II

(Acts whose publication is not obligatory)

COMMISSION

COMMISSION DIRECTIVE

of 7 June 1984

amending the Annexes to Council Directive 77/96/EEC on the examination for trichinae (trichinella spiralis) upon importation from third countries of fresh meat derived from domestic swine

(84/319/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 77/96/EEC of 21 December 1976 on the examination for trichinae (trichinella spiralis) upon importation from third countries of fresh meat derived from domestic swine (1), as last amended by Directive 83/91/EEC (2), and in particular Article 8 thereof,

Whereas recent studies have enabled certain methods to be devised for detecting trichinae in pigmeat; whereas the reliability of these methods from the point of view of health protection is equivalent to that of existing methods; whereas appropriate additions should, therefore, be made to Annex I to Directive 77/96/EEC;

Whereas, in order to facilitate the work of examination for trichinae, non-member countries and the Member States should be permitted to choose between the examination methods provided for;

Whereas certain technical adaptations must be made to the methods of examination for trichinae currently applied and as regards the conditions with which laboratories engaged in the detection of trichinae must comply;

Whereas the measures laid down by this Directive are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DIRECTIVE:

Article 1

Directive 77/96/EEC is hereby amended as set out in the Annex.

Article 2

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive not later than 1 January 1985. They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 7 June 1984.

For the Commission

Poul DALSAGER

Member of the Commission

(1) OJ No L 26, 31. 1. 1977, p. 67.
(2) OJ No L 59, 5. 3. 1983, p. 34.

ANNEX

A. Annex I is hereby amended as follows:

1. In point II (a):

— the 10th indent is replaced by the following:

'— a stereo-microscope (magnification 15 to 40 ×) with a suitable light source,'

— the last indent is replaced by the following:

'— digestive liquid made up as follows:

10 g of pepsin (80 u/g FIP: Fédération internationale de pharmacie), 5 ml HCl (at least 37 %) made up to a litre with tap-water.'

2. Point III is replaced by the following:

'III. METHOD USING THE ARTIFICIAL DIGESTION OF COLLECTIVE SAMPLES

(a) **Apparatus and reagents**

— knife and tweezers for collecting specimens,

— meat mincer with 2 to 3 mm diameter perforations,

— a 3 litre Erlenmeyer flask with a rubber or cotton-wool plug,

— a conical separation funnel of 2 000 ml capacity,

— an ordinary A-base stand of approximately 28 cm length with an 80 cm stem,

— a ring, diameter approximately 10 to 11 cm which can be fixed to the stand,

— a clamp with a flat vice (23 × 40 mm) which can be attached to the stand by means of a double coupling,

— a sieve (mesh size 177 microns) with an external diameter of 11 cm fitted with brass or stainless steel mesh,

— a funnel with an internal diameter of not less than 12 cm,

— 100 ml glass measuring cylinders,

— a stereo-microscope (magnification 15 to 40 ×) with a suitable light source, or a trichinoscope with horizontal table for the compressor with suitable light source,

— when using the trichinoscope: a larval counting basin is used which may be described as follows:

the larval counting basin is made from 3 mm thick acrylic plates, as follows:

(i) the bottom of the basin to be 180 × 40 mm, marked off into squares,

(ii) the sides to be 230 × 20 mm,

(iii) the end to be 40 × 20 mm. The bottom and the ends should be inserted between the sides, thus forming a basin with two small handles in both ends. The upper side of the bottom should be raised 7 to 9 mm from the base of the frame formed by the sides and the ends. The parts should be fixed by using glue appropriate for the material,

— a number of 9 cm diameter Petri dishes (when using stereo-microscope) marked on their undersides into 10 × 10 mm square examination areas using a pointed instrument,

— a number of 10 litre bins to be used when applying decontamination, such as formol treatment, to the apparatus and for the remaining digestive juice in the case of positive results,

— concentrated (37 %) hydrochloric acid,

— pepsin strength: 1 : 10 000 NF (US National Formulary)
corresponding to 1 : 12 500 BP (British Pharmacopoea)
corresponding to 2 000 FIP (Fédération internationale de pharmacie),

— a number of trays which can hold 50 samples of approximately 2 g each,

— a balance accurate to 0,1 g.

