemiological indicators are listed in Table 20.

Recommendations

- It is recommended that the Commission and the Member States define the harmonised requirements for the controlled housing conditions at farms related to the specific hazards. The scope of the food chain information is suggested to be extended to cover information on the use of partial depopulation (thinning) and the use of antimicrobials during the whole lifespan of the birds (starting from hatchery). The Commission and the Member States should define the detailed rules for the content of this food chain information.
- For some biological hazards addressed, there is a need for more research. In the case of ESBL-/AmpC-producing *E. coli* there is still a lack of information on factors placing poultry at risk of colonisation and the optimal analytical methods to be used in monitoring. In addition, more information would be welcome regarding the use of quantitative data on bacterial (*Campylobacter*, *E. coli*) counts to assess and categorise slaughterhouses. Furthermore, the usefulness and sensitivity of boot swabs for sampling of *Campylobacter* at poultry farms should be clarified. The Member States are invited to support research and studies on these subjects at the national level.
- The proposed epidemiological indicators will generate data that will provide information on the epidemiological situation in the EU and these data can be used to update the epidemiological indicators, when appropriate. It is recommended that the Member States report the data generated from implementation and monitoring of the indicators within the framework of annual reporting in accordance with Directive 2003/99/EC.
- The harmonised epidemiological indicators proposed by this report should be reviewed regularly in light of new information and the data generated from monitoring of them.

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

Conclusions

- Comparable data from the EU Member States were available for only a few of the proposed epidemiological indicators. This was the case for some indicators for *Salmonella* and *Campylobacter*, where such data were provided by annual reporting on zoonotic agents under Directive 2003/99/EC or from the EU-wide baseline surveys. These data are summarised in Chapter 8 of this report.

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

Conclusions

- For each epidemiological indicator the key elements of minimum monitoring or inspection requirements are defined. This includes the animal/carcass population to be targeted, the stage of the food chain where the sampling should take place, type and details of the specimen to be taken, diagnostic or analytical method to be used, and a case definition.
- A general description is provided on how to choose the sampling strategy for the different types of indicators and also specifically for each indicator. Guidance on sample size determination and sampling is given to aid the Member States in the implementation and monitoring of the indicators.

- If Commission or the Member States need further advice on the sampling schemes to be applied for the HEIs, EFSA can be requested to provide technical assistance in formulation of such schemes.

Recommendations

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

Table 20: Proposed harmonised epidemiological indicators for poultry meat inspection

Indicators (animal/ food category/other)	Food chain stage	Analytical /diagnostic method	Specimen
Salmonella			
HEI 1 <i>Salmonella</i> in breeding parent flocks	Farm	Microbiology (detection and serotyping)	Pooled faeces (e.g. boot swabs) possibly combined with dust samples
HEI 2 <i>Salmonella</i> in poultry flocks prior to slaughter	Farm	Microbiology (detection and serotyping)	Pooled faeces (e.g. boot swabs)
HEI 3 Controlled housing conditions at farm for laying hens and fattening flocks (including biosecurity)	Farm	Auditing	Not applicable
HEI 4 <i>Salmonella</i> in birds – carcasses after slaughter process and chilling	Slaughterhouse	Microbiology (detection and serotyping)	Neck and breast skin
Campylobacter			
HEI 1 <i>Campylobacter</i> in poultry flocks prior to slaughter	Farm	Microbiology – real-time PCR	Caecal droppings
HEI 2 - Controlled housing conditions at farm for flocks (including biosecurity)	Farm	Auditing	Not applicable
HEI 3 – Use of partial depopulation in the flock	Farm	Food chain information	Not applicable
HEI 4 – <i>Campylobacter</i> in birds –in-coming to slaughter process (evisceration stage)	Slaughterhouse	Microbiology - enumeration	Caecal content
HEI 5 – <i>Campylobacter</i> in birds – carcasses after slaughter process and chilling	Slaughterhouse	Microbiology - enumeration	Neck and breast skin
ESBL/AmpC <i>E. coli</i>			
HEI 1 ESBL/AmpC <i>E. coli</i> in elite, grand parent and parent breeding flocks producing chicks for meat production line	Farm	Microbiology, enumeration, molecular methods for characterization on a sub-sample	Pooled faeces (boot swabs)
HEI 2 ESBL/AmpC <i>E. coli</i> in incoming one day old birds for fattening purpose	Farm	Microbiology, enumeration, molecular methods for characterization on a sub-sample	Paper used in transport boxes
HEI 3 ESBL/AmpC <i>E. coli</i> in poultry flocks prior to slaughter	Farm	Microbiology, enumeration, molecular methods for characterization on a sub-sample	Pooled faeces (boot swabs)
HEI 4 Controlled housing conditions	Farm	Auditing	Not applicable
HEI 5 Use of antimicrobials during the whole life time of the flock (including in ovo, hatching, rearing, laying, all types of flocks)	Hatchery/farm	Food chain information (from hatchery to farm, from farm to slaughterhouse)	Not applicable
HEI 6 ESBL/AmpC <i>E. coli</i> in birds - carcasses after slaughter process and chilling	Slaughterhouse	Microbiology, enumeration, molecular methods for characterization on a sub-sample	Neck (and breast) skin
Generic <i>E.coli</i> indicator			
HEI1 Generic <i>E.coli</i> in birds – carcasses after slaughter process and chilling	Slaughterhouse	Microbiology – <i>E. coli</i> - enumeration	Neck skin/breast skin

