

## COMMISSION DECISION

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 the Member States**

(notified under document number C(2007) 3440)

(2007/516/EC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Decision 90/424/EEC of 26 June 1990 on expenditure in the veterinary field <sup>(1)</sup>, and in particular Article 20 thereof,

Whereas:

(1) Decision 90/424/EEC lays down procedures governing a financial contribution by the Community towards specific veterinary measures, including technical and scientific measures. It provides for the Community to undertake, or assist Member States in undertaking the technical and scientific measures necessary for the development of Community veterinary legislation and for the development of veterinary education or training.

(2) According to the Report of the European Food Safety Authority (EFSA) on Trends and Sources of zoonoses, zoonotic agents and antimicrobial resistance in the Community in 2005 <sup>(2)</sup>, a total of 194 695 cases of campylobacteriosis in humans were reported in 22 Member States. Broiler meat is considered the most common source of infection. Up to 66,4 % positive samples in broiler meat were reported. In broiler flocks, 0,2 to 86 % of the reported samples were positive.

(3) In addition, according to the EFSA report, a total of 168 929 cases of human salmonellosis were reported in 22 Member States in 2005. Typical contamination rates of fresh poultry meat vary from 4 to 10 %, being the highest rates of all foodstuffs analysed.

(4) The EFSA also indicates in its report that a relatively high proportion of *Campylobacter* and *Salmonella* isolates from animals and food were resistant to antimicrobials commonly used in treatment of human diseases. This specially applies to the case of resistance to fluoroquinolones in *Campylobacter* isolates from poultry, where up to 94 % of isolates were reported resistant to ciprofloxacin. Food-borne infections caused by these resistant bacteria pose a particular risk to humans due to possible treatment failure.

(5) In accordance with Commission Decision 2005/636/EC of 1 September 2005 concerning a financial contribution by the Community towards a baseline survey on the prevalence of *Salmonella* spp. in broiler flocks of *Gallus gallus* to be carried out in the Member States <sup>(3)</sup>, comparable information was collected with regard to the prevalence of *Salmonella* in such flocks. It is, however, very difficult to compare prevalence of *Campylobacter* in broiler flocks and broiler meat, and of *Salmonella* in broiler meat from different Member States as there is no harmonised monitoring.

(6) Under Article 5 of Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC <sup>(4)</sup>, coordinated monitoring programmes may be established, especially when specific needs are identified, to assess risks and to establish baseline values related to zoonoses and zoonotic agents at the level of Member States.

(7) Scientific experts in collaboration with the EFSA prepared technical specifications for a baseline study on a harmonised monitoring of *Campylobacter* in broiler flocks. Training was organised in 2006 for laboratory staff in all Member States on the detection methods for *Campylobacter* in such flocks and is scheduled in 2007 with regard to the enumeration method for *Campylobacter* on carcasses.

<sup>(1)</sup> OJ L 224, 18.8.1990, p. 19. Decision as last amended by Decision 2006/965/EC (OJ L 397, 30.12.2006, p. 22).

<sup>(2)</sup> The EFSA Journal (2006) 94.

<sup>(3)</sup> OJ L 228, 3.9.2005, p. 14.

<sup>(4)</sup> OJ L 325, 12.12.2003, p. 31. Directive as amended by Council Directive 2006/104/EC (OJ L 363, 20.12.2006, p. 352).

