



Report of
Task Force on Zoonoses Data Collection
on proposed technical specifications for a co-
ordinated monitoring programme for
***Salmonella* and *Campylobacter* in broiler**
meat in the EU¹

(Question N° EFSA-Q-2006-045)

Adopted by

The Task Force on 16-17 October 2006

¹ For citation purposes: Report of Task Force on Zoonoses Data Collection on proposed technical specifications for a coordinated monitoring programme for *Salmonella* and *Campylobacter* in broiler meat in the EU, *The EFSA Journal* (2006), 92, 1-33



Summary

The European Food Safety Authority was asked by the European Commission to prepare a proposal for the technical specifications of a coordinated monitoring programme on *Salmonella* and *Campylobacter* in broiler meat in the EU. Coordinated monitoring programmes may be established to assess risks or to establish base-line values related to zoonoses or zoonotic agents at the Member State level or at Community level.

The proposed technical specifications focus on sampling those categories of broiler meat, which enable the best characterisation of the health risk for consumers. According to the proposal, fresh broiler meat and meat preparations would be targeted at two different stages of the food chain: at slaughterhouses and at retail outlets serving the final consumer. The data from the slaughterhouses will give information on the contamination level of *Salmonella* and *Campylobacter* in broiler meat production, while the programme undertaken at retail will provide information on the exposure of consumers to *Salmonella* and *Campylobacter* via broiler meat.

Standardised analytical methods are proposed to be employed in the analyses of samples for detection of *Salmonella* and *Campylobacter*, and an enumeration method is also proposed to be used to obtain quantitative information on *Campylobacter* in the broiler meats.



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A. Background and terms of reference

Salmonella and *Campylobacter* were by far the most frequently reported causes of food-borne zoonoses in humans according to the Community Summary Report on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance in the European Union (EU) in 2004². Poultry meat was identified as one of the main sources of these human infections.

Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents³ aims to improve and co-ordinate the monitoring of zoonotic agents in the Community. The objective is to make the data collected easier to compile and compare, which will also enable better contribution to risk assessment of zoonotic agents.

When specific needs are recognized in the Community, Article 5 of the Directive allows the establishment of coordinated monitoring programmes, which typically take a form of one year surveys. These coordinated monitoring programmes may be established to assess risks or to establish base-line values related to zoonoses or zoonotic agents at the Member State level or at Community level. According to Article 13 of the Directive the Commission has to consult the European Food Safety Authority (EFSA) before establishing any coordinated monitoring programme.

Microbiological criteria for foodstuffs have been recently reviewed in the Community and the new Regulation (EC) No 2073/2005⁴ includes, among other things, new criteria for *Salmonella* in broiler meat. The Regulation does not lay down criteria for *Campylobacter*, but as it is a Regulation open to ongoing review they may also be considered in the future.

Additionally, programmes for the control of *Salmonella* in flocks of breeding hens and broilers are progressively implemented in accordance with Regulation (EC) No 2160/2003,⁵ and are expected to have an impact on the *Salmonella* contamination levels

² The EFSA Journal, (2005), 310, p.1

³ 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)

⁴ Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (OJ L 338, 22.12.2005, p.1)

⁵ 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)



in broiler meat. Control measures against other specified food-borne zoonotic agents, such as *Campylobacter*, and at other stages of production may also be considered within the framework of this Regulation.

The European Commission has asked EFSA to produce a proposal for technical specifications regarding harmonized monitoring schemes for *Salmonella* and *Campylobacter* in broiler meat. Subsequently the Commission and EFSA formulated a more detailed mandate to this task. It was considered particularly important to determine baseline values for *Salmonella* and *Campylobacter* in relevant categories of broiler meat in order to get a good picture of contamination levels in the Community and to enable the consideration of Community measures to combat these zoonotic agents in these foodstuffs. The specifications, especially regarding sample collection and analytical methods, are also recommended for undertaking continuous monitoring within the framework of Directive 2003/99/EC.

The task of preparing the technical specifications in EFSA was assigned to the Task Force on Zoonoses Data Collection. The Task Force set up a working group comprised of experts proposed by the Task Force members to develop a proposal for the specifications.

Terms of reference

The Task Force on Zoonoses Data Collection and its working group were asked to prepare a monitoring scheme for *Salmonella* and *Campylobacter* in broiler meat in the framework of Directive 2003/99/EC.

This scheme should be designed as a survey protocol and cover, in particular:

- monitoring of fresh meat (including carcasses and frozen meat), minced meat and meat preparations;
- sampling at slaughterhouses and retail level;
- domestic and imported products;
- collection of qualitative and quantitative analysis results; and
- verification of Community *Salmonella* criteria.

B. Rationale for the choices made in the proposal

While preparing the coordinated monitoring programme for *Salmonella* and *Campylobacter* in broiler meat, some decisions and choices regarding the scope and design of the programme were made. These decisions and their rationales are described below.

1. The choice of broiler meat categories to be sampled

In the EU, there is a large variety of different types of products of broiler meat origin on the market, ranging from fresh meat to various types of meat preparations. In this monitoring programme, the purpose is to target those broiler meat categories, which are supposed to be most contaminated with *Salmonella* and/or *Campylobacter*, and which at the same time have a relevant market share.

A substantial proportion of live broilers harbor *Salmonella* and *Campylobacter* bacteria in their intestinal contents without having clinical symptoms of disease, and the bacteria can be present on the skin and feathers of the birds. Broiler meat carcasses can become contaminated with the bacteria during slaughter. Fresh meat and meat preparations are thus likely to contain these bacteria. Both *Salmonella* and *Campylobacter* bacteria are destroyed when the meat is subject to thorough heat treatment or certain other types of further processing such as fermentation and curing. The health risk to the consumer is from the consumption of raw or undercooked meat, as well as cross-contamination of other ready-to-eat foods during food preparation.

Broiler meat products are not included in this programme because they have undergone further processing which often includes a bacteriocidal step, and thus they are relatively of lower risk for consumers. Minced meat is by definition a food category of its own, and was not included in the programme because its market share in Europe is understood to be relatively small.