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APPENDICES

Appendix 1. PROPOSED REQUIREMENTS FOR CONTROLLED HOUSING CONDITIONS AT FARMS

Throughout this report references to controlled housing conditions have been made and it will be beneficial to have a common understanding of what could be considered adequate requirements for poultry controlled housing conditions.

Those requirements could vary for different pathogens. Not all pathogens respond to biosecurity measures in the same way. There are differences in their tolerance to disinfectants and their survival in the environment and vaccination is not always available. In addition, not all requirements are applicable to free-range poultry production.

Good biosecurity including vaccination programmes have resulted in a drop in the incidence of *Salmonella* on poultry meat. Compared with *Salmonella*, *Campylobacter* is a far more difficult an organism to combat. *Campylobacter* is particularly difficult to control because it is ubiquitous in the environment and shows tolerance to a wide range of climatic conditions. Once it colonises a suitable host, for example poultry, it multiplies very rapidly but the evidence is that the rigorous application of biosecurity measures can and does prevent *Campylobacter* getting into the flock, although the epidemiology is poorly understood. Information on risk and protective factors for ESBL/AmpC *E. coli* is limited. The stocking of new animals, exposure to contaminated air, water, feed, insect or rodent vectors and human-to-animal and animal-to-animal transmission of ESBL/AmpC *E. coli* are known risk factors.

The main criteria for generic controlled housing condition are those that prevent entry and colonisation of the flock. Those criteria should be part of a farm management system that minimises entry and colonisation of poultry by pathogens. However, antimicrobials used in animal production, play an important role in the selection of ESBL/AmpC *E. coli*.

The criteria that could be part of a farm management system to be defined as controlled housing conditions are illustrated in Table 21 (question marks indicate the current uncertainty on the protective effect of the measures).

Table 21: Suggested criteria for defining controlled housing conditions for *Salmonella*, *Campylobacter* and ESBL-/AmpC-producing *E. coli*

Measures to protect...	<i>Salmonella</i>	<i>Campylobacter</i>	ESBL/ AmpC <i>E. coli</i>
The birds			
Day old chicks are obtained from breeding flocks and hatcheries complying with the relevant controls and monitoring programmes for <i>Salmonella</i> and other biological hazards	√		?
Flocks are managed on an "all-in all-out" basis.	√	√	√
Protocol for cleaning and disinfection between flocks	√	√	√
Protocol for the disinfection of equipment before and after using in each house	√	√	√
Litter is controlled to be free from contamination before use and it is protected against possible sources of contamination (bird/ vermin). It must also be removed and disposed appropriately	√	√	√
Health policy that prevents employees suffering from diarrhoea or vomiting entering the farm	√	√	√
Each poultry house			
Controls on feed for <i>Salmonella</i> . Careful handling of feed is also essential to avoid spillages that will attract pests	√		?

