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**BASELINE SURVEY ON THE PREVALENCE OF
SALMONELLA IN FLOCKS OF TURKEYS IN THE EU**

TECHNICAL SPECIFICATIONS

PRESENTED AT THE MEETING OF
THE STANDING COMMITTEE ON THE FOOD CHAIN AND ANIMAL HEALTH
ON 18 JULY 2006

BASELINE SURVEY ON THE PREVALENCE OF SALMONELLA IN FLOCKS OF TURKEYS IN THE EU

TECHNICAL SPECIFICATIONS

1. OBJECTIVES OF THE SURVEY

The objective is to estimate the prevalence of *Salmonella* spp in flocks/holdings of turkeys (*Meleagris gallopavo*) in the Member States of the European Union, taking into account the sampling frame below. The current technical specifications cover fattening turkeys and breeding turkeys.

The survey will concentrate on zoonotic *Salmonella*. *Salmonella Pullorum* and *Salmonella Gallinarum* are excluded from its scope.

The results of this survey found in the different Member States shall be comparable. The survey should cover a one year period commencing 1 October 2006.

2. SAMPLING FRAME

In fattening turkeys, the sampling frame should cover primarily holdings representing at least 80% of the total population of turkey meat finishing flocks. This can usually be achieved by including all holdings with at least 500 birds at a given time. The number of holdings sampled in holdings with either 500 to 4999 birds or >5 000 birds should be proportionate to number of animals in these 2 populations. Preferably, only one flock per holding should be sampled.

- For countries where the calculated number of holdings to be sampled (see Chapter 3) is higher than the number of holdings available with at least 500 birds, up to four flocks may be sampled on the same holding in order to achieve the calculated number of flocks
- Where possible these additional flocks from a single holding should be from different turkey houses and samples taken in different seasons. Larger holdings should be focussed on.
- If the number of flocks to be sampled is still not sufficient, more than four flocks may be sampled on the same holding, larger holdings being focused on.
- For countries where fewer than 80% of the meat turkeys are kept in holdings above 500 birds, progressively smaller holdings may initially be selected, focussing preferably on holdings with more than 250 birds.
- Selection of holdings should take into account the risk that certain initially selected holdings may not be sampled (e.g. because of early slaughtering or closing down the production in the holding) and therefore a slightly larger number of holdings should initially be considered or arrangements made for timely substitution of unsuitable holdings.
- The birds shall be sampled within 3 weeks before leaving of the last animals for slaughter.

With regard to breeding turkeys, all flocks on holdings with at least 250 birds should be sampled once during the survey within 9 weeks before depopulation.

As far as possible, sampling should be equally distributed over the year to cover the different seasons.

In order to organise such sampling, the authorities should organise a system enabling them to visit the flocks at the appropriate time.

Sampling shall be performed by the competent authority or under its supervision.

Definition of holding: A facility used for the breeding, rearing or fattening of turkeys.

Definition of flock as established in Article 2 of Regulation 2160/2003/EC:

All poultry of the same health status kept on the same premises or in the same enclosure and constituting a single epidemiological unit. In the case of housed poultry this includes all birds sharing the same airspace. In the case of free-range birds this includes all birds sharing a common airspace or outdoor area.

3. SAMPLE SIZE IN FATTENING FLOCKS AND SELECTION OF FLOCKS

Primarily, the number of flocks to be tested (sample size) has to be calculated. It is calculated on the basis of the following criteria:

- Target prevalence: 50%
- Desired confidence level: 95%
- Accuracy: 5%

Countries shall sample the number of flocks as calculated on the basis of the above criteria, and should not take into account the prevalence known in the country from other pre-existing measures. The calculation of the number of flocks to be sampled takes into account the population size (i.e. the total number of flocks). For finite populations the sample size will be smaller than for large (infinite) populations (the latter applies to populations above 100 000 units). Therefore, it may be necessary to adjust the sample size to the population size taking into account the following considerations:

Annex 1 provides the formula for the calculation of the approximate sample size required.

Calculation of the population size (total number of flocks in a MS)

It is expected that on average:

- 80% of holdings reported are considered as holdings with fattening turkeys
- there are 2 houses per holding
- there are 2.5 turkey flocks raised per house per year

Consequently, to establish the population size (total number of flocks), the number of holdings in a MS should be multiplied by the factor 5.

If detailed data are available in the country, a more detailed estimation of the population may be done.

