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COUNCIL DIRECTIVE 92/40/EEC
of 19 May 1992
introducing Community measures for the control of avian influenza

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COUNCIL DIRECTIVE 92/40/EEC

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introducing Community measures for the control of avian influenza

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 43 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 poultry is listed in Annex II of the Treaty; whereas the marketing of poultry constitutes an important source of revenue for the agricultural population;

Whereas, it is necessary to establish at Community level the control measures to be taken in the event of outbreak of the highly pathogenic form of avian influenza, caused by an influenza virus with specific characteristics, and hereinafter termed avian influenza, in order to ensure national development of the poultry sector and contribute to the protection of animal health in the Community;

Whereas an outbreak of avian influenza can quickly take on epizootic proportions, causing mortality and disturbances on a scale liable to reduce sharply the profitability of farming or poultry as a whole;

Whereas action must be taken as soon as the presence of the disease is suspected so that immediate and effective control measures can be implemented when its presence is confirmed;

Whereas it is necessary to prevent any spread of the disease as soon as an outbreak occurs, by carefully monitoring movements of animals and the use of products liable to be contaminated, and where appropriate, by vaccination;

Whereas diagnosis of the disease must be carried out under the auspices of responsible national laboratories, the coordination of which must be ensured by the Community reference laboratory;

Whereas common measures for the control of avian influenza form a basis for maintaining a unified standard with relation to animal health;

Whereas Article 3 of Council Decision 90/424/EEC of 26 June 1990 on expenditure in the veterinary field [4] applies in the event of the occurrence of avian influenza;

Whereas it is appropriate to confer upon the Commission the task of taking the necessary applicatory measures,

HAS ADOPTED THIS DIRECTIVE:

Article 1

This Directive defines the Community control measures to be applied in the event of an outbreak of avian influenza in poultry without prejudice to the Community provisions governing intra-Community trade.

This Directive shall not apply where avian influenza is detected in other birds; however, in this case, the Member State concerned shall inform the Commission of any measure it takes.

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(1) OJ No C 231, 5. 9. 1991, p. 4.
(2) OJ No C 326, 16. 12. 1991, p. 242.
(3) OJ No C 79, 30. 3. 1992, p. 8.
(4) OJ No L 224, 18. 8. 1990, p. 19. Decision amended by Decision 91/133/EEC (OJ No L 66, 13. 3. 1991, p. 81).

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Article 2

For the purpose of this Directive, the definitions given in Article 2 of Council Directive 90/539/EEC of 15 October 1990 on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs [1] shall apply as appropriate.

The following definitions shall also apply:

(a) *infected poultry* shall mean any poultry:

— in which the presence of avian influenza, within the meaning of Annex I, has been officially confirmed following an examination by an approved laboratory, or

— in the case of second and subsequent outbreaks, in which clinical signs or post-mortem lesions consistent with avian influenza are present;

(b) *poultry suspected of being infected* shall mean any poultry showing clinical signs or post-mortem lesions which are such that the presence of avian influenza may reasonably be suspected or any poultry in which the presence of influenza A virus of subtype H5 or H7 has been demonstrated;

(c) *poultry suspected of being contaminated* shall mean any poultry which may have been directly or indirectly exposed to the avian influenza virus, or influenza A virus of H5 subtype or H7 subtype;

(d) *competent authority* shall mean the competent authority within the meaning of Article 2 (6) of Directive 90/425/EEC [2];

(e) *official veterinarian* shall mean the veterinarian designated by the competent authority.

Article 3

Member States shall ensure that there is compulsory and immediate notification of the suspected presence of avian influenza to the competent authority.

Article 4

1. When poultry in a holding are suspected of being infected or contaminated with avian influenza, Member States shall ensure that the official veterinarian immediately activates official investigation arrangements to confirm or rule out the presence of the disease and, in particular, must take or have taken the samples necessary for laboratory examination.

2. As soon as the suspected infection is notified, the competent authority shall have the holding placed under official surveillance and shall in particular require that:

(a) a record be made of all categories of poultry on the holding showing in respect of each of the categories the numbers of poultry which have died, which show clinical signs, and which show no signs. The record shall be kept up-to-date to include birds born or dying during the period in which there is a suspicion. The data in the record shall be kept up-to-date and be produced on request, and may be checked at each visit;

(b) all poultry on the holding are kept in their living quarters or confined in some other place where they can be isolated and without contact with other poultry;

(c) no poultry enter or leave the holding;

(d) all movement

— of persons, other animals and vehicles to or from the holding,

[1] OJ No L 303, 31. 10. 1990, p. 6. Directive as last amended by Directive 91/496/EEC (OJ No L 268, 24. 9. 1991, p. 56).