2. Choice of two sampling stages

The coordinated monitoring programme has two distinct objectives, thus two different sampling stages of the food chain were chosen.

- The data from the slaughterhouse samples will give information on the *Salmonella* and *Campylobacter* contamination levels in broiler meat production in each Member State, which will reflect the incoming *Salmonella* and *Campylobacter* load and cross-contamination at the slaughterhouse.
- At the retail level, the data from samples will reflect the exposure to consumers via (raw) broiler meat. This information is useful when assessing the human health risk involved and typically needed in risk assessments.

3. Choice of the sample in the slaughterhouse

For comparability purposes it was necessary to decide a common point for sampling in the slaughterhouse. Sampling should preferably take place after chilling as scientific evidence has indicated that chilling can be an important step where both cross-



contamination and a reduction in bacterial levels can occur, resulting in a higher prevalence but a lower level of contamination on carcasses after chilling. Due to the variation in packing, processing and freezing methods in use in the processing plants, ‘after chilling but before any further processing’ was chosen as the best point to sample. This means that in some slaughterhouses samples will be taken before the end of the chilling process, for example, when breast caps are harvested before chilling is complete, or when carcasses are wrapped before immersion chilling or freezing.

A whole carcass is taken as the sample and sent to the laboratory where skin sampling for examination can be undertaken. A more practical proposal, as far as sample transport is concerned, would be a skin sample including neck skin of at least 27g removed from one side of a whole carcass at the slaughterhouse and sent to the laboratory. As this would require more expertise and facilities at the slaughterhouse, whole carcass sampling was preferred.

4. Explanation of how the retail sampling was decided

The retail market for broiler meat in the EU is diverse and varied. In order to obtain information concerning consumer exposure an attempt has been made to propose a scheme that will reflect the exposure of the majority of consumers in each Member State to broiler meat. To achieve this, the scheme targets a significant proportion of consumers by limiting sampling to major towns and cities and to the major retail outlets where the majority of broiler meat is sold. As information concerning the retail outlets for broiler meat was not available for every Member State it was decided that the identification of the major retail outlets would be left to the Member State following the rules provided.

Broiler meat can be purchased by the consumer as a wide range of products including chilled and frozen whole chickens and portions, meat preparations that include whole chicken with herbs added on the surface, boneless chicken meat marinated in a sauce and minced and seasoned chicken meat patties. There again, data on the comparative volume of each product type sold was not available for all Member States, so the protocol leaves the decision on which product types to include in the sampling frame to the Member States and provides rules to be followed. By using a combination of marketing or other data, visiting major outlets and purchasing products on display, the objective of reflecting the exposure of the majority of consumers should be achieved.

All broiler meat categories included in the monitoring scheme are raw meat and to be cooked by the consumer prior to consumption. The risk of the consumer of exposure to contaminated products is from undercooking and from cross-contamination of other ready-to-eat foods during preparation.



It is acknowledged that the approach described is not a truly random scheme as practical considerations had to be taken into consideration, such as only sampling at retail on weekdays and not during the weekend.

5. Rationale for use of the term ‘slaughter consignment’

Whilst the Community legislation on food hygiene refers to the concept of a ‘slaughter batch’ the definition of ‘batch’ in Regulation No 2073/2005 requires production in ‘a given place’. Groups of birds entering poultry slaughterhouses might include birds from several different poultry houses of different pathogen prevalences e.g. gathered at thinning, and arguably fall outside the tight definition of a batch. Article 14 of Regulation EC No 178/2002 provides for a concept of batch/lot/consignment as defined by potential for cross contamination e.g. by faecal contamination of crates of broilers on a transport vehicle. Contemporaneous Commission programmes are designed to investigate flock-level prevalences of these pathogens, so stratification of consignments arriving at slaughterhouses by house of origin was not deemed necessary for the present programme. For the purpose of clarity it is chosen to utilise the term ‘slaughter consignment’ defined for the purposes of this programme in Chapter 2.

6. Rationale for approach other than true randomisation

The most accurate characterisation of actual risk would arise from a truly random sample. In some instances this programme permits deviations from true randomisation, in the interests of appropriate targeting of limited resources. This programme requires sampling to be distributed evenly over a 12 month period. This should allow for observation on any variation in risk during the year.

Similarly this programme requires targeting of the largest slaughterhouses, the largest population centres, and the largest retail outlet channels. This approach might arguably result in the omission of minority production approaches with unique risk characteristics, e.g. poultry slaughter at a small scale on-farm or retail supply small market stalls, but very small numbers of samples representing these activities would not produce meaningful data. The ‘80%’ approach in this programme provides a reasonable indication of the exposure to these hazards on a population risk basis. Similarly, this programme also requires active attempts to sample retail fresh meat and meat preparation types representing the greatest contribution to the market share, and hence population risk rather than a truly random approach.

This programme also permits, where necessary, the exclusion of certain days from the sampling approach. From a risk assessment basis, this might be a relevant issue e.g. regarding build-up of contamination over a week’s production in a slaughterhouse, but might not be so relevant at the retail level with e.g. limited turnover of shelf contents. The



varying capacity of Member States to assure the quality of analysis performed on samples e.g. taken at the end of the working week was recognised, but reporting of any such exclusion criteria is recommended.

7. Sample size calculation

It is expected that the *Campylobacter* prevalence will be high in most of the Member States (possibly 50 % of the samples tested). The *Salmonella* prevalence is expected to be lower, and to contain more variation. There is no information available that provides an estimate of the likely prevalences for each of the Member States and thus it was decided to use the same number of samples for *Salmonella* and *Campylobacter* based on a 50% prevalence. The costs incurred will not increase significantly as it is possible to use the same primary sample when undertaking *Campylobacter* and *Salmonella* analyses. This approach also avoids the selection of a subset of samples for *Salmonella* analyses, which might cause an additional source of bias in the survey.

8. The inclusion of quantitative analyses for *Campylobacter*

In general, illness from bacterial pathogens follows a dose response with the risk of illness increasing with increasing numbers of bacteria in the food. For raw foods intended to be eaten cooked, the cross-contamination to ready-to-eat foods during preparation is also more likely when the contamination level is higher.

