Commission Regulation (EC) No 36/2005 of 12 January 2005 amending Annexes III and X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards epidemio-surveillance for transmissible spongiform encephalopathies in bovine, ovine and caprine animals (Text with EEA relevance)

COMMISSION REGULATION (EC) No 36/2005

of 12 January 2005

amending Annexes III and X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards epidemio-surveillance for transmissible spongiform encephalopathies in bovine, ovine and caprine animals

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies[(1)], and in particular Article 23 thereof,

Whereas:

(1) Regulation (EC) No 999/2001 lays down rules for the monitoring of transmissible spongiform encephalopathies (TSEs) in bovine, ovine and caprine animals.

(2) In its opinion of 4 and 5 April 2002 on a strategy to investigate the possible presence of bovine spongiform encephalopathy (BSE) in small ruminants, the Scientific Steering Committee (SSC) recommended a strategy for such investigation concerning the Community's small ruminant population.

(3) A panel of experts on strain typing has been assembled by the Community Reference Laboratory (CRL) for TSEs for further defining the strategy recommended by the SSC. The strategy includes firstly implementing a screening method of all confirmed TSE cases in small ruminants at the level of the national reference laboratories. Secondly, a ring trial with at least three different methods in selected laboratories under the heading of the CRL to be carried out on all cases in which the first screening test could not exclude BSE. Finally, mouse strain typing is required if the outcome of the molecular typing methods needs confirmation.

(4) It is necessary to ensure that brain material of an optimal quality and in sufficient quantity from positive scrapie cases is delivered to the laboratories carrying out confirmatory examinations.

(5) When molecular typing of confirmed scrapie cases reveals a BSE-like or unusual isolate, it is desirable that the competent authority should have access to brain material from other infected animals on the holding, to further assist the investigation of the case.

(6) Four laboratories have successfully participated in a ring trial conducted by the CRL between July 2003 and March 2004 to test their proficiency in using molecular typing methods. The CRL should organise proficiency testing for other laboratories in the use of one of these molecular typing methods before April 2005.

(7) In the meantime, in view of the necessity to extend and accelerate the monitoring of caprine animals following a suspect case found in a goat, and considering the information forwarded to the CRL panel of experts by the laboratories of certain Member States on their capacity to carry out molecular testing, those laboratories should be provisionally approved for such testing pending the results of the proficiency test.

(8) Member States are submitting monthly TSE reports on a voluntary basis in addition to the annual report required by Article 6(4) of Regulation (EC) No 999/2001. The information forwarded in the annual and monthly reports should be harmonised and additional information, in particular on the age distribution of tested bovine animals, should be provided in order to evaluate the prevalence of BSE in different age groups.

(9) Regulation (EC) No 999/2001 should therefore be amended accordingly.

(10) In view of the increasing urgency to differentiate BSE from scrapie, the amendments made by this Regulation should enter into force without delay.

(11) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS REGULATION:

Article 1

Annexes III and X to Regulation (EC) No 999/2001 are amended in accordance with the Annex to this Regulation.

Article 2

This Regulation shall enter into force on the day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 12 January 2005.

For the Commission

Markos KYPRIANOU

Member of the Commission

ANNEX

Annexes III and X to Regulation (EC) No 999/2001 are amended as follows:

1. In Annex III, Chapter A, Part II and III and Chapter B, Part I are replaced by the following:

II. MONITORING IN OVINE AND CAPRINE ANIMALS

1. General

Monitoring in ovine and caprine animals shall be carried out in accordance with the laboratory methods laid down in Annex X, Chapter C, point 3.2.(b).

2. Monitoring in ovine animals slaughtered for human consumption

Member States in which the population of ewes and ewe lambs put to the ram exceeds 750 000 animals shall test in accordance with the sampling rules laid down in point 4 a minimum annual sample of 10 000 ovine animals slaughtered for human consumption[(2)].

3. Monitoring in ovine and caprine animals not slaughtered for human consumption

Member States shall test in accordance with the sampling rules laid down in point 4 and the sample sizes indicated in table A and table B respectively, ovine and caprine animals which have died or been killed, but which were not:

— killed in the framework of a disease eradication campaign, or

— slaughtered for human consumption.