[2] Council Directive 90/425/EEC of 26 June 1990 (OJ No L 224, 18. 8. 1990, p. 29); last amended by Directive 91/496/EEC (OJ No L 268, 24. 9. 1991, p. 56).

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— of poultry meat or carcases, or of animal feed, implements, waste, droppings, manure litter or anything liable to transmit avian influenza be subject to authorization by the competent authority;

(e) eggs shall leave the holding with the exception of eggs sent directly to an establishment approved for the manufacture and/or processing of egg products under Article 6 (1) of Directive 89/437/EEC [1], and transported under an authorization which has been granted by the competent authority. Such authorization must meet the requirements laid down in Annex I;

(f) appropriate means of disinfection be used at the entrances and exits of buildings housing poultry and of the holding itself;

(g) an epizootiological inquiry be carried out in accordance with Article 7.

3. Until such time as the official measures laid down in paragraph 5 are enforced, the owner or keeper of any poultry in which disease is suspected shall take all reasonable action to ensure compliance with paragraph 2, except for (g) thereof.

4. The competent authority may apply any of the measures provided for in paragraph 2 to other holdings should their location, their configuration or contacts with the holding where the disesase is suspected give reason to suspect possible contamination.

5. The measures referred to in paragraphs 1 and 2 shall not be withdrawn until the suspicion of avian influenza has been ruled out by the official veterinarian.

Article 5

1. Once the presence of avian influenza has been officially confirmed on a holding, the Member States shall ensure that the competent authority requires, in addition to the measures listed in Article 4 (2), the following measures to be undertaken:

(a) all poultry on the holding shall without delay be killed on the spot. The poultry which have died or been killed and all eggs shall be destroyed. These operations shall be carried out in a way which minimizes the risk of spreading disease;

(b) any substance or waste, such as animal feed, litter or manures liable to be contaminated, shall be destroyed or treated appropriately. This treatment, carried out in accordance with the instructions of the official veterinarian, shall ensure the destruction of any avian influenza virus present;

(c) where poultry from the holding have been slaughtered during the presumed incubation period of disease the meat from those poultry shall wherever possible be traced and destroyed;

(d) hatching eggs laid during the presumed incubation period which have been moved from the holding shall be traced and destroyed; but poultry which have already hatched from the eggs shall be placed under official surveillance; table eggs laid during the presumed incubation period which have been moved from the holding shall whereever possible be traced and destroyed, unless they have previously been properly disinfected;

(e) after carrying out operations listed in subparagraphs (a) and (b) the buildings used for housing poultry, their surroundings, the vehicles used for transport and all equipment likely to be contaminated shall be cleaned and disinfescted in accordance with the provisions of Article 11;

(f) no poultry shall be reintroduced to the holding until at least 21 days after completion of operations provided for in subparagraph (e);

[1] OJ No L 212, 22. 7. 1989, p. 87. Directive as amended by Directive 89/662/EEC (OJ No L 395, 30. 12. 1989, p. 13).

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(g) an epizootiological inquiry shall be carried out in accordance with Article 7.

2. The competent authority may extend the measures provided for in paragraph 1 to other neighbouring holdings should their location, their configuration, or contact with the holding where the disease has been confirmed give reason to suspect possible contamination.

Article 6

In the case of holdings which consist of two or more separate flocks, the competent authority may, in accordance with criteria set by the Commission under the procedure laid down in Article 21, grant a derogation from the requirements of Article 5 (1), for healthy flocks of a holding which is infected, provided that the official veterinarian has confirmed that the operations carried out there are such that the flocks are completely separate as regards housing, keeping and feeding, so that the virus cannot spread from one flock to another.

Article 7

1. The epizootiological inquiry shall deal with:

— the length of time during which avian influenza may have existed on the holding,

— the possible origin of the avian influenza on the holding and the identification of other holdings on which there are poultry which may have become infected or contaminated from the same source,

— the movement of persons, poultry or other animals, vehicles, eggs, meat and carcases and any implement or substance likely to have carried avian influenza virus to or from the holding in question.