There is a need for quantitative data particularly for *Campylobacter* where the prevalence is expected to be high. As detailed in the opinion of EFSA Biological Hazards panel on *Campylobacter* in animals and foodstuffs,⁶ risk assessments have concluded that the human health risk posed by a foodstuff is related to the number of the *Campylobacter* bacteria in the food.

For *Salmonella* the human health risk is not only dependent on the initial number of bacteria present, especially as unlike *Campylobacter*, *Salmonella* is able to multiply in the food if the circumstances are favorable. Apart from this, quantitative analyses for *Salmonella* are very laborious. While quantitative data for *Salmonella* might be useful addition to scientific knowledge, it was concluded that the quantitative analyses for *Salmonella* might not make a contribution to risk assessment that would justify the increased resources needed for this particular analysis

⁶ The EFSA Journal (2005) 173 1-10



9. EN/ISO methods

In order to obtain comparable data among the Member States, established and standardized analytical methods have to be used. The EN ISO methods are already in use in many Member States. By using EN ISO 6579-2002 (E) “Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.”, comparison of the results to the data derived under Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs is guaranteed.

It would have been preferable to use the new Annex D to the EN/ISO *Salmonella* method standard, which is intended for detection of *Salmonella* spp. in animal faeces and in samples from the primary production stage, but as the Annex is still a draft and has not been validated for meat it was not appropriate to include it Annex D. Validation studies organised by the Community Reference Laboratories are currently ongoing and if these trials are successfully completed before the coordinated programme commences, then Annex D should be used.

10. Justification for recommending PCR for speciation of *Campylobacter*

PCR is the preferred method for *Campylobacter* speciation as phenotypical methods (e.g. detection of hippurate hydrolysis) bear a certain risk to give intermediate or incorrect test results.

11. Rationale for approach to describing reporting requirements

Section 8 provides details of the reporting requirements for the Member States. For some sections, Member States will be required to provide narrative overviews of the approach to this sampling programme in that state. The type of information which should be considered for inclusion is outlined by means of a series of bullet-points, which should provide guidance on the type of information that would be useful in data interpretation. Difficulty in obtaining the level of detail suggested by some bullet points is envisaged, e.g. descriptive account of the breakdown of market share of broiler meat types. Member States should endeavour to provide the best obtainable information. However, in some instances the information sought is of critical importance in subsequent data interpretation, e.g. number of birds slaughtered annually in that Member State, or absolute size of broiler meat market in the Member State.

Section 8 also provides details of the information required on each specific sample. The provision of a multi-level data dictionary format is envisaged to provide for rapid data entry with e.g. designation as retail meat preparation type of sample, prompting requirement for entry of subsequent information relevant only to meat preparations. Furthermore, it would be appropriate for the data dictionary to facilitate incorporation of



automated entry of non-varying data, e.g. entry of slaughterhouse code should automatically generate pre-entered chilling method at that slaughterhouse.

12. Elements of the programme, which could be used in continuous monitoring

Although the programme has been designed as a one year survey, most elements of it can be used in the other routine monitoring of broiler meat. This would in fact be recommendable in order to receive more harmonized data from the Member States for the Community Summary Report on zoonoses.

The elements which could be used the routine monitoring include:

- stratification of sampling in accordance with seasons;
- selection of marketing channels (retail outlets) to be included in the survey;
- type and details of samples to be taken (e.g. stage of slaughter line, the sampling techniques);
- instructions for sample transport and sample information;
- sampling procedure in the laboratory;
- analytical methods.

C. Proposal for a coordinated monitoring programme for *Salmonella* and *Campylobacter* in broiler meat in the EU

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1. Introduction

Article 5 of Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, foresees the establishments of coordinated monitoring programmes to assess risks or to establish base-line values related to zoonoses or zoonotic agents at the Member State level or at Community level, especially when specific needs are identified.

In order to get a picture of contamination levels in broiler meat and to enable the consideration of Community measures to combat *Salmonella* and *Campylobacter* in these foodstuffs, there is a need to determine baseline values for these pathogens in relevant categories of broiler meat.

The results from a baseline survey regarding *Salmonella* in broilers in the EU (Commission Decision 2005/636/EC⁷) should be considered when interpreting the data obtained in the proposed survey. Results from a baseline survey regarding *Campylobacter* in broilers which is to be performed should also be considered (Draft protocol SANCO/453/2006 (Ex SANCO/D/40029) – Working document rev2).

In this technical specification the coordinated monitoring programme takes the form of a one year baseline survey. The specifications, in particular with regard to sample collection and analytical methods, are also recommended for undertaking continuous monitoring within the framework of Directive 2003/99/EC.

2. Definitions

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

Broiler meat –meat from the species *Gallus gallus*, when bred and reared for meat production. Meat from culled laying hens is specifically excluded from this monitoring programme.

***Campylobacter* positive foodstuff** – a foodstuff where the presence of any thermophilic *Campylobacter* spp. is detected in a sample taken out of it.

⁷ Commission Decision 2005/636/EC, OJ L 228, 3.9.2005, p. 14



Carcase - the body of an animal after slaughter and dressing (Regulation (EC) No 853/2004⁸).

Coordinated monitoring programme – programme referred to in Article 5 of Directive 2003/99/EC, which may be established especially when specified need are identified, to assess risks or to establish base-line values related to zoonoses or zoonotic agents at the level of Member States or at Community level.

Country of origin – the country indicated in the health or identification mark referred to in Article 1 of Regulation (EC) No 853/2004 and Article 5 of Regulation (EC) No 854/2004⁹.

Fresh meat - meat that has not undergone any preserving process other than chilling, freezing or quick-freezing including meat that is vacuum-wrapped or wrapped in a controlled atmosphere (Regulation (EC) No 853/2004).

Meat - edible parts of the animals, including blood (Regulation (EC) No 853/2004).

Meat preparations - fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat (Regulation (EC) No 853/2004).

Meat products - processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat (Regulation (EC) No 853/2004). *Meat products are not covered in the monitoring programme set down in this document.*

Minced meat - boned meat that has been minced into fragments and contains less than 1 % salt (Regulation (EC) No 853/2004).

