COUNCIL DIRECTIVE

of 11 December 1984

amending Directive 64/432/EEC as regards brucellosis in respect of the buffered brucella antigen test, the micro-agglutination test and the milk ring test as applied to samples of milk

(84/644/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Articles 43 and 100 thereof,

Having regard to the proposal from the Commission (1),

Having regard to the opinion of the European Parliament (2),

Having regard to the opinion of the Economic and Social Committee (3),

Whereas Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals (4), as last amended by Directive 84/643/EEC (5), lays down common standards on anti-brucellosis measures applicable to animals intended for intra-Community trade;

Whereas it is necessary to indicate precisely that the slow tube serum agglutination test is the test to be carried out on bovine animals and swine before they enter into intra-Community trade;

Whereas, in order to ensure the continuity of free trade within the Community of bovine animals, it is necessary to adapt the technical provisions of the above Directive relating to brucellosis to take account of scientific progress:

Whereas new scientific knowledge and technical developments in the diagnosis and control of bovine brucellosis require adjustment of existing Community measures in this field,

HAS ADOPTED THIS DIRECTIVE:

Article 1

Directive 64/432/EEC is hereby amended as follows:

1. In Article 3 (3) (c), 'paragraph A' is added after 'Annex C'.

2. In Article 3 (4) (i), 'paragraph A' is added after 'Annex C'.

3. In Article 3 (6) (c), 'paragraph A' is added after 'Annex C'.

4. In Article 7 (1) C, 'paragraph A' is added after 'Annex C'.

5. In Article 7 (1) D, 'paragraph A' is added after 'Annex C'.

6. In Annex A.II.A.1 (c) (i):
   - in the first paragraph, 'paragraph A' is added after 'Annex C',
   - the second indent is replaced by the following:
     '— the sero-agglutination tests referred to in the first paragraph may be replaced by two official buffered brucella antigen tests or two micro-agglutination tests carried out in accordance with Annex C (D) and (G). These tests shall also be carried out at intervals of at least three months and at most 12 months.'

7. Annex A.II.A.1 (c) (ii) is replaced by the following:

   '(ii) are checked annually to establish that brucellosis is not present by three ring tests carried out at intervals of at least three months or two ring tests at an interval of at least three months and one serological test (sero-agglutination test or buffered brucella antigen test or plasma agglutination test or plasma ring test or micro-agglutination test) carried out at not less than six weeks after the second ring test. If ring tests are not carried out, two serological tests (sero-agglutination test or buffered brucella antigen test or plasma agglutination test or plasma ring test or micro-agglutination test) shall be carried out each year at intervals of at least three months and not more than six months.

   Where, in a Member State or region thereof in which all bovine herds are subject to official operations to combat brucellosis, not more than 1 % of bovine herds are infected, it shall be sufficient to carry out each year two ring tests at

(1) OJ No C 255, 23. 9. 1983, p. 3.
(2) OJ No C 342, 19. 12. 1983, p. 117.
(3) OJ No C 23, 30. 1. 1984, p. 23.
(4) OJ No 121, 29. 7. 1964, p. 1977/64.
(5) See page 27 of this Official Journal.

an interval of at least three months, or one serological test (sero-agglutination test or buffered brucella antigen test or plasma-agglutination test or plasma-ring test or micro-agglutination tests).

Where checks are carried out on bulk tanks, the number of tests referred to in the preceding subparagraphs shall be doubled and the intervals between the tests shall be halved.'

8. In Annex A.II.A.2 (c), the last paragraph is replaced by the following:

'The sero-agglutination tests referred to in 1 (c) (i), first indent, may be replaced by buffered brucella antigen tests carried out in accordance with Annex C (D) or micro-agglutination tests carried out in accordance with Annex C (G).'

9. In Annex C:

— paragraph C is replaced by the following:

'C. Ring test

1. The ring test must be made on the contents of each milk churn or on the contents of each bulk tank from the farm.

2. The standard antigen to be used must come from one of the institutes listed in paragraph A.9 (a) to (j). It is recommended that the antigens should be standardized according to the WHO/FAO recommendations.