2. In order to provide full coordination of all measures necessary to ensure eradication of avian influenza as quickly as possible and for the purpose of carrying out the epidemiological inquiry, a crisis unit shall be established.

The general rules concerning national crisis units and Community crisis units will be laid down by the Council, acting by a qualified majority proposal from the Commission.

Article 8

1. Where the official veterinarian has reason to suspect that poultry on any holding may have been contaminated as a result of the movement of persons, animals or vehicles or in any other way, that holding shall be placed under official control in accordance with paragraph 2.

2. The purpose of the official control shall be to detect immediately any suspicion of avian influenza, count the poultry and monitor their movements and, where appropriate, to take the action provided for in paragraph 3.

3. When a holding is subject to the official control under paragraphs 1 and 2, the competent authority shall prohibit removal of poultry from the holding other than for transport directly to a slaughterhouse under official supervision for the purpose of immediate slaughter. Before granting such authorization, the official veterinarian must have carried out a clinical examination of all the poultry to exclude presence of avian influenza on the holding. The movement restrictions referred to in this Article shall be imposed for a period of 21 days from the latest date of potential contamination; however, such restrictions must apply for a period of at least seven days.

4. Where it considers that conditions permit, the competent authority may limit the measures provided for in this Article to a part of the holding and to the poultry contained therein, provided that the poultry there have been housed, kept and fed completely separately by separate staff.

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Article 9

1. Once the diagnosis of avian influenza has been officially confirmed, the Member States shall ensure that the competent authority establishes around the infected holding a protection zone based on a minimum radius of three kilometres, itself contained in a surveillance zone based on a minimum radius of 10 kilometres. The establishment of the zones must take account of geographical, administrative, ecological and epizootiological factors relating to avian influenza, and of monitoring facilities.

2. The measures applied in the protection zone shall include:

(a) the identification of all holdings having poultry whithin the zone;

(b) periodic visits to all the holdings having poultry, a clinical examination of those poultry including, if necessary, the collection of samples for laboratory examination; a record of visits and findings must be kept;

(c) the keeping of all poultry in their living quarters or some other place where they can be isolated;

(d) the use of appropriate means of disinfection at the entrances and exits of the holding;

(e) the control of movements of persons handling poultry, poultry carcases and eggs and vehicles carrying poultry, carcases and eggs within the zone; in general, transport of poultry shall be prohibited, except for transit by major highways or railways;

(f) a prohibition on removing poultry and hatching eggs from the holding on which they are kept unless the competent authority has authorized the transport;

  (i) of poultry for immediate slaughter to a slaughterhouse preferably located in the infected area or, if that is not possible, to a slaughterhouse designated by the competent authority outside the infected area. The special health mark provided for in Article 5 (1) of Directive 91/494/EEC [1] must be applied to this poultrymeat;

  (ii) of day-old chicks or ready-to-lay pullets to a holding within the surveillance zone at which there are no other poultry. This holding must be placed under the official control provided for in Article 8 (2);

  (iii) of hatching eggs to a hatchery designated by the competent authority; before dispatch, eggs and their packing must be disinfected. Movements allowed in (i), (ii) and (iii) shall be directly executed, under official control. They shall be authorized only after the official veterinarian has carried out a health inspection of the holding. The means of transport used must be cleaned and disinfected before and after use;

(g) a prohibition on removing or spreading used litter or poultry manure without authorization;

(h) the prohibition of fairs, markets, shows or other gatherings of poultry or other birds.

3. The measures applied in the protection zone shall be maintained for at least 21 days after the carrying out of preliminary cleaning and disinfection operations on the infected holding in accordance with Article 11. The protection zone shall thereafter be part of the surveillance zone.

4. The measures applied in the surveillance zone shall include:

(a) the identification of all holdings having poultry within the zone;

(b) the control of poultry and hatching egg movement within the zone;

(c) a prohibition on the movement of poultry out of the zone during the first 15 days, except for movement directly to a slaughterhouse outside the surveillance zone designated by the competent

[1] OJ No L 268, 24. 9. 1991, p. 35.