Monitoring - system of collecting, analysing and disseminating data on the occurrence of zoonoses, zoonotic agents and antimicrobial resistance related thereto (Directive 2003/99/EC).

Random sample - sample in which the characteristics of the batch from which it is drawn are maintained. (*Codex General Guidelines on Sampling - CAC/GL 50, 2004*). It is

⁸ Regulation (EC) No 853/2004 of the European Parliament and of the Council, OJ L 139, 30.4.2004, p. 22

⁹ Regulation (EC) No 854/2004 of the European Parliament and of the Council, OJ L 139, 30.4.2004, p. 83



a sample which is taken under statistical consideration to provide representative data (Decision 98/179/EC¹⁰).

Retail - the handling and/or processing of food and its storage at the point of sale or delivery to the final consumer (Regulation (EC) No 178/2002¹¹). *In this document retail covers only shops, supermarkets and other similar outlets that serve directly the final consumer. It does not include distribution terminals or centres, catering operations, factory canteens, restaurants and other similar food service operations and wholesale outlets.*

Salmonella positive foodstuff – a foodstuff where the presence of any *Salmonella* spp. is detected in a sample taken out of it.

Sampling frame - complete list of all units of the population, which can be sampled.

Sample size - the number of units randomly chosen from the sampling frame.

Test portion weight - the weight (in gram) of the sample used in the laboratory for analysis.

Slaughter consignment - A group of birds delivered simultaneously to a slaughterhouse, and slaughtered sequentially as an identifiable group.

Shelf-life - the period preceding the “Use by” or the minimum durability date (Directive 2000/13/EC¹²).

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

Test portion - unit or portion of a matter which is sampled and intended to be analysed.

¹⁰ Commission Decision 98/179/EC, OJ L 65, 5.9.1998, p. 31

¹¹ Regulation (EC) No 178/2002 of the European Parliament and of the Council, OJ L 31, 1.2.2002, p. 1

¹² Directive 2000/13/EC of the European Parliament and of the Council, OJ L 124, 2.5.2000, p. 66



3. Objectives

The main objective of the monitoring programme is to establish the baseline prevalence of *Salmonella* and *Campylobacter* in broiler meat in the Community and the Member States. This prevalence will be measured in broiler meat carcasses produced in EU slaughterhouses and in fresh broiler meat and meat preparations on sale in retail outlets in the Member States. The data from the slaughterhouses will give information on the contamination level of *Salmonella* and *Campylobacter* in broiler meat production in each country, while the programme undertaken at retail will provide information on the exposure of consumers to *Salmonella* and *Campylobacter* via broiler meat.

The programme is designed so that results can be compared among Member States.

The programme also aims at gathering information on:

- seasonality patterns in *Salmonella* and *Campylobacter* contamination;
- contamination levels of different types of fresh broiler meats and broiler meat preparations;
- contamination levels of broiler meat produced in the EU and products from third countries; and
- contamination levels in various production and processing methods.

In addition quantitative data on *Campylobacter* in broiler meat will be collected.

The results of the programme will be made available and should, especially the quantitative data, be of value for risk assessments. These risk assessments will provide information that may be taken into consideration related to the Community microbiological criteria and other risk management options for broiler meat carcasses and meat preparations.

4. Sampling frame

4.1. The population

Two levels of populations of broiler meat are addressed in the programme: firstly the broiler meats in the European Community market and secondly Member State -specific broiler meats. In the former case the data will allow a prevalence to be estimated at the Community-level, whilst in the latter case prevalence can be estimated specifically for each Member State. For the Community prevalence estimation, weighting will be



implemented to take into account the size of populations of foodstuffs produced or on the market in each Member State.

Two points in the food chain will be sampled, that is, the slaughterhouse and at retail.

At the slaughterhouse level the object of study are broiler meat carcasses after chilling but before any further processing step (e.g. freezing or cutting) (see further section 6 on sample collection). Both the Community prevalence and Member State specific prevalence will be estimated.

At retail level the broiler meat prevalence will be estimated specifically for every Member State. The Community-level prevalence will be estimated if the necessary market figures are available. The broiler meat categories typically on the market and representing the exposure of the consumers in each Member State are targeted including meats derived from different production methods such as organic and free range and meats from different countries of origin.

Two different categories are sampled at retail level:

- 1) fresh broiler meat with and without skin (including both chilled and frozen, whole carcasses and portions) and,
- 2) meat preparations (including both chilled and frozen preparations which may be seasoned, marinated, in sauce, minced or breaded). For the diverse group of meat preparations, descriptions of typical examples of subcategories are given in section 6 for information.

4.2. Sampling design

The sampling plan of the programme is based on a multistage design. The first level is composed of the 25 strata (Member States). The second level represents the slaughterhouses and retail shops in the Member States. At the third level broiler meat categories to be sampled are selected.

As prevalence of foodstuffs contaminated with *Campylobacter* or *Salmonella* is expected to vary with season, this merits further stratification. For that purpose, a 12-month period is divided in 12 periods of 1 month. In each of those periods 1/12th of the total sample size should be taken.

4.2.1. Slaughterhouse

Apart from the stratification for seasons, the sampling must otherwise be based on random selection, regarding slaughterhouses, sampling days and carcasses to be sampled on a selected sampling day. All types and sizes of broiler carcasses need to be included in the population to be sampled if they are produced for human consumption. The central

competent authority is responsible for generating the randomization scheme and ensuring that it is implemented correctly.

In the Member State, all broiler slaughterhouses subject to Regulation (EC) No 853/2004 shall be included in the sampling frame. Then, for practical reasons, the largest slaughterhouses should be selected for sampling until their combined slaughter capacity covers at least 80 % of the annual kill in the country.

The allocation of the number of samples between the slaughterhouses should be made proportionally to the number of broiler chickens processed annually by the selected slaughterhouses. The samples must be distributed evenly over the 12 months of the survey.