TABLE A

Member State population of ewes and ewe lambs put to the ram	**Minimum sample size of dead ovine animals[a]**
> 750 000	10 000
100 000-750 000	1 500
40 000-100 000	500
< 40 000	100

a Sample sizes are set to take account of the size of the ovine populations in the individual Member States and are intended to provide achievable targets. The sample sizes of 10 000, 1 500, 500 and 100 animals will allow the detection of a prevalence of 0,03 %, 0,2 %, 0,6 % and 3 % respectively with a 95 % confidence.

TABLE B

Member State population of goats which have already kidded and goats mated	**Minimum sample size of dead caprine animals[a]**

a Sample sizes are set to take account of the size of the caprine populations in the individual Member States and are intended to provide achievable targets. The sample sizes of 5 000, 1 500, 500 and 50 animals will allow the detection of a prevalence of 0,06 %, 0,2 %, 0,6 % and 6 % respectively with a 95 % confidence. Where a Member State experiences difficulty in collecting sufficient numbers of dead caprine animals to reach its allotted sample size, it may choose to supplement its sample by testing caprine animals slaughtered for human consumption over the age of 18 months at the ratio of three caprine animals slaughtered for human consumption to one dead caprine animal.

> 750 000	5 000
250 000-750 000	1 500
40 000-250 000	500
< 40 000	50

a Sample sizes are set to take account of the size of the caprine populations in the individual Member States and are intended to provide achievable targets. The sample sizes of 5 000, 1 500, 500 and 50 animals will allow the detection of a prevalence of 0,06 %, 0,2 %, 0,6 % and 6 % respectively with a 95 % confidence. Where a Member State experiences difficulty in collecting sufficient numbers of dead caprine animals to reach its allotted sample size, it may choose to supplement its sample by testing caprine animals slaughtered for human consumption over the age of 18 months at the ratio of three caprine animals slaughtered for human consumption to one dead caprine animal.

4. Sampling rules applicable to the animals referred to in points 2 and 3

The animals shall be over 18 months of age or have more than two permanent incisors erupted through the gum.

The age of the animals shall be estimated on the basis of dentition, obvious signs of maturity, or any other reliable information.

The sample selection shall be designed with a view to avoid the over-representation of any group as regards the origin, age, breed, production type or any other characteristic.

Multiple sampling in the same flock shall be avoided, wherever possible.

The Member States shall put in place a system to check, on a targeted or other basis, that animals are not being diverted from sampling.

The sampling shall be representative for each region and season.

However, Member States may decide to exclude from the sampling remote areas with a low animal density, where no collection of dead animals is organised. Member States making use of this derogation shall inform the Commission thereof, and shall submit a list of those remote areas where the derogation applies. The derogation shall not cover more than 10 % of the ovine and caprine population in the Member State concerned.

5. Monitoring in infected flocks

From 1 October 2003, animals over 12 months or which have a permanent incisor erupted through the gum, and which are killed for destruction in accordance with the provisions of Annex VII, point 2(b)(i) or (ii) or point 2(c), shall be tested based on the selection of a simple random sample, in accordance with the sample size indicated in the following table.

Number of animals over 12 months or which have a permanent incisor erupted through the gum, killed for destruction in the herd or flock	**Minimum sample size**
70 or less	All eligible animals
80	68
90	73
100	78

120	86
140	92
160	97
180	101
200	105
250	112
300	117
350	121
400	124
450	127
500 or more	150

Where possible, the killing and subsequent sampling shall be delayed until the result of primary molecular testing carried out for the further examination of positive scrapie cases under the provisions of Annex X, Chapter C, point 3.2.(c)(i) is known.

6. Monitoring in other animals

In addition to the monitoring programmes set out in points 2, 3 and 4, Member States may on a voluntary basis carry out monitoring in other animals, in particular:

— animals used for dairy production,

— animals originating from countries with indigenous TSEs,

— animals which have consumed potentially contaminated feedingstuffs,

— animals born or derived from TSE infected dams.

7. Measures following testing of ovine and caprine animals

7.1. Where an ovine or caprine animal slaughtered for human consumption has been selected for TSE testing in accordance with point 2, its carcase shall not be marked with the health marking provided for in Chapter XI of Annex I to Directive 64/433/EEC until a negative result to the rapid test has been obtained.

7.2. Member States may derogate from point 7.1. where a system approved by the competent authority is in place in the slaughterhouse ensuring that all parts of an animal can be traced and that no parts of the animals tested bearing the health mark can leave the slaughterhouse until a negative result to the rapid test has been obtained.