- (8) The Task Force on Monitoring of Zoonoses Data Collection of EFSA adopted during its meeting on 16 and 17 October 2006 the report on proposed technical specifications for a coordinated monitoring programme for *Salmonella* and *Campylobacter* in broiler meat in the EU <sup>(1)</sup>.
- (9) The Task Force also adopted a Report including a proposal for a harmonised monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys and pigs and *Campylobacter jejuni* and *C. coli* in broilers <sup>(2)</sup> on 20 February 2007. The report makes recommendations on a harmonised monitoring scheme and harmonised methodology for susceptibility testing.
- (10) Pursuant to Article 7(3) and Annex II(B) of Directive 2003/99/EC, detailed rules should be laid down on the antimicrobial resistance monitoring of *Campylobacter jejuni* and *Campylobacter coli* in poultry. Data needs to be collected in order for such rules to be laid down. Therefore, testing of the antimicrobial resistance should be included in the survey in order to gather the necessary data.
- (11) Taking into account the high number of *Salmonella* and *Campylobacter* cases in humans, the importance of broilers and broiler meat as source of infection and the increasing concern on antimicrobial resistance development, comparable data on the prevalence of *Campylobacter* in broilers and broiler meat, *Salmonella* in broiler meat in the Member States should be collected to consider the need, feasibility, cost and benefit of Community-wide control measures.
- (12) The survey is to provide technical information necessary for the development of Community veterinary legislation including on the use of antimicrobials in zoonoses control programmes in poultry. Given the importance of collecting comparable data on the prevalence of *Salmonella* and *Campylobacter* in broilers and broiler meat and antimicrobial resistance of *Campylobacter* in broiler flocks in the Member States, they should be granted a Community financial contribution for implementing the specific requirements of the survey. It is appropriate to reimburse 100 % of the costs incurred on the laboratory testing, subject to a ceiling. All other costs incurred, such as costs for sampling, travel and administration should not be eligible for any Community financial contribution.
- (13) A financial contribution from the Community should be granted provided that the survey is carried out in accordance with Community law and subject to compliance with certain other conditions.
- (14) A financial contribution from the Community should be granted insofar as the actions provided for are effectively carried out and provided that the competent authorities furnish all the necessary information within the time limits provided for in this Decision.
- (15) For reasons of administrative efficiency all expenditure presented for a financial contribution by the Community should be expressed in euro. In accordance with Council Regulation (EC) No 1290/2005 of 21 June 2005 on the financing of the common agricultural policy <sup>(3)</sup>, the conversion rate for expenditure in a currency other than euro should be the rate most recently set by the European Central Bank prior to the first day of the month in which the application is submitted by the Member State concerned.
- (16) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS DECISION:

#### Article 1

##### Subject matter and scope

This Decision lays down rules on a financial contribution from the Community towards a survey to be carried out in the Member States on the prevalence of:

- (a) *Campylobacter* spp. in broiler flocks and their antimicrobial resistance; and
- (b) *Campylobacter* spp. and *Salmonella* spp. in broiler carcasses.

#### Article 2

##### Definitions

For the purposes of this Decision, the following definitions shall apply:

- (a) 'flock' means all poultry (such as broiler) 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, it includes all birds sharing the same airspace;

<sup>(1)</sup> The EFSA Journal (2007) 96, 1-46.

<sup>(2)</sup> The EFSA Journal (2006) 403, 1-62.

<sup>(3)</sup> OJ L 209, 11.8.2005, p. 1. Regulation as last amended by Regulation (EC) No 378/2007 (OJ L 95, 5.4.2007, p. 1).

(b) 'slaughter batch' means a delivery of broilers which have been raised in the same flock to a slaughterhouse on one single day;

(c) 'competent authority' means the authority or authorities of a Member State as designated under Article 3 of Regulation (EC) No 2160/2003 of the European Parliament and of the Council <sup>(1)</sup>.

#### Article 3

##### Zoonoses and zoonotic agents covered by the survey

The Member States shall carry out a survey to assess the prevalence of the following zoonoses and zoonotic agents in samples taken in slaughterhouses selected at random in accordance with Annex I:

(a) *Campylobacter* spp. in broiler flocks and their antimicrobial resistance;

(b) *Campylobacter* spp. in broiler carcasses;

(c) *Salmonella* spp. in broiler carcasses;

across the Community sampled in slaughterhouses. Only broilers produced from day one within the Member State shall be included in the survey.

#### Article 4

##### Performance of sampling and analyses

1. Sampling shall be performed by the competent authority or under its supervision in accordance with the technical specifications set out in Annex I.

2. National reference laboratories (NRLs) for *Salmonella* spp., *Campylobacter* spp. and antimicrobial resistance testing shall perform the relevant parts of the analyses of samples and isolates.

3. However, the competent authority may decide to designate other laboratories involved in official controls of *Salmonella* spp., *Campylobacter* spp. and antimicrobial resistance testing, to perform the analyses of samples and isolates.

In such cases, the NRLs shall provide support and training to the designated laboratories and ensure that they comply with rules on quality controls by arranging regular ring tests.