For each slaughterhouse, the day of the month when sampling shall take place is selected by choosing a number between 1 and 31 randomly. If the selected number is a slaughtering day in that month the sampling shall be carried out on that day. If not, then a new number is selected at random. Every month this selection process is repeated as many times as there are samples to be collected at that slaughterhouse in that month.

The slaughter consignment to be sampled on the chosen sampling day is selected randomly by using a randomisation sheet provided to the sampler. This has been generated using a maximum that exceeds the highest possible number of slaughter consignments on any given day. The carcass is then randomly sampled from the chosen consignment.

A randomisation scheme may then look as follows (Table 1):

Table 1: Randomisation table for January

Slaughterhouse code	Day of the month	Slaughter consignment
AXD	19	5
MLG	4	2
MLG	12	4
MLG	17	2
GHT	8	5
GHT	12	8



Details of the randomisation scheme are to be reported to the Commission (See Section 8). Sampling must be undertaken by the competent authorities or carried out under their supervision.

4.2.2. Retail

At the retail level the outlets that supply the majority (at least 80%) of the national market of the selected broiler meat food categories to the final consumers should be targeted.

The central competent authority must draw up a sampling plan following the rules described below and based on the best marketing data available. This marketing data or assistance with how to obtain the information may often be available from a national trade association. In the absence of marketing data, the best estimate of market shares should be used.

The competent authorities are responsible for choosing the retail outlets to be visited. Typical types of retail outlets that could be included for sampling are: supermarkets, butchers, poulterers, small shops, and street markets (e.g. farmer’s markets). The following rule shall be used to choose the types of retail outlets to be sampled and needs to be followed for each of the two main categories of broiler meats:

- if the biggest category of outlets (for example supermarkets) supply at least 80% of the broiler meat market then samples only need to be taken from those outlets. Where that is not the case, the second largest outlet category should be added and if needed the third and so on until at least 80% of the market is covered. The number of samples that should be taken from each retail outlet category that is included in the sampling plan should be proportionate to the market share of that category.

The broiler meat categories to be sampled are also selected based on the marketing data. The fresh broiler meat subcategories and broiler meat preparation subcategories are listed in descending order according to their market share. The categories having the largest market share are chosen for the sampling plan until at least 80 % of the market is covered using the same procedure as for choosing the retail outlet categories.

The number of major cities/towns to be sampled in each Member State must be at least 4. These cities/towns should cover at least 30% of the human population in the country. However, if already the largest 8 cities/towns are included in the plan the population coverage could be less than 30 %.

The sampling shall be evenly divided for all the weeks of each calendar month using different weekdays.

The retail sampling plan to be prepared by the central competent authority shall define, in line with the reporting requirements set down in Chapter 8, the following:



- The cities/towns to be included in the monitoring programme;
- The types of retail outlets covered and their share in sampling;
- The types of meat subcategories to be sampled within the two main categories of fresh meat and meat preparations;
- the number of samples taken from each meat category;
- the distribution of the samples throughout the year, weeks and weekdays.

5. Sample size (number of samples) calculation

The population size is the number of units of the population. In this study the population is considered to be infinite, as there are more than 100,000.

The sample size gives the number of foodstuffs to be tested. It is calculated on the basis of the following criteria, assuming simple random sampling:

- Annual expected prevalence (**p**): 50%
- Desired confidence level (**Z**): 95%, corresponding to a Z_{α} value of 1.96
- Accuracy (**L**): 5%
- Using these values and the formula:
$$n_{\infty} = \frac{(Z_{\alpha})^2 p(1-p)}{L^2}$$

In this survey an annual expected prevalence of 50% is used to calculate the sample size. For an infinite population the sample size is 384 based on the aforementioned criteria. All samples are to be tested for both *Salmonella* and *Campylobacter*.

Slaughterhouse

Based on the above calculation, a total of 384 broiler carcasses shall be sampled per Member State.

Retail

Two different types of broiler meats shall be sampled per Member State: 1) fresh meat and 2) meat preparations. The sample size is 384 for each of these two main categories.

When planning the programme, non-response must be anticipated, and taken into consideration by increasing the number of samples by approximately 10 %.

6. Sample collection in the slaughterhouse and at retail

6.1. Type and detail of sample

6.1.1. Slaughterhouse

The objective is to sample whole carcasses immediately after chilling but before further processing such as freezing, cutting or packaging. In some slaughterhouses this may mean that samples are taken after pre-chilling when this is the last step before further processing.

The sample collected must be placed in a separate plastic bag avoiding cross contamination and sent to the laboratory where skin sampling can be undertaken.

6.1.2. Retail

The objective is to collect samples of the categories of broiler meat taken at random from the customer display e.g. refrigerator shelves / freezer cabinets in the selected retail outlet. Where possible whole retail packs should be collected.

Samples which are packaged, must not show evidence of damage. If the label on the broiler meat is not clear or damaged then the sample should not be purchased. When fresh, un-packaged broiler meat or meat preparations are collected e.g. from smaller butchers, the sampling officer may need to inquire about the country of origin, best before date and other information which is normally on the label of packaged meat so that this can be recorded.

It is essential that cross-contamination be avoided during the collection of broiler meat samples. Precautions must therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage are not contaminated with the pathogens investigated in the programme.

Once collected each sample should be placed in a separate sampling bag to avoid the risk of cross contamination, and sent to the laboratory for testing.

The following should be noted in the sampling:

Fresh meat must not have any seasonings, stuffings or sauces in contact with the meat but may be included in a separate part of the retail pack such as a sachet.

A meat preparation has ingredients added to it but the cut surface retains the characteristics of fresh meat. In contrast, meat products which are not included in the survey, are produced from fresh meat but have been further processed and the cut surface no longer retains the characteristics of fresh meat.



Meat preparations are a diverse group and include breaded products containing raw chicken meat e.g. escalopes/goujons made from raw chicken meat, products made from seasoned minced chicken meat and whole pieces of meat such as marinated /stuffed/seasoned whole birds and portions. Some sample types e.g. goujons and burgers exist both as a meat preparation and as a meat product (when they have undergone partial heat treatment) and samplers must avoid sampling meat products. Information from trade associations may be useful if samplers need training on this aspect.