7.3. All parts of the body of a tested animal, including the hide, shall be retained under official control until a negative result has been obtained to the rapid test, except for animal by-products directly disposed of in accordance with Articles 4(2)(a), (b) or (e) of Regulation (EC) No 1774/2002.

7.4. Except for the material to be retained in conjunction with the records provided for in Chapter B, Part III of this Annex, all parts of the body of an animal found positive to the rapid test, including the hide, shall be directly disposed of in accordance with Articles 4(2)(a), (b) or (e) of Regulation (EC) No 1774/2002.

8. Genotyping

8.1. The prion protein genotype shall be determined for each positive TSE case in sheep. TSE cases found in resistant genotypes (sheep of genotypes which encode alanine on both alleles at codon 136, arginine on both alleles at codon 154 and arginine on both alleles at codon 171) shall immediately be reported to the Commission. Where possible, such cases shall be submitted for strain-typing. Where strain-typing of such cases is not possible, the herd of origin and all other herds where the animal has been kept shall be subjected to enhanced monitoring with a view to finding other TSE cases for strain-typing.

8.2. In addition to the animals genotyped under the provisions of point 8.1., the prion protein genotype of a minimum sample of ovine animals shall be determined. In the case of Member States with an adult sheep population of more than 750 000 adult animals, this minimum sample shall consist of at least 600 animals. In the case of other Member States the minimum sample shall consist of at least 100 animals. The samples may be chosen from animals slaughtered for human consumption, from animals dead-on farm or from live animals. The sampling should be representative of the entire ovine population.

III. MONITORING IN OTHER ANIMAL SPECIES

Member States may on a voluntary basis carry out monitoring for TSEs in animal species other than bovine, ovine and caprine animals.

CHAPTER B

REPORTING AND RECORDING REQUIREMENTS

I. REQUIREMENTS ON MEMBER STATES

A. Information to be presented by Member States in their annual report as provided for in Article 6(4)

1. The number of suspected cases placed under official movement restrictions in accordance with Article 12(1), per animal species.

2. The number of suspected cases subject to laboratory examination in accordance with Article 12(2), per animal species, including the results of the rapid and confirmatory tests (number of positives and negatives) and, with regard to bovine animals, an estimation of the age distribution of all tested animals. The age distribution should be grouped whenever possible as follows: “below 24 months”, distribution per 12 months between 24 and 155 months, and “above 155 months” of age.

3. The number of flocks where suspected cases in ovine and caprine animals have been reported and investigated pursuant to Article 12(1) and (2).

4. The number of bovine animals tested within each subpopulation referred to in Chapter A, Part (I), points 2.1., 2.2., 2.3., 3.1., 4.1., 4.2., 4.3. and 5. The method for the sample selection, the results of the rapid and confirmatory

tests and an estimation of the age distribution of the tested animals grouped as set out in point 2 shall be provided.

5. The number of ovine and caprine animals and flocks tested within each subpopulation referred to in Chapter A, Part II, points 2, 3 and 5 together with the method for sample selection and the results of the rapid and confirmatory tests.

6. The geographical distribution, including the country of origin if not the same as the reporting country, of positive cases of BSE and scrapie. The year, and where possible the month of birth shall be given for each TSE case in bovine, ovine and caprine animals. TSE cases which have been considered atypical and the reasons why shall be indicated. For scrapie cases, the results of the primary molecular testing with a discriminatory immuno-blotting, referred to in Annex X, Chapter C, point 3.2.(c)(i), shall be reported.

7. In animals other than bovine, ovine and caprine, the number of samples and confirmed TSE cases per species.

8. The genotype, and where possible the breed, of each ovine animal either found positive to TSE or sampled in accordance with Chapter A, Part II, points 8.1. and 8.2.

B. Reporting periods

The compilation of reports containing the information referred to in A and forwarded to the Commission on a monthly basis or, with regard to the information referred to in point 8 on a quarterly basis, may constitute the annual report as required by Article 6(4), provided that the information is updated whenever additional information becomes available.

2. In Annex X, Chapter C is replaced by the following:

CHAPTER C

Sampling and laboratory testing

1. Sampling

Any samples intended to be examined for the presence of a TSE shall be collected using the methods and protocols laid down in the latest edition of the Manual for diagnostic tests and vaccines for Terrestrial Animals of the International Office for Epizooties (IOE/OIE) (“the Manual”). In the absence of OIE methods and protocols, and to ensure that sufficient material is available, the competent authority shall ensure the use of sampling methods and protocols in accordance with guidelines issued by the Community Reference Laboratory. In particular the competent authority shall try to collect part of the cerebellum and the whole brain stem of small ruminants and shall keep at least half of the collected tissues fresh but not frozen until the result of the rapid or confirmatory test is negative.