(b) **Collection of specimens**

1. In the case of whole carcases, a specimen to be taken of approximately 2 g from a pillar of the diaphragm at the transition to the sinewy part. In the absence of diaphragm pillars, a specimen of the same size to be taken from the rib part of the breastbone part of the diaphragm, from the lingual muscle or the jaw muscle or the abdominal muscles.

2. For cuts of meat, a sample of approximately 2 g of skeletal muscle to be taken, containing little fat and, where possible, near to bones or tendons.

(c) **Method**

1. (i) *Complete pools* (100 samples at a time)

Approximately 1 g of sample shall be taken from each of the 100 individual samples from the pigs. The pooled sample is put once through the mincer.

The minced meat shall be placed in the 3 litre Erlenmeyer flask together with 7 g of pepsin, approximately 2 litres of tap-water heated to about 40 to 41 °C and 25 ml of concentrated hydrochloric acid. The mixture shall be shaken to dissolve the pepsin.

The pH of the solution will be about 1,5 to 2.

— For digestion, the Erlenmeyer flask shall be incubated at 40 to 41 °C for approximately four hours. The flask shall be regularly shaken during the time of incubation, at least twice every hour.

— The digested solution shall be filtered through the sieve into the conical 2 litre separation funnel and left undisturbed on the stand for at least one hour.

— A total volume of approximately 45 ml should be run off into a measuring cylinder and divided between three Petri dishes, the bottoms of which should be marked off into squares, with 15 ml in each dish.

— Each Petri dish shall be minutely examined for larval trichinae under the stereo-microscope.

— Where larval counting basins are employed, the 45 ml shall be distributed between two larval counting basins and examined under the trichinoscope.

The larvae appear as identifiable organisms in the deposit and often, when the water is lukewarm, rolling and unrolling movements of the "spiral" may be observed.

— Digests should be examined as soon as they are ready. Under no circumstances should examination be postponed until the following day.

If the digests are unclear or not examined within 30 minutes of their preparation, they should be clarified as follows. The final sample of 45 ml is poured into a measuring cylinder and allowed to stand for 10 minutes. At the end of this time, 30 ml of supernatant fluid is removed by suction and the remaining 15 ml is made up to 45 ml with tap-water. After a further settling period of 10 minutes, 30 ml of supernatant fluid is removed by suction and the remaining 15 ml is poured into a Petri dish or larval counting basin for examination. The measuring cylinder should be washed with 10 ml of tap-water and these washings should be added to the sample in the Petri dish or larval counting basin for examination.

(ii) *Pools of less than 100 samples*

Up to 15 single samples could be added to a total pool of 100 samples and be examined together with these samples. If more than 15 samples and less than 100 samples are examined, the digestion fluid should be reduced proportionately.

2. In the case of a positive or doubtful result following the examination of a collective sample, a further 20 g sample should be taken from each pig in accordance with (b) above. The 20 g samples from five pigs should be pooled and examined by the method described above. In this way samples from 20 groups of five pigs will be examined. When trichinellae are detected in a pooled sample from five pigs, further 20 g samples should be collected from the individual pigs in the group and each should separately be examined using the method described above.'

3. The following points IV, V and VI are added:

'IV. THE MECHANICALLY ASSISTED POOLED SAMPLE DIGESTION METHOD/ SEDIMENTATION TECHNIQUE

(a) **Apparatus and reagents**

— knife or scissors for cutting specimens,

— trays marked off with 50 squares each of which can hold samples of approximately 2 g of meat,

— a Stomacher Lab-blender 3 500 Thermo model,

— plastic bags suitable for the Stomacher Lab-blender,

— conical separation funnels of 2 litre capacity, preferably fitted with teflon safety plugs,

— stands, rings and clamps,

— sieves, mesh size 177 microns, external diameter 11 cm with stainless steel mesh,

— funnels with an internal diameter not less than 12 cm, to support the sieves,

— 100 ml glass measuring cylinders,

— a 25 ml dispenser,

— beakers of 3 litre capacity,

— spoon or glass rod for stirring the digestion fluid in the beaker,

— a plastic syringe and tube for suction,

— a measuring spoon for 6 g,

— a thermometer accurate to ± 0,5 °C within the range 1 to 100 °C,

— a vibrator, e.g. an electric shaver with the head removed,

— a relay which will switch on and off at one minute interval,

— a trichinoscope with a horizontal table or a stereo-microscope, with a suitable light source,