6.2. Sample information

All relevant information available from the sample should be recorded on a sampling form produced by the competent authority to enable the data requirements in section 8 to be fulfilled.

It is essential to identify the pack-producer codes on retail products (and identification marks/supplier codes when possible) from each sample so that the origin of the chicken can be determined retrospectively as the country of origin of a chicken pack is not always apparent from examining the label. The co-operation of retailers may be required to permit identification of imported chicken.

Each sample and its sample form should be labelled with a unique number which should be used from sampling to testing. The competent authority must arrange for the issue and use a unique numbering system.

6.3. Transport of samples

The chilled samples must be kept at between +2 to +8°C and free from external contamination during transportation. Frozen and chilled samples must be transported separately. A temperature indicator should be placed with the samples in the container to monitor compliance with these requirements.

All chicken samples should ideally reach the laboratory within 24 hours of sampling. In exceptional situations (e.g. long journeys, weekends and public holidays) this period may be extended to 80 hours.

Frozen samples may be transported frozen or transported chilled allowing defrosting to take place during transport. In the latter case, the temperature of the sample must not exceed +8°C. Once defrosting has commenced samples must not be refrozen.

7. Sampling in the laboratory and analytical methods

7.1. Laboratories

National Reference Laboratories for *Salmonella* and *Campylobacter* are the laboratories where normally all types of analyses described below (detection, identification, typing and quantification) shall take place. However, the competent authorities may decide to designate other laboratories involved in official control of *Salmonella* and *Campylobacter* to perform the analyses. In such case, national reference laboratories shall provide support to the designated laboratories and ensure the quality of these by arranging regular ring tests. The *Campylobacter* analyses may need specific experience and training which could be provided by the National Reference Laboratory for *Campylobacter*.

The laboratories participating in testing shall have proven experience of using the required methods, have a quality assurance system complying with EN/ISO standard 17025, and be subjected to the supervision of the relevant national reference laboratories.

7.2. Receipt of samples

On receipt of the samples, laboratories shall check the information recorded by the sampler and complete the relevant sections of the sample form. For samples from retail outlets, digital photographs or photocopies of the label of the packaging may be taken and stored electronically with the appropriate sample number to capture all product information. The photograph or photocopy should be of a high resolution so that the labelling details are clear.

Chilled chicken samples need to be held at +2 to +8°C in the laboratory and the laboratory sampling procedure should begin within 24 hours of arrival at the laboratory. If the transport period was 72 -80 hours from sampling, then the sample shall be tested on receipt. All retail samples must always be tested before their use-by dates.

All frozen whole and portioned chickens and meat preparations should be thawed out under controlled conditions prior to testing and held at +2 to +8°C until testing commences. Once defrosting has commenced samples must not be refrozen.

7.3. Sample preparation

All samples received shall be examined to ensure that the transport packaging is intact before testing.

It is essential that handlers take care to avoid cross contamination between samples and from the surrounding environment at all stages (e.g. by wearing gloves and changing them between each sample of chicken and by sanitising the surface of the packaging before unwrapping it).

Fresh (chilled and defrosted) whole birds from slaughterhouse or at retail

Wearing disposable gloves, the chicken is removed from its sample bag and any retail wrapping, taking care not to contaminate the outer surface of the chicken. Using a sterile instrument and an aseptic technique, the neck skin if present is removed together with the skin from one side of the carcass, avoiding any fat, to make a 27 g test portion and then placed into a stomacher bag (or pulsifier).

Fresh (chilled and defrosted frozen) chicken portions with skin

Wearing disposable gloves, the chicken is removed from its retail wrapping, taking care not to contaminate the outer surface of the chicken. Using a sterile instrument and an aseptic technique, a 27 g of skin is removed (if there is less than 27 g of skin on the chicken, all of it is removed, the amount shall be weighed and recorded, and subsequently a thin surface muscle slice shall be added as described below).

Fresh (chilled and defrosted frozen) chicken portions without skin or with only a small amount of skin

Using a sterile technique a thin surface muscle slice or slices are removed from the surface and added to any skin sample to make a 27 g test portion. The slice should be taken in a way that includes as much as possible the surface of the meat.

Chilled or defrosted frozen meat preparations

A 27 g sample from one retail pack is to be examined. For meat preparations made from whole pieces of meat such as marinated chicken portions or oven ready stuffed and seasoned whole birds, the sample should be taken from the surface, including skin if present, but scraping away any sauce or non meat components as the presence of seasonings marinades etc may interfere with the analysis. For meat preparations made from minced poultry meat the 27 g should be taken throughout the sample as a cross section.

Primary samples are discarded once laboratory analyses have been initiated. When the analysis is aborted e.g. due to unacceptable deviations in the analysis process, new samples must be obtained. The EN/ISO analytical method must be followed and it will be the responsibility of the NRL to ensure that deviations are not permitted.

7.4. Initial suspension

A total of 27 g are needed to perform analyses for *Salmonella* and *Campylobacter* from one sample in parallel. The 27 g test portion is transferred to 9 volumes (243 ml) buffered peptone water (BPW) and treated in a stomacher or pulsifier for approximately 1 min. Foaming should be avoided by removing the air as much as possible from the stomacher

bag. The buffered peptone water should be brought to room temperature before adding the test portion (27g sample).

This initial suspension is used as follows:

- 10 ml (~1g) must be transferred to 90 ml enrichment medium for *Campylobacter* detection (see 7.5.2).
- 10 ml (~1g) are transferred to an empty sterile tube. 1ml is used for the enumeration of *Campylobacter* on selective plates (see 7.5.2).

The rest of the initial suspension (250 ml ~ 25g) is used for the detection of *Salmonella* (see 7.5.1).

7.5. Detection, identification and quantification methods

All types of samples are analysed qualitatively for the presence of both *Salmonella* and *Campylobacter* spp. Quantitative determinations are carried out for *Campylobacter* only.

7.5.1. *Salmonella*

Detection of *Salmonella*

The detection of *Salmonella* spp. shall be done according to EN/ISO 6579-2002 (E). “Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.”