The samples shall be correctly marked as to the identity of the sampled animal.

2. Laboratories

Any laboratory examination for TSE shall be carried out in laboratories approved for that purpose by the competent authority.

3. Methods and protocols

3.1. Laboratory testing for the presence of BSE in bovine animals

(a) Suspect cases

Samples from bovine animals sent for laboratory testing pursuant to the provisions of Article 12(2) shall be subject to a histopathological examination as laid down in the latest edition of the Manual, except where the material is autolysed. Where the result of the histopathological examination is inconclusive or negative or where the material is autolysed, the tissues shall be subjected to an examination by one of the other diagnostic methods laid down in the Manual (immunocytochemistry, immuno-blotting or demonstration of characteristic fibrils by electron microscopy). However, rapid tests cannot be used for this purpose.

If the result of one of those examinations is positive, the animals shall be regarded a positive BSE case.

(b) BSE monitoring

Samples from bovine animals sent for laboratory testing pursuant to the provisions of Annex III, Chapter A, Part I (Monitoring in bovine animals) shall be examined by a rapid test.

When the result of the rapid test is inconclusive or positive, the sample shall immediately be subject to confirmatory examinations in an official laboratory. The confirmatory examination shall start by a histopathological examination of the brainstem as laid down in the latest edition of the Manual, except where the material is autolysed or otherwise not suitable for examination by histopathology. Where the result of the histopathological examination is inconclusive or negative or where the material is autolysed, the sample shall be subjected to an examination by one of the other diagnostic methods referred to in (a).

An animal shall be regarded a positive BSE case, if the result of the rapid test is positive or inconclusive, and either

— the result of the subsequent histopathological examination is positive, or
— the result of another diagnostic method referred to in (a) is positive.

3.2. Laboratory testing for the presence of TSE in ovine and caprine animals

(a) Suspect cases

Samples from ovine and caprine animals sent for laboratory testing pursuant to the provisions of Article 12(2) shall be subject to a histopathological examination as laid down in the latest edition of the Manual, except where the material is autolysed. Where the result of the histopathological examination is inconclusive or negative or where the material is autolysed, the sample shall be subjected to an examination by immunocytochemistry, immuno-blotting or demonstration of characteristic fibrils by electron microscopy, as laid down in the Manual. However, rapid tests cannot be used for this purpose.

If the result of one of those examinations is positive, the animal shall be regarded a positive scrapie case.

(b) Scrapie monitoring

Samples from ovine and caprine animals sent for laboratory testing pursuant to the provisions of Annex III, Chapter A, Part II (Monitoring in ovine and caprine animals) shall be examined by a rapid test.

When the result of the rapid test is inconclusive or positive, the brainstem shall immediately be sent to an official laboratory for confirmatory examinations by immunocytochemistry, immuno-blotting or demonstration of characteristic fibrils by electron microscopy, as referred to in (a). If the result of the confirmatory examination is negative or inconclusive, additional confirmatory testing shall be carried out according the guidelines of the Community Reference Laboratory.

If the result of one of the confirmatory examination is positive, the animal shall be regarded a positive scrapie case.

(c) Further examination of positive scrapie cases

(i) Primary molecular testing with a discriminatory immuno-blotting

Samples from clinical suspect cases and from animals tested in accordance with Annex III, Chapter A, Part II, points 2 and 3 which are regarded as positive scrapie cases following the examinations referred to in points (a) or (b), or which display characteristics which are deemed by the testing laboratory to merit investigation, shall be forwarded for further examination by a primary molecular typing method to:

— Agence Française de Sécurité Sanitaire des Aliments, Laboratoire de pathologie bovine, 31, avenue Tony Garnier, BP 7033, F-69342, Lyon Cedex, France, or

— Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom, or

— to a laboratory, appointed by the competent authority, which has participated successfully in proficiency testing organised by the Community Reference Laboratory for the use of a molecular typing method, or

— on a provisional basis until 1 May 2005, the laboratories approved for this purpose by the CRL panel of experts.