3. The antigen may be stained only with hematoxylin or tetrazolium; hematoxylin should preferably be used.

4. If no preservation is used then the reaction test must be carried out between 18 and 24 hours of taking the sample from the cow. If milk is to be tested later than 24 hours after sampling, then preservation must be used, formalin or mercuric chloride may be used as preservatives and if either of these are used the test must be carried out within the following 14 days after the day of sampling. Formalin may be added to give a final concentration in the milk sample of 0,2 % and, in such cases, the ratio between the amount of milk and the solution of formalin must be at least 10 to 1. A solution of mercuric chloride may be used instead of formalin to give a final concentration in the milk of 0,2 % and, in such cases, the ratio between the amount of milk and the solution of mercuric chloride must be 10 to 1.

5. The reaction must be carried out using one of the following methods:

— on a column of milk at least 25 mm high and on a volume of milk of 1 ml to which 0,03 ml of one of the standardized stained antigens has been added,

— on a column of milk at least 25 mm high and on a volume of milk of 1 ml to which 0,05 ml of one of the standardized stained antigens has been added,

— on a volume of milk of 8 ml which 0,08 ml of one of the standardized stained antigens has been added,

— on a column of milk at least 25 mm high and on a volume of milk of 2 ml to which 0,05 ml of one of the standardized stained antigens has been added.

6. The mixture of milk and antigens must be incubated at 37 °C for not less than 45 minutes and not more than 60 minutes. The test must be assessed within 15 minutes of removal from the incubator.

7. The reaction must be assessed according to the following criteria:

(a) negative reaction: coloured milk, colourless cream;

(b) positive reaction: milk and cream identically coloured or colourless milk and coloured cream.';

— a new paragraph G shall be added:

'G. Micro-agglutination test

1. Diluents are made up of 0,85 % physiological saline solution phenolized at 0,5 %.

2. The antigen shall be prepared as described under points 6, 7 and 8 of Annex C (A) and shall be titrated as described under point 5 of Annex C (A). At the moment the antigen is used safranin O shall be added at 0,02 % (final dilution).

3. The standard serum is the same as that under point 1 of Annex C (A).

4. The standard serum must be supplied by the Bundesgesundheitsamt, Berlin.

5. The micro-agglutination test shall be carried out on plates bearing wells with conical bottoms of a volume of 0,250 ml. The test shall be carried out as follows:

(a) Predilution of the serum: 0,050 ml of each serum to be tested are added to each well containing 0,075 ml of diluent. The mixtures are shaken for 30 seconds.

(b) Gradual serum dilution: prepare at least three dilutions for each serum. To this end from the predilutions (1 : 2,5) one takes 0,025 ml of each serum and transfers them to a well containing 0,025 ml of diluent. In this way the first dilution reaches a strength of 1 : 5 and the following dilutions are carried out by doubling.

(c) Addition of antigen: 0,025 ml of antigen is added to each well containing the different serum dilutions. After being shaken for 30 seconds the plates are closed with their respective lids and kept at 37 °C for 20 to 24 hours in a humidified atmosphere.

(d) Reading the results: assessment of the aspect of the sedimentation of the antigen is made by examining the bottom of the well reflected in a concave mirror placed above it. If there is a negative reaction, the antigen forms a sediment in the form of a compact button with clear edges and having an intense red colour. If there is a positive reaction, on the other hand, a diffused pink veil is formed that is evenly distributed. The different percentages of agglutination are determined by comparison with antigen checks indicating 0, 25, 50, 75 and 100 % agglutination. The title of each serum is expressed in International Units of Agglutination per ml. There should be included in the test, controls with negative and positive serum diluted so as to contain 30 International Units of Agglutination per ml.'

Article 2

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive by 30 September 1985. They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 11 December 1984.

For the Council

The President

A. DEASY