The laboratories designated in accordance with the third paragraph of this Article which perform testing must comply with the following conditions:

(a) they must have proven experience of using the methods required for the testing;

(b) they must have a quality assurance system complying with EN/ISO standard 17025;

(c) they must be subjected to the supervision of the relevant NRLs.

#### Article 5

##### Conditions for paying a Community financial contribution

1. The Community financial contribution towards the costs for sampling and analyses shall be paid to the Member States up to the maximum total amount for the co-financing set out in Annex II.

2. The Community financial contribution provided for in paragraph 1 shall be paid to the Member States provided that the survey is implemented in accordance with the relevant provisions of Community law, including rules on competition and on the award of public contracts, and subject to compliance with the following conditions:

(a) the laws, regulations and administrative provisions required to implement the survey must enter into force by 31 December 2007 at the latest;

(b) a progress report containing the information listed in Part E(1) to Annex I and covering the first three months of the survey shall be submitted to the Commission by 31 May 2008 at the latest;

(c) a final report on the implementation of the survey containing all information in points 1 and 2 of Part E to Annex I, together with supporting evidence for the costs incurred by the Member States for the sampling and analyses and the results attained during the period from 1 January 2008 to 31 December 2008 shall be submitted to the Commission by 28 February 2009 at the latest; and the evidence as to costs incurred must comprise at least the information set out in Annex III;

(d) the survey must be implemented effectively.

<sup>(1)</sup> OJ L 325, 12.12.2003, p. 1. Regulation as last amended by Council Regulation (EC) No 1791/2006 (OJ L 363, 20.12.2006, p. 1).

3. Failure to submit the final report referred to in paragraph 2(c) by 28 February 2009 at the latest shall entail a progressive reduction of the financial contribution to be paid, amounting to 25 % of the total amount by 30 March 2009, 50 % by 30 April 2009 and 100 % by 30 May 2009.

#### Article 6

##### Maximum amounts to be reimbursed

The maximum amounts of the Community financial contribution towards the costs to be reimbursed to the Member States for sampling and analyses covered by the survey shall not exceed the following:

- (a) EUR 20 for each detection testing of *Campylobacter* and *Salmonella* spp;
- (b) EUR 30 for each confirmation, speciation and enumeration of *Campylobacter* spp. isolates and the serotyping of *Salmonella* spp. isolates;
- (c) EUR 30 for antimicrobial testing of *Campylobacter* isolates from broiler flocks.

#### Article 7

##### Collection of data, assessment and reporting

1. The competent authority responsible for preparing the yearly national report pursuant to Article 9(1) of Directive 2003/99/EC shall collect and assess the results of the sampling and analyses with regard to the prevalence of *Salmonella* and *Campylobacter* carried out pursuant to Article 4 of this Decision and shall report all necessary data and its assessment thereof by the Member States to the Commission by 28 February 2009 at the latest. The results from antimicrobial resistance testing will be reported before the end of May 2009 in the frame of the annual reporting in accordance with Article 9(1) of Directive 2003/99/EC.

2. The Commission shall forward those results obtained during the implementation of the survey together with the national aggregated data and the assessments thereof by the Member States to the European Food Safety Authority, which shall examine them.

Any use of the data submitted by the Member States for the purpose other than the survey shall be subject to prior agreement of the Member States.

3. National aggregated data and results shall be made available publicly in a form that ensures confidentiality.

#### Article 8

##### Conversion rate for expenditure

Where a Member State's expenditure is in a currency other than the euro, the Member State concerned shall convert it into euro by applying the most recent exchange rate set by the European Central Bank prior to the first day of the month in which the application is submitted by the Member State.

#### Article 9

##### Application

This Decision shall apply from 1 January 2008.

#### Article 10

##### Addressees

This Decision is addressed to the Member States.

Done at Brussels, 19 July 2007.

For the Commission  
Markos KYPRIANOU  
Member of the Commission

## ANNEX I

## TECHNICAL SPECIFICATIONS REFERRED TO IN ARTICLE 4

## PART A

**Sampling frame**

To avoid age-related effects the monitoring shall be carried out on slaughter batches at the slaughterhouse.

As prevalence of *Campylobacter* spp. has been shown to vary significantly depending on the season, this merits stratification. For that purpose a 12-month period must be divided in 12 periods of one month. In each of those periods 1/12th of the total sample size must be taken.