Provision of dedicated clothing and footwear (overalls, boots and masks) for each poultry house	√	√	√
Available boot-dips with an effective disinfectant (has to be approved, at the right concentration, of sufficient quantity and replaced when it becomes ineffective). Dirt from boot cleats must be removed after visiting a poultry house	√	√	?
Hand washing facilities with warm water, soap and disposable towels or hot air hand drier. Also hand sanitizer to be applied to clean hands	√	√	√
Hygiene barriers could be physical barriers located immediately before the entrance to the house so that to gain access to the house personnel must pass through the barrier	√	√	√
The area inside the hygiene barrier must be kept clean and be sanitised regularly including dedicated footwear inside the hygiene barrier Pests, vermin and wild birds should be controlled with an effective pest policy including secure bait boxes and houses that prevent access to wild birds and flies.	√	√	√
Fly control nets and insect killers should be strategically located to minimise access to the poultry house by flies and insects		√	
The farm			
A visitor's policy to keep their number to a minimum and to have control and records of visitors to the site, including their recent medical history or any visits to other poultry sites. Special consideration should be taken with maintenance staff (engineers) and their maintenance tools	√	√	√
Control of vehicles allowing only essential vehicles on the farm and ensuring that their wheels and wheel arches are disinfected before entering and when leaving the farm. Containers are cleaned and disinfected before catching and loading	√	√	?
Poultry house surrounds should be hard surfaces and clear of any vegetation or rubbish that could attract vermin and pests. The formation of water puddles should be avoided using run-offs and drainage for surface water	√	√	√
Under controlled housing conditions no pets or livestock should be allowed beyond the poultry's farm perimeter	√	√	√
Disposal and storage of waste should be properly handled and controlled to avoid cross contamination of 'clean' areas of the farm, with subsequent flocks or, in mixed farms, with waste from other animal species. Containers for waste should prevent the access of vermin and leakages and should be capable of being cleaned and disinfected	√	√	√
Training of farm staff should also be part of the controlled housing conditions to ensure that staff are fully aware of the bio security standards and good farming/ hygiene practices	√	√	√
Water quality plan including regular tests for bacteriological quality	√	√	√

Appendix 2. FOOD CHAIN, RISK AND PROTECTIVE FACTORS, POSSIBLE HARMONISED EPIDEMIOLOGICAL INDICATORS AND THEIR EVALUATION

1. Identification of potential epidemiological indicators for *Salmonella* in poultry

Table 22: Potential epidemiological indicators for *Salmonella* in poultry

Farm (including contribution from wildlife)	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Buy-in chicks from <i>Salmonella</i> positive breeding flocks and hatcheries	Data on prevalence of <i>Salmonella</i> in breeding flocks/hatcheries readily available from control programmes in breeding flocks of <i>Gallus gallus</i> and turkeys	Data readily available from <i>Salmonella</i> control programmes	<i>Salmonella</i> status of supply breeding parent flock
Risk factor 2 Feed (possibly <i>Salmonella</i> positive)	Some data available from the industry and official controls of feed and poultry <i>Salmonella</i> programmes	Possible to gather	<i>Salmonella</i> prevalence in feed or occurrence in feed mill
Risk factor 3 On farm and housing conditions (biosecurity)	Data on <i>Salmonella</i> in broilers (<i>Gallus gallus</i>) readily available.	Data readily available from audits of farms.	Housing and on-farm conditions
Transport to slaughterhouse	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Loading and transport – cross-contamination, cleanliness of crates	Data available from research and studies on impact of transport on <i>Salmonella</i> prevalence	It is possible to obtain such data. There is no monitoring at present.	Microbiology on transport vehicles and crates
Slaughterhouse	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 External contamination of in-coming birds and infected birds	Data available from the EU baseline study and from literature.	It is possible to obtain such data	<i>Salmonella</i> status of the in-coming birds/ slaughter batches
Risk factor 2 Slaughterhouse capacity	Slaughterhouse capacity data available from industry	It is possible to obtain such data	