If the calculated population size turns out to be less than 100 000 flocks, the sample size should be adjusted taking into account the calculated population size.

Stratification

In each Member State, the population of turkeys should be stratified according to holding size and region. It should be representative of the population of turkey production flocks, thus covering a representative number of holdings, flocks and all four seasons.

Annex 2 provides an estimation of the number of flocks to be sampled, broken down by the categories of holding size.

4. SAMPLES

The same sampling procedure should be applied for breeding and fattening turkey.

For all production types the same sampling approach can be applied. For free range flocks, samples should only be collected in the area inside the house.

There should be 5 pooled samples taken in any selected flock. Each pooled sample should comprise faecal material fixed to a pair of boot swabs (or sock samples which are considered equivalent).

This type of sample is considered equivalent to 300 faeces samples picked up individually and combined to 5 pools. This sampling procedure will theoretically provide 95% confidence of detection of 1% within flock prevalence assuming the test is 100% sensitive.

In small flocks (e.g. flocks below 100 turkeys), where it is not possible to use boot swabs/socks because it is not possible to access the houses, these may be replaced by hand drag swabs – where the boot swabs or socks are worn over gloved hands and rubbed over surfaces contaminated with fresh faeces, or if not feasible, by other sampling techniques for faeces. As far as possible, 5 separate samples should be taken.

Only staff trained in standard sampling procedures should collect samples.

Details of sampling procedures

Five pairs of ‘boot’ or ‘sock’ swabs, must be taken from each flock to be sampled at farm.

1. Boot swabs are a commercial absorptive paper/fabric overboot. Sock swabs comprise a 20 cm length of Tubegrip bandage which is bunched up around the ball of the foot. Sock swabs should be turned by 90° to expose a clean surface three times during the sampling run but boot swabs are not turned. Tubegrip ‘socks’ can also be used as boot swabs if they are extended over the whole foot rather than folded over the ball of the foot.
2. Ensure that there can be no contamination of swabs prior to use or no contact with disinfectant, for example, plastic overboots should be put on after walking through disinfectant boot-dips and not before.
3. Enter house to be sampled and put on a pair of new plastic overboots before putting on the boot/sock swabs. It is normally easier to put on subsequent pairs of overboots on top of previous pair. Ensure that a new pair of plastic overboots is used for each sample and that there can be no cross contamination of boot swabs via hands when changing overboots or boot swabs. It is best to use new disposable gloves for each change of overboots and boot swabs.
4. Before putting on the boot/sock swabs: moisten the surface of the boot/sock-swabs or socks with maximum recovery diluent (MRD: 0.8% sodium chloride, 0.1% peptone in sterile deionised water), or sterile water or any other diluent approved by the National Reference Laboratory. The use of farm water containing antimicrobials or additional disinfectants should be avoided. The easiest way to moisten boot swabs is to pour liquid inside before putting on over the plastic overboots or into the inside of the boot swab after putting it on. Alternatively boot swabs or socks may be autoclaved with diluent within autoclave bags or jars before use. Diluent can also be applied after boots are put on using a spray or wash bottle.
5. Divide the floor area of the house into five equal sectors for sampling. If the house is internally subdivided, ensure that all sections are represented in the sampling in a proportionate way.

6. Move about within the chosen sector so that at least 100 steps per pair of boot/sock swabs is covered, ensuring that all parts of the sector are sampled, including littered and non-littered areas but not including any outdoor areas in free-range flocks.
7. On completion of sampling in the chosen sector carefully remove the boot/sock swabs so as not to dislodge adherent material. Boot swabs should be inverted to retain material. Place in bag or pot and ensure this is labelled. It is possible to gather boot swabs into sterile jars which can subsequently be used for culture of the sample.
8. Samples shall be sent by fast mail or courier to the relevant laboratory.

5. TESTING

National reference laboratories for salmonella are the laboratories where detection and serotyping shall take place. In case the National Reference Laboratory does not have the capacity to perform analyses or if it is not the laboratory that performs detection routinely, the competent authorities may decide to designate a limited number of other laboratories involved in official control of salmonella to perform the analyses. These laboratories should have proven experience of using the required detection method and have a quality assurance system complying with ISO standard 17025 and be submitted to the supervision of the national reference laboratory. At the laboratory, samples should be kept refrigerated until examination, which should be begun within 48 hours of receipt.