The sampling must otherwise be based on a random selection, both regarding slaughterhouses, sampling days each month and which batches are to be sampled on a selected sampling day. In particular, the randomisation scheme shall guarantee a selection of slaughter batches proportionate to the number of flocks fattened according to the different production types (conventional, free-range, organic). In addition, the *Salmonella* spp. or *Campylobacter* spp. status, if known at slaughter, must not bias the randomisation. The competent authority shall take responsibility for generating the randomization scheme and ensuring it is implemented correctly. An example of a randomisation procedure is provided in the Report of the EFSA Task Force on Monitoring of Zoonosis Data Collection on proposed technical specifications for a coordinated monitoring programme for *Salmonella* and *Campylobacter* in broiler meat in the EU. Details of the randomisation scheme shall be reported to the Commission.

## PART B

**Sample size****1. Primary sample size**

- (a) The primary sample size gives the number of slaughter batches to be tested.
- (b) At least 384 slaughter batches shall be sampled. Non-response shall be anticipated by sampling about 10 % more than the indicated numbers.
- (c) By way of derogation from point (b), the following numbers of slaughter batches <sup>(1)</sup> shall be sampled in Estonia, Latvia and Luxembourg:
  - (i) in Estonia, at least 96 slaughter batches;
  - (ii) in Latvia, at least 120 slaughter batches;
  - (iii) in Luxembourg, at least 12 slaughter batches.

**2. Secondary sample size**

The secondary sample size gives the number of individual broiler chickens per slaughter batch to be sampled. That number shall be 10 birds for the detection of *Campylobacter* in caeca and one bird for the detection of *Campylobacter* and *Salmonella* on carcasses. These caeca samples and carcass sample must be from the same slaughter batch.

## PART C

**Specimen collection, handling and analysis for the detection and antimicrobial testing of *Campylobacter* spp. in broiler flocks****1. Collection and transport**

*Campylobacters* are relatively fragile organisms, which die quickly outside the host gut. Therefore, care shall be taken to ensure that samples are taken appropriately and analysed quickly. Extreme temperatures have to be avoided and transport shall be as fast as possible.

Samples to be collected shall be intact caeca. Caecal samples shall be taken at the time of evisceration.

<sup>(1)</sup> Estimation: number of holdings (four in Estonia, five in Latvia) × two flocks per holding × two slaughter batches per flock × six rounds per year. In Luxembourg, only broilers from three small flocks are slaughtered. A slaughter batch from each of them will be sampled each trimester.

Only staff trained in standard sampling procedures shall collect samples. The main objective shall be to minimise external contamination from caecal content while sampling. That is best achieved by careful manual traction at the junction with the intestine. One intact caecum shall be taken per bird, and samplers shall verify that the caecum is full or it shall be disregarded. Birds shall preferably be sampled at random throughout the batch (avoiding the first part of the batch to be slaughtered), collecting samples from non-consecutive birds. The 10 caeca collected may be placed in a single sterile bag/pack for transport.

All relevant information available from the sample must be recorded on a sampling form produced by the competent authority to enable the reporting requirements in Part E to be fulfilled. Each sample and its sample form must be labelled with a unique number which must be used from sampling to testing. The competent authority must arrange for the issue and use of a unique numbering system. The same identifier of the slaughter batch shall be used as the one for the carcass sample.

Caecal samples shall be transported as intact caeca to the laboratory within 24 hours (i.e. overnight postage or courier) and analysed there immediately. In cases where that cannot be managed, the samples shall be kept refrigerated at least until transported to the laboratory and they shall be analysed no later than 72-80 hours after sampling. At the laboratory, samples that cannot be tested on the day of arrival shall be kept refrigerated until analysis.

At the laboratory, the caecal contents shall be aseptically removed and pooled to one composite sample.

## 2. *Diagnostic method*

### 2.1. *Culture*

Direct culture on a selective medium provides a good estimate of the prevalence of *Campylobacters*. Direct culture of the sample shall be carried out on a selective medium suitable for *Campylobacter*, (i.e. modified *Campylobacter* blood free selective medium (CCDA); Karmali; or Preston Agar).

The plates shall be incubated at  $41,5 \pm 1$  °C, in a micro-aerobic atmosphere, for at least 48 +/- 2 hours. Growth may be detected after 24 hours.