Risk factor 3 Cross contamination either by live birds or by equipment	Data available from literature	It is possible to obtain such data	Prevalence of <i>Salmonella</i> on the carcass (skin)
Risk factor 4 Evisceration of birds (<i>Salmonella</i> contamination from intestines to carcass surface)	Data available from the literature	It is possible to obtain such data	Prevalence of <i>Salmonella</i> on the carcass (skin)
Risk factor 5 Type and time of chilling of the carcasses	Data available from the EU baseline survey and from literature	It is possible to obtain such data from the slaughterhouse	Prevalence of <i>Salmonella</i> on the carcass (skin)
Processing of meat and products thereof	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Cross contamination during processing	Data available from literature and national surveillance/ monitoring	It is possible to obtain such data	Detection of <i>Salmonella</i> on fresh meat products (with and without skin)
Retail	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Fresh poultry meat with skin Risk factor 2 Cross contamination at retail	Some prevalence data available from literature and national surveillance/ monitoring	It is possible to obtain such data	Detection of <i>Salmonella</i> on fresh meat products and carcasses (with and without skin)
Consumer	Availability of human incidence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Handling in the kitchen and cross contamination	Limited data available	Difficult to obtain	
Risk factor 2 Undercooking of poultry meat	Limited data available	Difficult to obtain	
Risk factor 3 Temperature abuses	Limited data available	Difficult to obtain	

Table 23: Evaluation of suggested indicators for *Salmonella* in poultry

Indicators (animal/ food category/other)	Food chain stage	Analytical /diagnostic method	Specimen	Weighting factor				Total points
				30 %	40 %	15 %	15 %	
				Quality of indicator (0,1,2)	Appropriateness of indicator (0,1,2)	Data availability (0,1,2)	Feasibility /cost (0,1,2)	
<i>Salmonella</i> status of the breeding flocks	Farm	Microbiology	Pooled faecal sample	2	1	2	2	1,6
<i>Salmonella</i> status poultry flocks before slaughter	Farm	Microbiology	Pooled faecal sample	2	2	2	2	2
Housing conditions at farms	farm	Auditing	Biosecurity	1	2	2	2	1,7
<i>Salmonella</i> in feed	Farm/ feed mill	Microbiology	feed	1	1	2	1	1,15
<i>Salmonella</i> contamination of transport vehicles	Transport	Microbiology	Swabs	1	1	0	0	0,7
<i>Salmonella</i> in in-coming birds	Slaughterhouse	Microbiology	Skin, ceacal content	1	1	1	0	0,85
<i>Salmonella</i> on carcasses after slaughter process after chilling	Slaughterhouse	Microbiology	Neck skin	2	2	2	2	2
<i>Salmonella</i> in/on fresh poultry meat	Processing plant, retail	Microbiology	Meat, surface of the meat, skin	1	0	1	1	0,6

0= bad, 1 = moderate, 2 = good

Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity)

Appropriateness of indicator = how well the indicator correlates to human health risk caused by the hazard and to possibility/need to amend meat inspection method

Data availability = is there already data available or is it easy to get the data needed

Feasibility= how laborious is the sampling and testing procedure and how much the sampling/testing would cost or is the data already available (no additional sampling/testing needed)

2. Identification of potential epidemiological indicators - risk and protective factors related to *Campylobacter* in poultry

Table 24: Potential epidemiological indicators for *Campylobacter* in poultry

Farm (including contribution from wildlife)	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Outdoor access including free range and outdoor flocks	Data on prevalence of <i>Campylobacter</i> available for the different production systems from literature and national surveillance	Data possible to obtain	<i>Campylobacter</i> status of the flocks at the farm Auditing of on-farm housing conditions for biosecurity
Risk factor 2 On farm and housing conditions (biosecurity)		Data readily available from audits of farms.	Auditing of on-farm housing conditions for biosecurity
Risk factor 3 Age of the birds	Data available such as EU baseline survey on broilers	Data possible to obtain	<i>Campylobacter</i> status of the flocks at the farm
Risk factor 4 Thinning (partial depopulation)	Data available from literature	Data possible to obtain	<i>Campylobacter</i> status of the flocks at the farm Auditing of on-farm housing conditions for biosecurity Food chain information regarding whether the flock of origin was thinned earlier
Risk factor 5 Seasonality	Data available from literature and national monitoring and surveillance	Data possible to obtain	<i>Campylobacter</i> status of the flocks at the farm
Transport to slaughterhouse	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Loading and transport – cross-contamination, cleanliness of crates	Data from literature	Data possible to obtain	Microbiology on transport vehicles and crates
Risk factor 2 Time in transit	Data from literature	Data possible to obtain	
Risk factor 3 Time in lairage	Data not readily available	Data possible to obtain	
Slaughterhouse	Availability of prevalence data	Data availability to divide population to groups between	Suggested epidemiological indicator (HEI)