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authority. The special health mark provided for in Article 3 of Directive 91/494/EEC must be applied to this poultry meat;

(d) a prohibition on the movement of hatching eggs out of the surveillance zone unless to a hatchery designated by the competent authority. Before dispatch the eggs and their packing must be disinfected;

(e) a prohibition on the movement of used litter or poultry manure out of the zone;

(f) a prohibition of fairs, markets, shows or other gatherings of poultry and other birds;

(g) without prejudice to the provisions of (a) and (b), the prohibition of transport of poultry except for transit by major highways or railways.

5. The measures applied in the surveillance zone shall be maintained for at least 30 days after the carrying out of preliminary cleaning and disinfection operations on the infected holding in accordance with Article 11.

6. Where the zones are situated in the territory of more than one Member State, the competent authorities of the Member States concerned shall cooperate in establishing the areas described in paragraph 1. However, if necessary, the protection zone and the surveillance zone shall be established by the procedure provided for in Article 21.

Article 10

Member States shall ensure that:

(a) the competent authority determines the arrangements allowing them to trace the movement of eggs and poultry;

(b) the owner or keeper of poultry is required to supply the competent authority, in response to any request by that authority, with information concerning poultry and eggs entering or leaving his holding;

(c) all persons engaged in the transport or marketing of poultry and eggs are able to supply the competent authority with information concerning the movements of poultry and eggs which they have transported or marketed and to furnish all the details concerning such information.

Article 11

Member States shall ensure that:

(a) the disinfectants to be used and their concentrations are officially approved by the competent authority;

(b) the cleaning and disinfection operations are carried out under official supervision, in accordance with:

(i) instructions given by the official veterinarian,

(ii) the procedure for cleaning and disinfecting an infected holding, as laid down in Annex II.

Article 12

Collection of samples and laboratory testing to detect the presence of avian influenza virus shall be carried out in accordance with Annex III.

Article 13

Member States shall ensure that the competent authority takes all the necessary measures for persons established in the protection and surveillance zones to be informed of the restrictions in force and make all necessary arrangements for the appropriate implementation of the measures in question.

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Article 14

1. Member States shall ensure that, in each Member State there is designated:

(a) a national laboratory at which facilities and expert personnel shall be maintained to permit assessment of the pathogenicity of influenza virus isolates, in accordance with Annex III, Chapter 7, and identification of influenza A viruses of H5 or H7 subtypes;

(b) a national laboratory at which reagents for use in regional laboratories are tested;

(c) a national institute or laboratory at which authorized vaccines may be tested in order to verify their conformity with the specifications laid down in the marketing authorization.

2. The national laboratories listed in Annex IV shall be responsible for coordinating standards and methods of diagnosis, use of reagents and testing of vaccines.

3. The national laboratories listed in Annex IV shall be responsible for coordinating the standards and diagnostic methods laid down in each avian influenza diagnostic laboratory within the Member State. To this end:

(a) they may provide diagnostic reagents to national laboratories;

(b) they shall control the quality of all diagnostic reagents used in that Member State;

(c) they shall arrange comparative tests periodically;

(d) they shall hold isolates of avian influenza virus from cases confirmed in that Member State;

(e) they shall ensure the confirmation of positive results obtained in regional diagnostic laboratories.

4. The national laboratories listed in Annex IV shall liaise with the Community reference laboratory referred to in Article 15.

Article 15

The Community reference laboratory for avian influenza is mentioned in Annex V. Without prejudice to the provisions of Decision 90/424/EEC, and in particular Article 28 thereof, the powers and duties of the laboratory shall be those appearing in the said Annex.

Article 16

Vaccination against avian influenza with vaccines authorized by the competent authority may only be used to supplement the control measures carried out when the disease appears and in accordance with the following provisions:

(a) the decision to introduce vaccination to supplement control measures shall be taken by the Commission in collaboration with the Member State concerned, acting in accordance with the procedure laid down in Article 21. This decision shall have particular regard to:

— the concentration of poultry in the affected area,

— the characteristics and composition of the vaccine to be used,

— the procedures for supervision of the distribution, storage and use of vaccines,

— the species and categories of poultry which shall be subject to vaccination,

— the areas in which vaccination shall be carried out.

However, by way of derogation from the first subparagraph, the decision to introduce emergency vaccination around the outbreak may be taken by the Member State concerned, following notification to the Commission, provided the fundamental interests of the Community are not jeopardized. Such decision will be re-examined

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immediately within the Standing Veterinary Committee in accordance with the procedure provided for in Article 21;

(b) where a Member State is authorized, in accordance with point (a), to have recourse to emergency vaccination on a limited part of its territory the status of the remainder of the territory shall not be affected, provided that the immobilization measures for the vaccinated animals are effective during a period to be determined in accordance with the procedure laid down in Article 21.