The micro-aerobic atmosphere may be obtained in commercially available micro-aerobic incubators (gas mixture 10 % CO<sub>2</sub>/6 % O<sub>2</sub>). In the absence of such incubators, micro-aerobic culture systems can be used i.e. gas jars. Commercial gas pack systems providing the appropriate micro-aerobic atmosphere are available.

Suitable positive and negative controls shall be included for each batch of samples cultured.

### 2.2. *Confirmation and speciation of the genus Campylobacter*

Isolation and confirmation of *Campylobacter* organisms should be undertaken as described in ISO 10272-1:2006(E). At least one *Campylobacter* isolate per batch must be speciated using phenotypic methods as described in ISO 10272-1:2006(E) or published molecular methods such as Polymerase Chain Reaction (PCR) techniques. The method used shall be indicated. The isolate speciated should be used for subsequent antimicrobial testing.

If a laboratory is less experienced in speciation, it shall store the isolate as set out in 2.4 pending additional training or send it to a more experience laboratory in consultation of the Community reference laboratory for *Campylobacter*.

### 2.3. *Quality control*

For quality assurance, a proportion of *Campylobacter* spp. isolates with a maximum of eight isolates shall be sent to the Community reference laboratory for *Campylobacter* for confirmation and speciation.

A proportion of those isolates shall be sent to that laboratory either in one batch or on a quarterly basis. If isolates are to be transported between laboratories, appropriate conditions (for example charcoal swabs) shall be used.

### 2.4. *Storage*

At least one isolate per positive sample shall be stored at the NRLs using the normal method for NRL culture collection, as long as it ensures viability of the strains for a minimum of two years.

### 2.5. Antimicrobial resistance testing

The number of *Campylobacter* isolates to be included in the antimicrobial resistance monitoring per Member State shall be 170. Not more than one isolate per *Campylobacter* species from the same slaughter batch shall be included in the monitoring.

In those Member States where, in any given year, a lower number of isolates than the target sample size is available, all these isolates shall be included in the antimicrobial resistance monitoring.

In those Member States where a higher number of isolates is available all isolates, or a representative random selection equal or larger than the target sample size, shall be included.

Member States shall test at least the antimicrobials that are specified in Table 1, using the cut-off values given and an appropriate concentration range to determine the susceptibility of *Campylobacter*.

Table 1

	Antimicrobial	Cut-off value (mg/L) R >
<i>Campylobacter</i> <i>Jejuni</i>	Erythromycin	4
	Ciprofloxacin	1
	Tetracycline	2
	Streptomycin	2
	Gentamicin	1
<i>Campylobacter</i> <i>Coli</i>	Erythromycin	16
	Ciprofloxacin	1
	Tetracycline	2
	Streptomycin	4
	Gentamicin	2

Dilution methods shall be performed according to the methods described in CLSI guidelines M31-A3 — Third Edition, Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals and M100-S16, Performance Standards for Antimicrobial Susceptibility testing; Sixteenth International Supplement.

## PART D

### Specimen collection, handling and analysis for the detection of *Campylobacter* spp. and *Salmonella* Spp. in broiler carcasses

#### 1. Collection and transport

One whole carcass per slaughter batch shall be collected 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 shall be placed in a separate sterile plastic bag, avoiding cross-contamination and be sent to the laboratory where skin sampling shall be undertaken.

Cross-contamination by other carcasses or caeca samples must be avoided during the collection of the carcasses. 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 survey.

All relevant information available from the sample shall be recorded on a sampling form produced by the competent authority to enable the recording requirements in Part E to be fulfilled.

Each sample and its sample form shall be labelled with a unique number which shall be used from sampling to testing. The competent authority must arrange for the issue and use of a unique numbering system. The same identifier of the slaughter batch shall be used as the one for the caeca samples.

The samples shall be kept at between + 2 to 8 °C and free of external contamination during transportation.

All samples shall ideally reach the laboratory within 24 hours of sampling. In exceptional situations (for example, long journeys, weekends and public holidays) that period may be extended to 80 hours.

In the case that different laboratories are used for *Campylobacter* and *Salmonella* testing then the laboratory testing *Campylobacter* should take preference in receipt of the sample.