Processing boot/sock swabs in the laboratory

1. It is possible to gather boot/sock swabs at farm into sterile jars which can subsequently be used for culture of the sample.
2. On arrival at the laboratory sterilise the outside of the jar by spraying with 70% alcohol and leave for at least one minute before adding a minimum of 225 ml of room temperature Buffered Peptone Water (BPW). If swabs are transported in bags, carefully evert the bags so that the boot swabs and any loose litter material is emptied into a minimum amount of 225 ml BPW.

Swirl the jar gently then place in the incubator.

Detection method:

The method recommended by the Community Reference Laboratory (CRL) for Salmonella in Bilthoven, the Netherlands, shall be used. This method is described in the current version of draft Annex D of ISO 6579 (2002): 'Detection of Salmonella spp. in animal faeces and in samples of the primary production stage'. In this method, a semi-solid medium (MSRV) is used as the single selective enrichment medium.

Serotyping:

At least one isolate from each positive sample shall be typed in the National Reference Laboratory for Salmonella. The National Reference Laboratory for Salmonella shall follow the Kaufmann-White scheme.

For quality assurance, a proportion of the non-typable isolates shall be sent to the CRL, with a maximum of 16 non-typable isolates. A proportion of these isolates should be sent to the CRL on a quarterly basis.

Storage of strains:

Strains isolated shall be stored, using the normal method for NRL culture collection, as long as it ensures the integrity of the strains for a minimum of 2 years.

Phagotyping:

It is strongly recommended that at least one isolate of *S. Enteritidis* and *S. Typhimurium* from each positive holding should be phagetyped, using the protocol defined by HPA Colindale, London.

Testing of Anti-microbial susceptibility:

For epidemiological purposes, it is recommended that, where possible, one isolate per serotype per flock is used for anti-microbial susceptibility testing. As far as possible, quantitative methods should be implemented and CLSI (previously NCCLS) standards should be used.

6. REPORTING

A flock is considered positive for the purpose of this survey if the presence of *Salmonella* spp. is detected in at least one of the samples.

The national authority responsible for the preparation of the yearly national report on the monitoring of *Salmonella* spp. in animals pursuant to Article 9 of Directive 2003/99/EC shall collect and assess the results and report all necessary data and its assessment to the Commission. All serotypes (which are investigated as an objective of this survey) shall be reported separately, including untypable serotypes.

Complete data for all sampled flocks shall be reported by the Member States to the Commission, containing the data mentioned below. The format according to which these data should be presented should be established by the Commission in consultation with Member States and involved parties as appropriate (CRL -Salmonella and EFSA).

The relevant data collected for the purpose of the survey shall be supplied by Member States to the European Food Safety Authority, at the request of the Commission. Any use of the data submitted by the Member States for 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. Preparation of the Community report will be done in consultation between the Commission, EFSA, CRL-Salmonella and Member States.

A progress report on the state of play with the implementation of the survey for covering the sampling period October-December 2006, including organisational matters, raw and compiled data and any difficulties encountered, shall be provided to the Commission before the end of February 2007. A final report together with the supporting financial documents shall be provided by 15 November 2007.

The following information should be at least reported to the Commission. Information under part 2 will be detailed in a data dictionary and reported electronically.

1. Overall description on the implementation of the programme

- Description of the turkey population under the survey stratified according to holding size and separating between flocks with birds reared for breeding, reared for fattening, fattening and breeding flocks, and between different production types.
- Description of randomisation procedure, including notification system and regional distribution of holdings sampled; the origin of information on the size of the holdings
- Sample size calculated and realized
- Details of authorities and laboratories involved in sampling/testing/typing
- Overall results of the survey in particular: prevalence of *Salmonella* spp.; prevalence of *Salmonella* Typhimurium, *S. Enteritidis*, *S. Virchow*, *S. Hadar*, *S. Infantis* and other serotypes
- If additional sampling is performed, details on samples and testing methods should be provided as well as comments on the results.

