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ANNEX

Testing scheme necessary to verify the achievement of the Union target as referred to in Article 1(2)

1. SAMPLING FRAME

The sampling frame shall comprise all flocks of fattening and breeding turkeys within the framework of the national control programmes provided for in Article 5 of Regulation (EC) No 2160/2003.

2. MONITORING IN TURKEYS

2.1. Frequency of sampling

- (a) Food business operators shall sample all flocks of fattening and breeding turkeys in the following way:
- (i) sampling of flocks of fattening and breeding turkeys shall take place within three weeks before slaughter. The competent authority may authorise sampling in the last six weeks prior to the date of slaughter in case the turkeys are either kept more than 100 days or fall under organic turkey production according to Commission Regulation (EC) No 889/2008⁽¹⁾;
- (ii) sampling of flocks of breeding turkeys shall take place:
- in rearing flocks: at day-old, at four weeks of age and two weeks before moving to the laying phase or laying unit,
 - in adult flocks: at least every third week during the laying period at the holding or at the hatchery,
 - on the holding in the case of flocks of breeding turkeys laying hatching eggs intended for the trade within the Union.
- (iii) The competent authority may decide to implement one of the options referred to in the second indent of point (ii) to the whole testing scheme for all flocks. However, sampling of breeding flocks laying hatching eggs intended for the trade within the Union must take place on the holding.
- (iv) By way of derogation from the second indent of point (ii), if the Union target has been achieved for at least two consecutive calendar years in the whole Member State, sampling at the holding may be extended to take place every four weeks, at the discretion of the competent authority. However, the competent authority may decide to keep or revert to a three-week testing interval in the case of detection of the presence of the relevant *Salmonella* serotypes in a breeding flock on the holding and/or in any other case deemed appropriate by the competent authority.
- (b) The sampling by the competent authority shall at least provide for:
- (i) sampling of flocks of breeding turkeys:
- once a year, all flocks with at least 250 adult breeding turkeys between 30 and 45 weeks of age and all holdings with elite, great grand parents and grand parent breeding turkeys; the competent authority may decide that this sampling may also take place at the hatchery; and
 - all flocks on holdings in case of detection of *Salmonella* Enteritidis or *Salmonella* Typhimurium from samples taken at the hatchery by food

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business operators or within the frame of official controls, to investigate the origin of infection;

- (ii) sampling of flocks of fattening turkeys shall be done once a year, at least in one flock on 10 % of the holdings with at least 500 fattening turkeys;
- (iii) sampling may be done on a risk basis and additionally each time the competent authority considers it necessary;
- (iv) a sampling carried out by the competent authority may replace the sampling by the food business operator as referred to in point (a).

2.2. Sampling protocol

2.2.1. General instructions for sampling

The competent authority or the food business operator shall ensure that samples are taken by persons trained for that purpose.

Sampling of flocks of breeding turkeys shall be done in accordance with point 2.2 of the Annex to Commission Regulation (EU) No 200/2010⁽²⁾.

For sampling of flocks of fattening turkeys at least two pairs of boot swabs shall be taken per flock. Boot swabs are put on the boots and the sample is taken by walking around in the poultry house. Swabs from one flock of turkeys may be pooled into one sample.

Before putting on the boot swabs, their surface shall be moistened by:

- (a) the application of maximum recovery diluents (MRD: 0,8 % sodium chloride, 0,1 % peptone in sterile deionised water); or
- (b) the application of sterile water; or
- (c) the application of any other diluents approved by the national reference laboratory referred to in Article 11(3) of Regulation (EC) No 2160/2003; or
- (d) being autoclaved in a container together with diluents.

The way to moisten boot swabs shall be to pour the liquid inside before putting them on or to shake them in a container of diluent.

It shall be ensured that all sections in a house are represented in the sampling in a proportionate way. Each pair of boot swabs must cover about 50 % of the area of the house.