		which the risk varies	
Risk factor 1 External contamination of in-coming birds and colonised birds	Some data available from literature	It is possible to receive such data	Quantification of <i>Campylobacter</i> on skin or in ceecal content
Risk factor 2 Plucking of birds	Some data available from literature	Surveys on sampling of surface of carcasses easy to carry out. Data available from food business operator's monitoring (microbiological process criterion)	Quantification of <i>Campylobacter</i> on carcass skin
Risk factor 3 Evisceration of birds (<i>Campylobacter</i> contamination from intestines to carcass surface)	Data available		
Risk factor 4 Variability in the sizes of birds plucking and evisceration	Some data available from literature		
Risk factor 5 Chilling system	Limited data available from literature		
Risk factor 6 Cross contamination of following batches during slaughter and chilling	Limited data available including data from the EU baseline survey on <i>Campylobacter</i> in broilers		
Processing of meat and products thereof	Availability of prevalence data		
Risk factor 1 Removal of skin	Limited data available	Data possible to obtain	Detection of <i>Campylobacter</i> on fresh meat products (with and without skin)
Risk factor 2 Presence of skin to meat preparations	Data are scarce	Possible to receive data by auditing	Detection of <i>Campylobacter</i> on fresh meat products (with and without skin)
Risk factor 3 Crust freezing- reduce <i>Campylobacter</i> counts	Data available	Possible to receive data by auditing	
Risk factor 4 Cross contamination	Limited data available	Data possible to obtain	Detection of <i>Campylobacter</i> on fresh meat products (with and without skin)
Retail	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Cross contamination (including other foodstuffs) during handling of unpacked poultry meat	Data available. Limited data from some Member States on contamination on the outside of chicken packaging.	Possible to receive data by auditing	

Consumer	Availability of human incidence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Undercooking of poultry meat	Data available	Possible to receive data by auditing	
Risk factor 2 Cross contamination (including other food stuffs) during handling in the kitchen	Data available	Possible to receive data by auditing	

Table 25: Evaluation of suggested indicators for *Campylobacter* in poultry

Indicators (animal/ food category)	Food chain stage	Analytical /diagnostic method	Specimen	Weighting factor				Total points
				30 %	40 %	15 %	15 %	
				Quality of Indicator (0,1,2)*	Appropriateness of Indicator (0,1,2)*	Data availability (0,1,2)*	Feasibility/ cost (0,1,2)*	
<i>Campylobacter</i> in poultry flocks prior to slaughter	Farm	Microbiology PRC	Caecal droppings/	2	2	2	1	1,85
Controlled housing conditions at farm for flocks (including biosecurity)	Farm	Auditing and food chain information	N/A	1	2	2	2	1,7
Use of partial depopulation in the flock	Farm	Auditing and food chain information	N/A	2	2	2	2	2
<i>Campylobacter</i> contamination of transport vehicles	Transport	Microbiology	Swabs	1	1	0	0	0,7
<i>Campylobacter</i> in birds – carcasses in evisceration	Slaughter-house	Microbiology - (enumeration)	Caecal content	2	2	2	1	1,85
<i>Campylobacter</i> in birds – carcasses after chilling	Slaughter-house	Microbiology (enumeration)	Neck and breast skin	2	2	2	2	2
<i>Campylobacter</i> in/on fresh poultry meat	Processing	Auditing	N/A	1	0	2	2	0,9
<i>Campylobacter</i> in/on fresh poultry meat	Retail	Auditing	N/A	1	0	1	1	0,6
<i>Campylobacter</i> in/on fresh poultry meat	Processing plant, retail	Microbiology	Meat, surface of the meat, skin	1	0	1	1	0,6

0= bad, 1 = moderate, 2 = good

Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity)

Appropriateness of indicator = how well the indicator correlates to human health risk caused by the hazard and to possibility/need to amend meat inspection method

Data availability = is there already data available or is it easy to get the data needed