Serotyping of *Salmonella*

At least one isolate from each positive sample shall be typed by the National Reference Laboratory for *Salmonella*, using the Kaufmann-White scheme.

For quality assurance, a proportion of the non-typeable isolates shall be sent to the Community Reference Laboratory (CRL) for *Salmonella*, with a maximum of 16 non-typeable isolates. A proportion of these isolates should be sent to the CRL on a quarterly basis.

Phage typing of *Salmonella*

For *S. Enteritidis* and *S. Typhimurium* it is recommended that at least one isolate from each positive sample should be phage typed, using the protocol defined by HPA (Health Protection Agency) Colindale, London.

7.5.2. *Campylobacter*

Detection of *Campylobacter*

The detection of *Campylobacter* spp. shall be done according to EN/ISO 10272-1:2006(E) “Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. Part1: Detection method”. The second

isolation media used has to be recorded. Confirmation should be done by phenotypical methods as described in EN/ISO 10272-1:2006(E). Speciation (optional) by the use of recognised PCR techniques, i.e. validated and published methods, is recommended. The method used should be indicated.

For quality assurance, a proportion of *Campylobacter* spp. isolates with a maximum of 16 isolates shall be sent to the CRL *Campylobacter* for confirmation and speciation. A proportion of these isolates should be sent to the CRL on a quarterly basis. If isolates are to be transported between laboratories, appropriate conditions (e.g. charcoal swabs) should be used.

Quantification of *Campylobacter*

The quantitative detection of *Campylobacter* spp. shall be done according to EN 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”. Starting with 10 ml initial suspension (see 7. initial suspension) 0,1 ml of this initial suspension and of further dilutions thereof shall be examined to allow the enumeration of up to 10⁶ cfu/g. In addition, 1 ml of the undiluted initial suspension has to be examined to obtain a limit of enumeration of 10 cfu/g. All plate determinations shall be done in duplicate.

7.6. Estimation of measurement uncertainty (MU) for quantitative *Campylobacter* determination

To enable correct comparison and judgement of data (for future risk assessment) the uncertainty of measurement of the abovementioned quantitative determination method should be estimated for each laboratory. To estimate the measurement uncertainty the technical specification ISO/TS 19036:2006 shall be used with the exception that the parallel dilutions from the initial suspension is applied for estimation of the MU. The MU is derived from the intralaboratory standard deviation of reproducibility. Data on MU estimation shall be collected from May to September in order to ensure positive samples. A total of 12 positive samples shall be examined in duplicate and parallel dilutions prepared from the 10 ml initial suspension. Data will be derived from samples of carcasses taken from slaughterhouses only. Raw data on MU estimation will be reported separately as part of the overview of the Sampling Programme and Results (see chapter 8).

7.7. Storage of isolates

In order to allow for e.g. later testing for antimicrobial susceptibility storage of a representative subset of isolates is recommended. All or at least a subset of 170 isolates of *Campylobacter* and *Salmonella* respectively deriving from the analyses should be obtained. One isolate per positive sample shall be stored. The *Campylobacter* isolate



obtained from the quantitative analysis should be preferred. The isolates should be stored at the National Reference Laboratories (NRLs) using the normal method for NRL culture collection, as long as it ensures viability of the strains for a minimum of 2 years.

8. Reporting

8.1. General provisions

The competent authority responsible for the preparation of the yearly national report on zoonoses pursuant to Article 9 of Directive 2003/99/EC shall collect and evaluate the results and report them to the Commission.

The Commission shall forward the result to the European Food Safety Authority, which shall examine and report on them. Any use of the data submitted by the Member States for the purposes other than the objective of this study will be subject to prior agreement of the Member States.

National aggregated data and results will be made available publicly in a form that ensures confidentiality regarding the establishments and products sampled.

The information to be reported by Member States, as far as the information is available or accessible, is outlined in subsections 8.2 to 8.8, and consists of two broad categories, description of the programme (8.2 to 8.5); and individual data for each sample (8.6 to 8.8).

The description of the programme should provide an overview of the sampling programme in its entirety in that Member State and the overall results obtained (8.2). Member States should also provide more detailed descriptive overviews of the slaughterhouse (8.3) and the retail (8.4) sampling programmes respectively, as well as the laboratory analytic approach (8.5) in that Member State. These descriptions will be submitted once by each Member State, and should take the form of a textual account of the sampling planned, the sampling actually realised, and the results obtained. Sections 8.2 to 8.5 provide some headings to indicate the type of information to be submitted in this context.

Individual data should be submitted for each sample tested as part of the sampling programme. This information shall be submitted in a form of raw data using a ‘Data Dictionary’ and data collection forms established and provided by the Commission. Some information such as details of analysis and results obtained will be required for all samples (8.6). There will also be specific information required depending whether the sample was collected from a slaughterhouse (8.7), or at retail, as fresh meat, or a meat

preparation (8.8). Sections 8.6 to 8.8 will be converted into a ‘Data Dictionary’ format to facilitate reporting of these data.

8.2. Overview of the sampling programme and results

- Member State name
- Date of beginning and finishing of the sampling and analysis
- Number of samples obtained and analysed:
 - From slaughterhouses
 - From retail outlets - fresh meat
 - From retail outlets - meat preparations
- Overall results:
 - Prevalence, and serovars of *Salmonella* on carcasses at slaughterhouses and in fresh broiler meat and meat preparations at retail level
 - Prevalence, (species) and quantity of *Campylobacter* on carcasses at slaughterhouses and in fresh broiler meat and meat preparations at retail level

8.3. Overview of slaughterhouse sampling programme

- Description of broiler production in that Member State
 - Number of broilers slaughtered annually (most up to date information available)
 - Proportion reared in other Member States
 - Approach to use *Salmonella* vaccination in broiler meat production
 - Principle breeds used in broiler meat production
 - Intensive housed versus outdoor access free range/organic housing
 - Normal slaughter age of broilers (approximate range)
- Number of broiler slaughterhouses in the Member State (subject to Regulation 853/2004)
- Slaughterhouses planned to be sampled and slaughterhouses actually sampled
 - Number of slaughterhouses included
 - Proportion of the national kill represented
- Description of randomisation procedure for slaughterhouse sampling
 - Day of week randomisation
 - Any exclusion criteria e.g. late in week
 - Time of day/consignment randomisation
- Comment on overall representativeness of the slaughterhouse sampling programme