On completion of sampling, the swabs shall be carefully removed from the boots so as not to dislodge adherent material. Boot swabs may be inverted to retain material. They shall be placed in a bag or pot and labelled.

The competent authority may decide to increase the minimum number of samples in order to ensure representative sampling on a case-by-case evaluation of epidemiological parameters, such as biosecurity conditions, the distribution or size of the flock.

If the competent authority approves one pair of boot swabs may be replaced by a dust sample of 100 g collected from multiple places throughout the house from surfaces with visible presence of dust. As an alternative, one or several moistened fabric swab(s) of a combined surface of at least 900 cm² may be used to gather dust from multiple surfaces throughout the house. Each swab shall be well coated with dust on both sides.

2.2.2. Specific instructions for certain types of holdings

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- (a) For free range flocks of turkeys, samples shall only be collected inside the house.
- (b) Where access to the houses is not possible due to limited space in flocks with less than 100 turkeys, and it is therefore not possible to use boot swabs when walking around, they may be replaced by the same kind of hand fabric swabs that are used for dust, where the swabs are rubbed over surfaces contaminated with fresh faeces, or if this is not feasible, by other sampling techniques for faeces fit for the intended purpose.

2.2.3. *Sampling by the competent authority*

The competent authority shall satisfy itself by conducting further tests and/or documentary checks as appropriate to verify that results are not altered through the presence of antimicrobials or other substances inhibiting the growth of bacteria.

Where the presence of *Salmonella* Enteritidis and *Salmonella* Typhimurium is not detected but antimicrobials or bacterial growth inhibitory effects are detected, the flock shall be considered to be an infected flock of turkeys for the purpose of the Union target referred to in Article 1(2).

2.2.4. *Transport*

Samples shall be sent without undue delay either by via express mail or courier, to the laboratories referred to in Articles 11 and 12 of Regulation (EC) No 2160/2003. During transport they shall be protected from heat over 25 °C and exposure to sunlight.

Where it is not possible to send the samples within 24 hours from the time of sampling they shall be stored refrigerated.

3. LABORATORY ANALYSES

3.1. **Preparation of the samples**

At the laboratory samples shall be kept refrigerated until examination, which shall be started within 48 hours following receipt and within 96 hours after sampling.

The pair(s) of boot/sock swabs shall be carefully unpacked to avoid dislodging adherent faecal material, pooled and placed in 225 ml buffered peptone water (BPW) which has been pre-warmed to room temperature. The boot/sock swabs shall be fully immersed in BPW and therefore more BPW may be added if necessary.

The dust sample shall preferably be analysed separately. However for fattening flocks, the competent authority may decide to allow it to be pooled with the pair of boot/sock swabs for analysis.

The sample shall be swirled to fully saturate it and culture shall be continued by using the detection method set out in point 3.2.

Other samples (e.g. from breeding flocks or hatcheries) shall be prepared in accordance with point 2.2.2 of the Annex to Regulation (EU) No 200/2010.

If standards of European Committee for Standardisation ('CEN') or the International Organisation for Standardisation ('ISO') on the preparation of faeces for the detection of *Salmonella* are agreed on, they shall be applied and replace the provisions on the preparation of samples set out in this point.

3.2. **Detection method**

The detection method recommended by the EU reference laboratory for *Salmonella* in Bilthoven, the Netherlands, shall be used.

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That method is described in the Annex D to EN/ISO 6579 (2002): 'Detection of *Salmonella* spp. in animal faeces and in samples of the primary production stage'.

In that detection method, a semi-solid medium (modified semi-solid Rappaport-Vassiliadis medium, MSRV) is used as the single selective enrichment medium.

3.3. Serotyping

For breeding flocks of turkeys, at least one isolate from each positive sample shall be serotyped, following the White-Kauffmann-Le Minor scheme.

For fattening flocks of turkeys, at least one isolate from each positive sample taken by the competent authority shall be serotyped, following the White-Kauffmann-Le Minor scheme.