(ii) Ring trial with additional molecular testing methods

Samples from scrapie cases in which the presence of BSE cannot be excluded according to the guidelines issued by the Community Reference Laboratory by the primary molecular testing referred to in (i), shall be forwarded immediately to the laboratories listed in point (d) after consultation with the Community Reference Laboratory, and with all the relevant information available. They shall be submitted to a ring trial with at least:

— a second discriminatory immuno-blotting,

— a discriminatory immunocytochemistry, and

— a discriminatory ELISA (Enzyme linked ImmunoSorbent Assay)

carried out in the laboratories approved for the relevant method as listed in point (d). Where samples are unsuitable for immunocytochemistry, the Community Reference Laboratory will direct appropriate alternative testing within the ring trial.

The results shall be interpreted by the Community Reference Laboratory assisted by a panel of experts including a representative of the relevant National Reference Laboratory. The Commission shall be informed immediately about the outcome of that interpretation. Samples indicative for BSE by three different methods and samples inconclusive in the ring trial shall be further analysed by a mouse bioassay for final confirmation.

Further testing of samples taken from infected flocks on the same holding in accordance with the provisions of Annex III, Chapter A, Part II, point 5, shall be carried out in accordance with the advice of the Community Reference Laboratory, after consultation with the relevant National Reference Laboratory.

(d) Laboratories approved for performing further examination by molecular typing methods

The laboratories approved for further molecular typing are:

Agence Française de Sécurité Sanitaire des Aliments

Laboratoire de pathologie bovine

31, avenue Tony Garnier

BP 7033

F-69342 Lyon Cedex

Centre CEA Fontenay-aux-Roses, BP 6

F-92265 Fontenay-aux-Roses Cedex

Service de Pharmacologie et d'Immunologie

Centre CEA Saclay, bâtiment 136

F-91191 Gif-sur-Yvette Cedex

Veterinary Laboratories Agency

Woodham Lane

New Haw

Addlestone

Surrey KT15 3NB

United Kingdom

3.3. Laboratory testing for the presence of TSEs in species other than those referred to in points 3.1. and 3.2.

Where methods and protocols are established for tests carried out to confirm the suspected presence of a TSE in a species other than bovine, ovine and caprine, they shall include at least a histopathological examination of brain tissue. The competent authority may also require laboratory tests such as immunocytochemistry, immuno-blotting, demonstration of characteristic fibrils by electron microscopy or other methods designed to detect the disease associated form of the prion protein. In any case at least one other laboratory examination shall be carried out if the initial

histopathological examination is negative or inconclusive. At least three different examinations shall be carried out in the event of the first appearance of the disease.

In particular, where BSE is suspected in a species other than bovine animals, samples shall be submitted for strain-typing, where possible.

4. Rapid tests

For the purposes of carrying out the rapid tests in accordance with Article 5(3) and Article 6(1), the following methods shall be used as rapid tests:

— immuno-blotting test based on a western blotting procedure for the detection of the protease-resistant fragment PrP^{Res} (Prionics-Check Western test),

— chemiluminescent ELISA test involving an extraction procedure and an ELISA technique, using an enhanced chemiluminescent reagent (Enfer test),

— sandwich immunoassay for PrP^{Res} carried out following denaturation and concentration steps (Bio-Rad TeSeE test, the former Bio-Rad Platelia test),

— microplate based immunoassay (ELISA) which detects protease-resistant PrP^{Res} with monoclonal antibodies (Prionics-Check LIA test),

— automated conformation-dependent immunoassay comparing the reactivity of a detection antibody to the protease-sensitive and protease-resistant forms of PrP^{Sc} (some fraction of the protease-resistant PrP^{Sc} is equivalent to PrP^{Res}) and to PrP^{C} (InPro CDI-5 test).

The producer of the rapid tests must have a quality assurance system in place agreed by the Community Reference Laboratory, which ensures that the test performance does not change. The producer must provide the test protocol to the Community Reference Laboratory.

Modifications to rapid tests or to test protocols may only be made following advance notification to the Community Reference Laboratory, and provided that the Community Reference Laboratory finds that the modification does not reduce the sensitivity, specificity or reliability of the rapid test. That finding shall be communicated to the Commission and to the National Reference Laboratories.

5. Alternative tests

(To be defined)

(**1**) OJ L 147, 31.5.2001, p. 1. Regulation as last amended by Commission Regulation (EC) No 1993/2004 (OJ L 344, 20.11.2004, p. 12).

(**2**) The minimum sample size has been calculated to detect a prevalence in slaughtered animals of 0,03 % with a 95 % confidence.

Changes to legislation:
There are currently no known outstanding effects for the Commission Regulation (EC) No 36/2005.