## 2. Sampling in the laboratory and the analytical methods

### 2.1. 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.

Samples shall be held at + 2-8 °C in the laboratory and the laboratory sampling procedure shall begin as soon as possible after the arrival of the samples at the laboratory and in any case within 72-80 hours from the time of sampling.

### 2.2. Sample preparation

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

Handlers must avoid cross-contamination between samples and from the surrounding environment at all stages.

Wearing disposable gloves, the chicken shall be removed from its sample bag, taking care not to contaminate the outer surface of the chicken.

Using a sterile instrument and aseptic technique, the neck skin shall be removed, if present, together with the skin from one side of the carcass avoiding any fat to make a 27 g test portion and placed into a stomacher bag (or pulsifier).

### 2.3. Initial suspension

The 27 g test portion shall be transferred to NINE volumes (243 ml) buffered peptone water (BPW), brought to room temperature before adding. The mixture shall be treated in a stomacher or pulsifier for approximately ONE minute (27 g are needed to perform analyses for *Salmonella* spp. and *Campylobacter* spp. from one sample in parallel). Foaming shall be avoided by removing the air from the stomacher bag as much as possible.

This initial suspension shall be used as follows:

- (a) 10 ml (~1g) shall be transferred to 90 ml enrichment medium for *Campylobacter* spp. detection;
- (b) 10 ml (~1g) shall be transferred to an empty sterile tube; 1 ml is used for the enumeration of *Campylobacter* spp. on selective plates.

The rest of the initial suspension (250 ml ~ 25g) shall be used for the detection of *Salmonella* spp.

### 2.4. Detection, identification methods for *Salmonella* spp.

#### 2.4.1. Detection of *Salmonella* spp.

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

#### 2.4.2. Serotyping of *Salmonella* spp.

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 for *Salmonella*, with a maximum of 16 non-typeable isolates. A proportion of those isolates shall be sent to that laboratory on a quarterly basis.

#### 2.4.3. Phage typing of *Salmonella* spp.

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

### 2.5. Detection, identification and quantification methods for *Campylobacter* spp.

#### 2.5.1. Detection of *Campylobacter* spp.

Isolation and confirmation of *Campylobacter* organisms should be undertaken as described in ISO 10272-1:2006(E). At least one *Campylobacter* isolate per batch must be speciated using phenotypic methods as described in ISO 10272-1:2006(E) or published molecular methods such as Polymerase Chain Reaction (PCR) techniques. The method used shall be indicated.

For quality assurance, a proportion of *Campylobacter* spp. isolates with a maximum of eight isolates shall be sent to the Community reference laboratory for *Campylobacter* for confirmation and speciation.

A proportion of those isolates shall be sent to that laboratory on a quarterly basis. If isolates are to be transported between laboratories, appropriate conditions (for example charcoal swabs) shall be used.

#### 2.5.2. Quantification of *Campylobacter* spp.

The quantitative detection of *Campylobacter* spp. shall be carried out according to 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 0,1 ml of this initial suspension and of further dilutions thereof shall be examined to allow the enumeration of up to  $10^6$  cfu/g. In addition, 1 ml of the undiluted initial suspension shall be examined to obtain a limit of enumeration of 10 cfu/g. All plate determinations shall be done in duplicate.

To enable correct comparison and judgement of data (for future risk assessment) the measurement uncertainty (MU) of the quantitative determination method shall be estimated for each laboratory.

To estimate the MU 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 intra-laboratory 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. Raw data on MU estimation shall be reported separately as part of the overall description on the implementation of the survey as set out in Part E.

### 3. Storage of isolates

In order to allow for e.g. later testing for antimicrobial susceptibility storage of a representative subset of isolates is recommended. One isolate per positive sample shall be stored. The *Campylobacter* isolate obtained from the quantitative analysis must be preferred. The isolates must be stored at the NRLs using the normal method for NRL culture collection, as long as it ensures viability of the strains for a minimum of two years.

## PART E

### Reporting

Reports shall be made including at least the following information:

#### 1. Overall description on the implementation of the survey:

— slaughterhouses: total number per country and the number that were sampled,

- primary sample size realised,
- description of the stratification and randomisation procedures,
- description of the quality control activities, inclusive a report on the 12 MU estimations per laboratory for *Campylobacter* quantification,
- overall results.

2. Specific information with regard to prevalence data

Member States shall submit the results of the investigation in the form of raw data using a data dictionary and data collection forms provided by the Commission.