— larval counting basin (when using trichinoscope): the larval counting basin is made from 3 mm thick acrylic plates as follows:

(i) the bottom of the basin to be 180 × 40 mm, marked off into squares,

(ii) the sides to be 230 × 20 mm,

(iii) the end to be 40 × 20 mm. The bottom and the ends should be inserted between the sides, thus forming two small handles in both ends. The upper side of the bottom should be raised 7 to 9 mm from the base of the frame formed by the sides and the ends. The parts should be fixed by using glue appropriate for the material,

— a number of 9 cm diameter Petri dishes (when using stereo-microscope) marked on their undersides into 10 × 10 mm square examination areas using a pointed instrument,

— 17,5 % hydrochloric acid solution,

— pepsin strength 1 : 10 000 NF (US National Formulary)
corresponding to 1 : 12 500 BP (British Pharmacopoea)
corresponding to 2 000 FIP (Fédération internationale de pharmacie),

— a number of 10 litre bins to be used when applying decontamination, such as formol treatment, to the apparatus and for the remaining digestive juice in the case of positive results,

— a balance accurate to 0,1 g.

(b) **Collection of specimens**

1. In the case of whole carcases, a specimen to be taken of approximately 2 g from a pillar of the diaphragm at the transition to the sinewy part. In the absence of diaphragm pillars, a specimen of the same size to be taken from the rib part or the breastbone part of the diaphragm, from the jaw muscle or the abdominal muscle.
2. For cuts of meat, a sample of approximately 2 g of skeletal muscle to be taken, containing little fat and, where possible, near to bones or tendons.

(c) **Method**

1. *Digestion procedure*

(i) Complete pools (100 samples at a time)

— The Stomacher Lab-blender 3 500 should be fitted with a double plastic bag and the temperature control set at 40 to 41 °C

— One and a half litres of water preheated to about 32 to 35 °C is poured into the inner plastic bag and the water heated to 40 to 41 °C.

— 25 ml of 17,5 % hydrochloric acid is then added to the water in the Stomacher.

— 100 samples of approximately 1 g each (at 25 to 30 °C) taken from each of the individual samples, in accordance with (b), are then added.

— 6 g pepsin is finally added. This order of addition should be strictly adhered to in order to avoid decomposition of the pepsin.

— The Stomacher is then allowed to pound the content of the bag for 25 minutes.

— The pastic bag is then removed from the Stomacher and the digestion fluid is filtered through the sieve into a 3 litre beaker.

— The plastic bag is washed with approximately 100 ml of water, which is then used to rinse the sieve and finally added to the filtrate in the beaker.

Up to 15 single samples could be added to a total pool of 100 samples and be examined together with these samples.

(ii) Smaller pools less than 100 samples

— The Stomacher Lab-blender 3 500 should be fitted with a double plastic bag and the temperature control set at 40 to 41 °C.

— A digestion fluid is prepared by mixing about one and a half litres of water and 25 ml of 17,5 % hydrochloric acid. 6 g of pepsin is added and the whole mixed at a temperature of 40 to 41 °C. This order of addition should be strictly adhered to in order to avoid decomposition of the pepsin.

— Of the digestion fluid, a volume corresponding to 15 ml per gram of sample is measured (e.g. for 30 samples the volume required is 30 × 15 ml or 450 ml) and transferred to the inner of the two plastic bags together with the meat samples of approximately 1 g (at 25 to 30 °C) taken from each of the individual samples in accordance with (b).

— Water at a temperature of approximately 41 °C is poured into the outer bag to a total volume in the two bags of one and a half litres.

— The Stomacher is then allowed to pound the content of the bag for 25 minutes.

— The plastic bag is then removed from the Stomacher and the digestion fluid is filtered through the sieve into a 3 litre beaker.

— The plastic bag is washed with approximately 100 ml of water, which is then used to rinse the sieve and finally added to the filtrate in the beaker.

2. *Recovery of larvae by sedimentation*

— Ice (300 to 400 g of ice flakes, scaly ice or crushed ice) is added to the digestion fluid, bringing its volume up to about 2 litres. The digestion fluid is then stirred until the ice has melted.

In the case of smaller pools (see 1 (ii)), the amount of ice should be reduced correspondingly.

— The chilled digestion fluid is transferred to a 2 litre separation funnel, equipped with a vibrator in an extra clamp.