Food business operators shall at least ensure that for all isolates none of them belong to the serotypes *Salmonella* Enteritidis or *Salmonella* Typhimurium including monophasic strains with the antigenic formula 1,4,[5],12:i:-.

3.4. Alternative methods

With regard to samples taken on the initiative of the food business operator, the methods of analysis provided for in Article 11 of Regulation (EC) No 882/2004 of the European Parliament and of the Council⁽³⁾, may be used instead of the methods for the preparation of samples, detection method and serotyping provided for in points 3.1, 3.2 and 3.3 of this Annex, if validated in accordance with EN/ISO 16140.

3.5. Storage of strains

Laboratories shall ensure that at least one isolated strain of *Salmonella* spp. per flock and per year can be collected by the competent authority and stored for possible future phage typing or anti-microbial susceptibility testing, using the normal methods for culture collection, which must ensure integrity of the strains for a minimum of two years from the date of analysis.

The competent authority may decide that isolates of *Salmonella* spp. from sampling by food business operators shall also be stored for future phagotyping or antimicrobial susceptibility testing to provide for isolates to be tested in accordance with Article 2 of Commission Decision 2007/407/EC⁽⁴⁾.

4. RESULTS AND REPORTING

4.1. Calculation of prevalence for the verification of the Union target

A flock of turkeys shall be considered positive for the purpose of verifying the achievement of the Union target, where the presence of *Salmonella* Enteritidis and/or *Salmonella* Typhimurium (other than vaccine strains, but including monophasic strains with the antigenic formula 1,4,[5],12:i:-) was detected in the flock.

Positive flocks of turkeys shall be counted only once per round, irrespective of the number of sampling and testing operations and only be reported in the year of the first positive sampling. The prevalence shall be calculated separately for flocks of fattening turkeys and flocks of adult breeding turkeys.

4.2. Reporting

4.2.1. Reporting shall include:

- (a) the total number of flocks of fattening and adult breeding turkeys which were tested at least once during the year of reporting;

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- (b) the total number of fattening and adult breeding flocks positive with any *Salmonella* serotype in the Member State;
 - (c) the number of flocks of fattening and adult breeding turkeys tested positive at least once for *Salmonella* Enteritidis and *Salmonella* Typhimurium including monophasic strains with the antigenic formula 1,4,[5],12:i:-;
 - (d) the number of fattening and adult breeding turkey flocks positive for each *Salmonella* serotype or for *Salmonella* unspecified (isolates that are untypable or not serotyped).
- 4.2.2. *The information referred to in (a) to (d) of point 4.2.1 shall be provided separately for the sampling within the overall national Salmonella control programme for:*
- (a) the food business operators sampling as provided for in point 2.1(a); and
 - (b) the competent authorities sampling as provided for in point 2.1(b).
- 4.2.3. *The results of the tests shall be considered relevant food chain information as provided for in Section III of Annex II to Regulation (EC) No 853/2004 of the European Parliament and of the Council⁽⁵⁾.*

At least the following information shall be made available to the competent authority from each flock of turkeys tested:

- (a) holding reference, remaining unique in time;
- (b) flock reference, remaining unique in time;
- (c) month of sampling;
- (d) number of birds per flocks.

The results and any additional relevant information shall be reported as part of the report on trends and sources provided for in Article 9(1) of Directive 2003/99/EC of the European Parliament and of the Council⁽⁶⁾.

The food business operator shall notify the competent authority of the detection of *Salmonella* Enteritidis and *Salmonella* Typhimurium including monophasic strains with the antigenic formula 1,4,[5],12:i:- without undue delay. The food business operator shall instruct the analysing laboratory to act accordingly.

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- (1) OJ L 250, 18.9.2008, p. 1.
- (2) OJ L 61, 11.3.2010, p. 1.
- (3) OJ L 165, 30.4.2004, p. 1.
- (4) OJ L 153, 14.6.2007, p. 26.
- (5) OJ L 139, 30.4.2004, p. 55.
- (6) OJ L 325, 12.12.2003, p. 31.

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