2. Complete data on each holding and flock sampled and corresponding tests results:

- Reference of the holding
- Holding production group (breeding, fattening)
- Holding production type
 - Free range (animals have access to outside)
 - Free range, organic (animals have access to outside and are registered with a recognised Organic Standard Regulatory Organisation)
 - Conventional (animals are kept inside houses)
- Size (category) of the holding
- Number of turkeys in the holding at any given time – maximum capacity,
- Number of turkeys in the holding – at the time of sampling
- Number of flocks in the holding - maximum capacity at any given time
- Number of crops (flock cycles, each new cycle being accounted for) per year
- Number of turkeys in the flock tested
- Flock production type : type of the flock tested, i.e. conventional/free range standard/free range organic
- Date and place of sampling (xx/yy/ zz; house)
- Age of turkeys at sampling (in days)
- Expected slaughter age (in days)
- Reference of samples
- Type of samples taken: boot/sock swab; other
- In case of additional sampling, specify details separately
- *Salmonella* vaccination status of the turkeys
- Use of antimicrobials during previous 2 weeks

The following information should be collected in Member States for each sample tested in the laboratory:

- ID of the laboratory (in case several laboratories are involved)
- Reference of the holding/flock from which the samples were taken
- Means of transport of samples
- Date of start of testing
- Type of specimen for additional samples

Annex 1

Approximate sample size required

Frankena K, Noordhuizen JP, Willeberg P, van Voorthuysen PF, Goelema JO. EPISCOPE: computer programs in veterinary epidemiology. Vet Rec 1990; 126: 573-576.

Approximate sample size required

The aim is to estimate the sample size required with the desired width of confidence limits: $x \pm w$

Using the normal approximation for an infinite population:

$$w = z_{\alpha} \sqrt{\frac{p(1-p)}{n}}$$

with p =prevalence and z_{α} = value of the standardized normal distribution corresponding to the probability α .

By resolving this for n , we obtain

$$n_{\infty} = \left(\frac{z_{\alpha}}{w} \right)^2 p(1-p).$$

For a finite population:

$$\frac{1}{n} = \frac{1}{n_{\infty}} + \frac{1}{N}$$

with

N =size real population

n_{∞} =size sample with infinite population

n = simple sample with finite population

Annex 2: Summary of population and sampling

Table 1: turkeys population (EUROSTAT 2003, unless more updated information was forwarded by the country)

| Country | Time | Animals in | | | Total Animals | % of animals on holdings | | Holdings with | | | Total holdings > 500 |
|-----------------|-------------|-------------------|----------------------|-------------------|-------------------|-----------------------------|------------|----------------|--------------|--------------|----------------------------|
| | | holdings < 500 | holdings 500-4999 | 5000&> | | >499 | >499 | < 500 | 500-4999 | 5000&> | |
| Belgique/België | 2005 | 1.620 | 21.350 | 213.868 | 236.838 | 99% | 90% | 9 | 9 | 24 | 33 |
| Česká Republika | 2005 | 20.000 | 90.000 | 810.000 | 920.000 | 98% | 88% | 3 | 37 | 69 | 106 |
| Danmark | 2003 | 0 | 20.000 | 360.000 | 380.000 | 100% | 95% | 50 | 1 | 14 | 15 |
| Deutschland | 2003 | 30.000 | 290.000 | 550.000 | 870.000 | 97% | 63% | 1.710 | 120 | 550 | 670 |
| Eesti | 2005 | 29 | 0 | 0 | 29 | 0% | 0% | 130 | 0 | 0 | 0 |
| Ellas | 2006 | | | | 259.800 | 0% | 0% | | | | 42 |
| España | 2003 | | 115.680 | 2.424.732 | 2.540.412 | 100% | 95% | | 37 | 158 | 195 |
| France | 2003 | 230.000 | 3.220.000 | 32.380.000 | 35.830.000 | 99% | 90% | 14.680 | 1.130 | 2.510 | 3.640 |
| Ireland | 2003 | 8.000 | 92.000 | 1.100.000 | 1.200.000 | 99% | 92% | 384 | 105 | 53 | 158 |
| Italia | 2006 | 70.000 | 260.000 | 11.210.000 | 11.540.000 | 99% | 97% | 190 | 44 | 804 | 848 |
| Kypros | 2003 | | | | | | | | 2 | | 2 |
| Latvija | 2003 | 20.000 | 0 | 0 | 20.000 | 0% | 0% | 3.940 | 0 | 0 | 0 |
| Lietuva | 2006 | 21.088 | 12.760 | 147.757 | 181.605 | 88% | 81% | 3.391 | 5 | 9 | 14 |
| Luxembourg | 2003 | | | | 0 | | | 50 | 0 | 0 | 0 |
| Magyarország | 2003 | 140.000 | 250.000 | 3.520.000 | 3.910.000 | 96% | 90% | 22.530 | 100 | 140 | 240 |
| Malta | 2005 | 0 | 0 | 1.200 | 1.200 | 100% | 100% | 0 | 0 | 1 | 1 |
| Nederland | 2003 | 0 | 40.000 | 1.070.000 | 1.110.000 | 100% | 96% | 0 | 10 | 70 | 80 |
| Österreich | 2005 | 67.000 | 90.000 | 705.000 | 862.000 | 92% | 82% | n.a. | 27 | 78 | 105 |
| Polska | 2003 | 1.060.000 | 280.000 | 10.200.000 | 11.540.000 | 91% | 88% | 101.170 | 110 | 480 | 590 |
| Portugal | 2003 | 40.000 | 20.000 | 950.000 | 1.010.000 | 96% | 94% | 11.580 | 10 | 30 | 40 |
| Slovenija | 2003 | 10.000 | 130.000 | 170.000 | 310.000 | 97% | 55% | 1.310 | 40 | 20 | 60 |
| Slovensko | 2003 | 10.000 | 20.000 | 160.000 | 190.000 | 95% | 84% | 1.380 | 10 | 10 | 20 |
| Suomi/Finland | 2003 | 1.184 | 91.202 | 403.654 | 496.040 | 100% | 81% | 1 | 41 | 44 | 85 |
| Sverige | 2005 | 0 | 5.200 | 137.150 | 142.350 | 100% | 96% | 0 | 2 | 9 | 11 |
| United Kingdom | 2003 | 60.000 | 510.000 | 6.790.000 | 7.360.000 | 99% | 92% | 3.550 | 250 | 210 | 460 |
| EU 25 | 2003 | 1.788.921 | 5.558.192 | 73.303.361 | 80.910.274 | 97% | 91% | 166.058 | 2.090 | 5.283 | 7.415 |
| Norway | 2004 | | | | 0 | | | | 9 | 62 | 71 |
| Switzerland | 2003 | | | | | | | | | | |
| Bulgaria | 2003 | 890.000 | 0 | 0 | 890.000 | 0% | 0% | 87.950 | 0 | 0 | 0 |
| Romania | 2003 | 3.090.000 | 0 | 0 | 3.090.000 | 0% | 0% | 639.580 | 0 | 0 | 0 |