8.4. Overview of retail sampling programme

- Description of broiler meat market in Member State
 - Overall absolute market size for broiler meat
 - Market share of different types of retail outlets (supermarket, small shops, butchers, poulterers, street markets including farmers’ markets) for fresh meat and meat preparations
 - Market share of imported (intra-Community trade and imports from 3rd countries) and domestic production for fresh meat and meat preparations
 - Market share of frozen, never-frozen, previously frozen fresh meat and meat preparations
 - Market share of fresh broiler meat and subcategories of fresh broiler meat
 - Market share of broiler meat preparations and subcategories of broiler meat preparations
 - Market share of different broiler production types for fresh broiler meat and meat preparations (intensive, outdoor access (free range/organic))
- Retail outlets planned to be sampled, and retail outlets actually sampled
 - Type of outlet categories covered: e.g. supermarkets, butchers, etc
 - Geographical distribution of sampling – cities/towns covered (% of human population covered)
- Fresh meat planned to be sampled and fresh meat actually sampled
 - Proportions of different sub-categories of fresh meat
 - Whole carcasses, portions, fragmented.
 - Proportions pre-packed vs. not packed
 - Proportions of modified atmosphere packed vs. packed
 - Proportions frozen vs. never frozen
 - Proportions skin-on vs skinless
 - Proportions domestic vs imported
 - Proportion intensive vs. outdoor access produced meat
- Meat preparations planned to be sampled, and meat preparations actually sampled
 - Proportions of different categories of meat preparations
 - Breaded, marinated etc
 - Proportions pre-packed vs. not packed
 - Proportions modified atmosphere packed vs. packed
 - Proportions frozen vs. never frozen
 - Proportions domestic vs. imported
 - Proportion intensive vs. outdoor access produced meat
- Description of randomisation procedure for retail sampling
 - Day of week randomisation
 - Any exclusion criteria e.g. late in week
- Comment on overall representativeness of the slaughterhouse sampling programme

8.5. Overview of laboratory analysis

- For each laboratory involved in *Salmonella* analysis
 - Laboratory identifier code*
 - NRL for this organism
 - Stipulate which variant of ISO 6579-2002 (Second Plate) used
- For each laboratory involved in *Campylobacter* analysis
 - Laboratory identifier code*
 - NRL for this organism
 - Speciation method used for *Campylobacter*
 - Report Measurement of Uncertainty estimations for *Campylobacter* quantification
 - 12 MU results for each laboratory

8.6. Individual sample data: all samples

- Code of the laboratory involved in initial analysis*
- Type of sample
 - Slaughterhouse carcass
 - Retail fresh meat
 - Retail meat preparation
- Production type
 - Intensive housed
 - Outdoor access (Organic/Free range)
- Date of sample collection
- Date laboratory analysis begun
- Detection of *Salmonella*
 - Qualitative result (positive/negative)
- Serotyping of *Salmonella*
 - Serotyping performed Yes/No (Y/N)
 - Serovar(s) detected (may be more than one)
- Detection of *Campylobacter*
 - Qualitative result (positive/negative)
- Speciation of *Campylobacter*
 - Speciation performed Y/N
 - Species identified (may be more than one)
- Quantification of *Campylobacter*
 - Quantification performed Y/N
 - Quantitative results (cfu per g)

8.7. Individual sample data: slaughterhouse sample

- Code of slaughterhouse*
- Throughput of slaughterhouse
 - No of birds slaughtered per year
(Most up to date information available)
- Type of chilling method used at that slaughterhouse
 - Air
 - Immersion
 - Spray
- Sample collection point
 - Immediately following chilling
 - Immediately after pre-chilling and before further processing
- Age of bird
 - Best estimate or range of consignment
- Result of thinning Y/N
 - From first thinning
 - From subsequent thinning or house clear-out

8.8. Individual sample data: retail sample

- Code of the town*
- Code of the shop*
- Type of retailer
 - Supermarket
 - Butcher / Poulterer
 - Small Shop /Independent retailer
 - Street market / Farmers’ market
- Use by date
- Country of origin*
 - As ascertained with reference to the Identification Mark on packaging or commercial documentation
- Whole carcase, anatomically recognisable portion, or fragmented meat
 - Portion type
 - Breast
 - Leg/thigh
 - Wing
 - Offal e.g. gizzards or livers
 - Other e.g. necks
- Skin present (Y/N)
- Frozen or never frozen
- Pre-packaged (Y/N)



- Modified atmosphere
- Vacuum-packed
- other
- Further information on meat preparations
 - Minced, fragmented or comminuted (Y/N)
 - Dusted, seasoned (Y/N)
 - Breaded, battered or coated (Y/N)
 - Stuffed (Y/N)
 - Marinated (Y/N)
 - Sauce over (Y/N)

* It is envisaged that Member States would adapt an approach of requiring sampling officers to record detailed information on the specific establishments where foods are sampled or produced. For the purposes of reporting information pertaining to individual samples in this programme to the Commission, these data could be anonymised by e.g. assignment of unique codes to the establishments and regions in which sampling took place, and only reporting these anonymous codes. Similarly for retail samples the Identification Mark referring to the establishment in which the food was produced, would be recorded by the sampling officer, but only the country of origin would be reported to the Commission.



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E. Acknowledgments

The Task Force on Zoonoses Data Collection wishes to acknowledge the contribution of the working group that prepared the draft programme: Thomas Alter, Enne De Boer, Lieven De Zutter, Merete Hofshagen, Mary Howell, Osek Jacek, Sandra Jelovcan, Charalambos Kakoyiannis, Mogens Madsen, Micheál O’Mahony, Eva Olsson Engvall, Antonia Ricci, Gilles Salvat, Kris De Smet, Maija Hatakka, Pia Makela, Frank Boelaert, and Sergio Potier Rodeia.