Feasibility= how laborious is the sampling and testing procedure and how much the sampling/testing would cost or is the data already available (no additional sampling/testing needed)

3. Identification of potential epidemiological indicators for ESB/AmpC producing *E.coli*

Table 26: Potential epidemiological indicators for ESBL/AmpC producing *E. coli* in poultry

Farm (including contribution from wildlife)	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 ESBL/AmpC producing <i>E. coli</i> in elite/grand parent and parent breeding flocks	Limited data available from literature	It is possible to obtain such data	ESBL/AmpC producing <i>E. coli</i> carrier status of elite/grand parent, parent breeding flocks for fattening purpose
Risk factor 2 Presence of ESBL/AmpC producing <i>E. coli</i> in hatchery environment	Limited data available from literature	It is possible to obtain such data	ESBL/AmpC producing <i>E. coli</i> in incoming one day old birds for fattening purpose
Risk factor 3 ESBL/AmpC producing <i>E. coli</i> in day-old birds	Limited data available from literature	It is possible to obtain such data	
Risk factor 4 Recent or present use of third generation cephalosporins in hatchery (<i>in ovo</i> or in day-old)		It is possible to obtain such data	
Risk factor 5 On farm and housing conditions (biosecurity)		Data readily available from audits of farms.	Auditing of farms for housing conditions
Risk factor 6 Use of antimicrobials in the flock		Food chain information	Use of antibiotics during the whole life time of the flock
Risk factor 7 Use of third generation cephalosporins on other animal species in the farm	Limited data available	Data readily available from audits of farms	ESBL/AmpC <i>E. coli</i> in poultry flocks prior to slaughter (all flocks to be tested)
Risk factor 8 Detection of ESBL/AmpC producing <i>E. coli</i> in birds of previous flocks from the same farm prior to slaughter	Limited data available	Records of previous analysis kept in farm or administration	Auditing of farms for housing conditions and documentation
Transport to slaughterhouse	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Loading and transport – cross-contamination, cleanliness of crates	Data available from research and studies on impact of transport on ESBL/AmpC	It is possible to obtain such data. There is no monitoring at present.	Microbiology on transport vehicles and crates.

	producing <i>E.coli</i> prevalence.		
Slaughterhouse	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 External contamination of in-coming birds	Limited data available for ESBL/AmpC producing <i>E. coli</i>	Surveys on sampling of surface of carcasses	ESBL/AmpC producing <i>E. coli</i> of the in-coming birds/ slaughter batches
Risk factor 2 Cross contamination either by live birds or by equipment	Limited data available for ESBL/AmpC producing <i>E. coli</i>	Surveys on sampling of surface of carcasses	ESBL/AmpC producing <i>E. coli</i> in carcasses after slaughter process and chilling
Risk factor 3 Evisceration of birds (ESBL/AmpC producing <i>E. coli</i> contamination from intestines to carcass surface)	Limited data available for ESBL/AmpC producing <i>E. coli</i>	Surveys on sampling of surface of carcass	ESBL/AmpC producing <i>E. coli</i> in carcasses after slaughter process and chilling
Processing of meat and products thereof	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Cross contamination during processing	Limited data available for ESBL/AmpC producing <i>E. coli</i>	It is possible to obtain such data	
Retail	Availability of prevalence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Cross contamination at retail	Some prevalence data available from literature and national surveillance/ monitoring	It is possible to obtain such data	ESBL/AmpC producing <i>E. coli</i> and commensal <i>E. coli</i> in poultry meat at retail
Consumer	Availability of human incidence data	Data availability to divide population to groups between which the risk varies	Suggested epidemiological indicator (HEI)
Risk factor 1 Handling in the kitchen and cross contamination	Limited data available	Difficult to obtain	
Risk factor 2 Undercooking of poultry meat	Limited data available	Difficult to obtain	
Risk factor 3 Temperature abuses	Limited data available	Difficult to obtain	