Table 2: Sample size

| Country | Number* of flocks of fattening turkeys (>500) | Flocks to be sampled | | | Breeding flocks |
|-----------------|---|----------------------|---------------------------|--------------|-----------------|
| | | Total** | Slaughter turkey holdings | | |
| | | | 500-4999 | > 5000 | |
| Belgique/België | 165 | 115 | 31 | 84 | all once |
| Česká Republika | 530 | 223 | 78 | 145 | all once |
| Danmark | 75 | 63 | 4 | 59 | all once |
| Deutschland | 2.680 | 336 | 60 | 276 | all once |
| Eesti | 0 | 0 | 0 | 0 | all once |
| Ellas | 168 | 117 | 117 | | all once |
| España | 975 | 276 | 52 | 223 | all once |
| France | 14.560 | 374 | 116 | 258 | all once |
| Ireland | 632 | 239 | 159 | 80 | all once |
| Italia | 4.240 | 352 | 18 | 334 | all once |
| Kypros | 8 | 8 | 8 | | all once |
| Latvija | 0 | 0 | 0 | 0 | all once |
| Lietuva | 70 | 59 | 21 | 38 | all once |
| Luxembourg | 0 | 0 | 0 | 0 | all once |
| Magyarország | 960 | 274 | 114 | 160 | all once |
| Malta | 5 | 5 | 0 | 5 | all once |
| Nederland | 320 | 175 | 22 | 153 | all once |
| Österreich | 420 | 201 | 52 | 149 | all once |
| Polska | 2.360 | 330 | 62 | 269 | all once |
| Portugal | 160 | 113 | 28 | 85 | all once |
| Slovenija | 240 | 148 | 98 | 49 | all once |
| Slovensko | 80 | 66 | 33 | 33 | all once |
| Suomi/Finland | 340 | 180 | 87 | 93 | all once |
| Sverige | 55 | 48 | 9 | 39 | all once |
| United Kingdom | 1.840 | 318 | 173 | 145 | all once |
| EU 25 | 30.883 | 4.020 | 1.343 | 2.677 | all once |
| Norway | 284 | | 60 | | all once |
| Switzerland | 0 | | | | all once |
| Bulgaria | 0 | | | | all once |
| Romania | 0 | | | | all once |

* See calculation of population size

** Calculation: see Annex 1