That data shall include at least the following information:

- name/code of the slaughterhouse,
- identifier of the slaughter batch,
- name/code of the holding (farm) of origin of the slaughter batch,
- holding size if known,
- *Salmonella* vaccination status of the flock if known,
- age of the broilers at sampling (slaughter),
- information regarding if this was the first or a subsequent batch to be slaughtered from the flock (preceding thinning or not),
- production type (i.e. conventional, free-range, organic),
- results of previous *Salmonella* and *Campylobacter* testing in the same flock,
- date of sampling,
- number of birds slaughtered per year in that slaughterhouse,
- type of chilling method used (air, immersion, spray),
- details of transport protocol (as specified: Y/N),
- date received at laboratory,
- date of testing,
- identification of the laboratory,
- type of sample,
- description of the culture methods used, in particular the selective medium/media,
- *Campylobacter* isolate: Method used for speciation,

- *Campylobacter*: result of the bacteriological testing, including speciation from the caecal sample,
- *Campylobacter*: result of the bacteriological testing, including speciation and quantification from the carcass sample,
- *Salmonella*: result of bacteriological testing and serotyping,
- time between sampling and analysis (per 12 hour period).

3. Specific information with regard to antimicrobial resistance testing of *Campylobacter* isolates of caecal samples.

The results of the antimicrobial resistance monitoring shall be assessed and reported, in accordance with Article 9 of Directive 2003/99/EC, in the yearly report on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance.

Without prejudice to the provisions of Annex IV of Directive 2003/99/EC the following information shall be reported:

- origin of isolates i.e. baseline study, control programme, passive surveillance,
  - number of isolates susceptibility tested per *Campylobacter* species,
  - number of isolates found to be resistant per antimicrobial per *Campylobacter* species, and
  - number of fully-susceptible isolates and number of isolates resistant to 1, 2, 3, 4 and > 4 antimicrobials listed in Table 1 per *Campylobacter* species.
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## ANNEX II

**Maximum Community financial contribution to the Member States**

(EUR)

Member State	Maximum total amount for co-financing of sampling and analyses
Belgium — BE	58 092
Bulgaria — BG	58 092
Czech Republic — CZ	58 092
Denmark — DK	58 092
Germany — DE	58 092
Estonia — EE	14 688
Ireland — IE	58 092
Greece — EL	58 092
Spain — ES	58 092
France — FR	58 092
Italy — IT	58 092
Cyprus — CY	58 092
Latvia — LV	18 360
Lithuania — LT	58 092
Luxembourg — LU	1 836
Hungary — HU	58 092
Malta — MT	58 092
Netherlands — NL	58 092
Austria — AT	58 092
Poland — PL	58 092
Portugal — PT	58 092
Romania — RO	58 092
Slovenia — SI	58 092
Slovakia — SK	58 092
Finland — FI	58 092
Sweden — SE	58 092
United Kingdom — UK	58 092
Total	1 429 092

## ANNEX III

**Certified financial report on the implementation of a survey on the prevalence of *Campylobacter* spp. in broiler flocks and their antimicrobial resistance and on the prevalence of *Campylobacter* spp. and *Salmonella* spp. in broiler carcasses**

Reporting period: ..... to .....

**Statement on costs incurred on the survey and eligible for Community financial contribution:**

Reference number of Commission Decision providing Community financial contribution:

.....  
 .....

Costs incurred related to functions at/by	Number of tests	Total costs of testing incurred during reporting period (national currency)
Bacteriological detection of <i>Campylobacter</i> spp.		
Bacteriological detection of <i>Salmonella</i> spp.		
Confirmation of <i>Campylobacter</i> spp.		
Speciation of <i>Campylobacter</i> isolates		
Enumeration of <i>Campylobacter</i> isolates		
Serotyping of <i>Salmonella</i> isolates		
Antimicrobial resistance testing of <i>Campylobacter</i> isolates		

**Declaration by the beneficiary**

We certify that:

- the above costs are genuine and have been incurred in carrying out the tasks laid down in this Decision and were essential for the proper performance of those tasks;
- all supporting documents supporting for the costs are available for audit purposes;
- no other Community contribution was requested for this programme.

**Date:** .....

**Person financially responsible:** .....

**Signature:** .....

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