Table 27: Evaluation of suggested indicators for ESBL/AmpC producing *E. coli*

Indicators (animal/ food category/other)	Food chain stage	Analytical /diagnostic method	Specimen	30 %	40 %	15 %	15 %	Total points
				Weighting factor				
				Quality of indicator (0, 1, 2)	Appropriateness of indicator (0,1,2)	Data availability (0,1,2)	Feasibility /cost (0,1,2)	
ESBL/AmpC producing <i>E. coli</i> carrier status of elite/grand parent, parent breeding flocks for fattening purpose	Farm	Microbiology	Pooled faeces	1	2	2	2	1,7
ESBL/AmpC producing <i>E. coli</i> in incoming one day old birds for fattening purpose	Paper used in transport boxes	Microbiology	Paper used in transport boxes	2	2	1	1	1,7
ESBL/AmpC producing <i>E. coli</i> in poultry flocks prior to slaughter (all flocks to be tested)	Farm	Microbiology	Pooled faeces	2	2	2	2	2
Controlled housing conditions	Farm	auditing	biosecurity	1	2	2	2	1,7
Use of antibiotics during the whole life time of the flock (including in ovo, hatching, rearing, laying, all types of flocks)	Hatchery/farm	information on food chain	information on food chain	2	2	2	2	2
ESBL/AmpC producing <i>E. coli</i> contamination of transport vehicles	Transport	Microbiology	Swabs	1	1	0	0	0,7
ESBL/AmpC producing <i>E. coli</i> in birds – carcasses in evisceration	Slaughterhouse	Microbiology -	Caecal content	1	1	2	1	1,15
ESBL/AmpC producing <i>E. coli</i> in carcasses after slaughter process and chilling	Slaughterhouse	Microbiology	Neck skin	2	2	2	2	2
ESBL/AmpC producing <i>E. coli</i> in/on fresh poultry meat	retail	Microbiology	Meat, surface of the meat, skin	1	0	2	2	0,9

0= bad, 1 = moderate, 2 = good

Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity);

Appropriateness of indicator = how well the indicator correlates to human health risk caused by the hazard and to possibility/need to amend meat inspection method;

Data availability = is there already data available or is it easy to get the data needed;

Feasibility= how laborious is the sampling and testing procedure and how much the sampling/testing would cost or is the data already available (no additional sampling/testing needed)

4. Evaluation of suggested indicators for general process hygiene

Table 28: Evaluation of suggested indicators for general process hygiene

Indicators (animal/ food category/other)	Food chain stage	Analytical /diagnostic method	Specimen	Weighting factor				Total points
				30 % Quality of indicator (0, 1, 2)	40 % Appropriateness of indicator (0,1,2)	15 % Data availability (0,1,2)	15 % Feasibility /cost (0,1,2)	
<i>E.coli</i> on carcasses after slaughter process before chilling	Slaughterhouse	Microbiology	Neck skin	2	1	2	1	1,45
<i>E.coli</i> on carcasses after slaughter process after chilling	Slaughterhouse	Microbiology	Neck skin	2	2	2	2	2
Enterobacteriaceae on carcasses after slaughter process after chilling	Slaughterhouse	Microbiology	Neck skin	2	1	2	1	1,45

0= bad, 1 = moderate, 2 = good

Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity)

Appropriateness of indicator = how well the indicator correlates to human health risk caused by the hazard and to possibility/need to amend meat inspection method

Data availability = is there already data available or is it easy to get the data needed

Feasibility= how laborious is the sampling and testing procedure and how much the sampling/testing would cost or is the data already available (no additional sampling/testing needed)

Appendix 3. PICTURES ILLUSTRATING SAMPLING OF CAECAL DROPPINGS AT FARM

The following pictures²⁰ illustrate the sampling of faecal droppings for *Campylobacter* at farm level, as proposed for HEI 1 'Campylobacter in poultry flocks prior to slaughter'.



²⁰ The two pictures presented here were kindly provided by Prof. Lieven De Zutter. They were taken in a broiler farm during sampling of caecal droppings for *Campylobacter*.

ABBREVIATIONS

AmpC	AmpC β -lactamases
BPW	Buffered Peptone Water
CVO	Chief Veterinary Officer
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ESBL	Extended spectrum β -lactamases
EU	European Union
HEI	Harmonised Epidemiological Indicator
MS	Member State
PCR	Polymerase Chain Reaction
RTE	Ready to Eat
ToR	Term of Reference
TBX	Tryptone Bile X-glucoronide
USDA	United States Department of Agriculture