Article 17

1. Each Member State shall draw up a contingency plan, specifying the national measures to be implemented in the event of an outbreak of avian influenza.

This plan must allow access to facilities, equipment, personnel and all other appropriate materials necessary for the rapid and efficient eradication of the outbreak.

2. The criteria to be applied for drawing up the plan are laid down in Annex VI.

3. Plans drawn up in accordance with the criteria listed in Annex VI shall be submitted to the Commission not later than six months after this Directive is brought into application.

4. The Commission shall examine the plans in order to determine whether they permit the desired objective to be attained and shall suggest to the Member State concerned any amendments required in particular to ensure that they are compatible with those of the other Member States.

The Commission shall approve the plans, if necessary amended, in accordance with the procedure laid down in Article 21.

The plans may subsequently be amended or supplemented, in accordance with the same procedure, to take into account developments in the situation.

Article 18

1. Commission experts may, in collaboration with the competent authorities, and insofar as is necessary to ensure uniform application of this Directive, make on-the-spot checks. In order to do this, they may check a representative percentage of establishments to see whether the competent authorities are checking that these establishments are fulfilling the requirements of this Directive. The Commission shall inform the Member States of the result of the checks carried out.

A Member State in whose territory a check is being carried out shall give all the necessary assistance to the experts in carrying out their duties.

The general provisions for implementing this Article shall be determined in accordance with the procedure laid down in Article 21.

Article 19

The detailed conditions governing the Community's financial contribution to the measures connected with the application of this Directive are laid down in Decision 90/424/EEC.

Article 20

The Annexes shall be amended, as and when required, by the Council acting by a qualified majority acting on a proposal from the Commission in particular in order to take into account developments in research and in diagnostic procedures.

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Article 21

1. Where the procedure laid down in this Article is to be followed, the Standing Veterinary Committee, set up by Decision 68/361/EEC [1], hereinafter referred to as the 'the Committee', shall be informed without delay by its Chairman either on his own initiative or at the request of the representative of a Member State.

2. The representative of the Commission shall submit to the committee a draft within a time limit which the chairman may lay down according to the urgency of the matter. The opinion shall be delivered by the majority laid down in Article 148 (2) of the Treaty in the case of decisions which the Council is required to adopt on a proposal from the Commission. The votes of the representatives of the Member States within the committee shall be weighted in the manner set out in that Article. The chairman shall not vote.

3. (a) The Commission shall adopt the measures envisaged if they are in accordance with the opinion of the committee.

(b) If the measures envisaged are not in accordance with the opinion of the committee, or if no opinion is delivered, the Commission shall, without delay, submit to the Council a proposal relating to the measures to be taken. The Council shall act by a qualified majority.

If, on the expiry of a period of three months from the date of referral to the Council, the Council has not acted, the proposed measures shall be adopted by the Commission save where the Council has decided against the said measures by a simple majority.

Article 22

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive before 1 January 1993. They shall forthwith inform the Commission thereof.

When Member States adopt these measures, they shall contain a reference to this Directive or shall be accompanied by such reference on the occasion of their official publication. The methods of making such a reference shall be laid down by the Member States.

Article 23

This Directive is addressed to the Member States.

[1] OJ No L 265, 18. 10. 1968, p. 23.

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ANNEX I

AUTHORIZATION TO REMOVE EGGS FROM A HOLDING SUBJECT TO THE CONDITIONS OF ARTICLE 4 (2) (e) OF THIS DIRECTIVE

The authorization issued by the competent authority to transport eggs from a suspect holding subject to the provisions of Article 4 (2) (e) to an establishment approved for the manufacture and processing of egg products in accordance with the provisions of Article 6 (1) of Directive 89/437/EEC, hereinafter called the designated establishment, must meet the following conditions:

1. in order to be allowed to be removed from a suspect undertaking, eggs must:
   (a) comply with the requirements laid down in Chapter IV of the Annex to Directive 89/437/EEC;
   (b) be sent directly from the suspect undertaking to the designated establishment; each consignment must be sealed before dispatch by the official veterinarian of the suspect holding and must remain sealed throughout transport to the designated establishment;
2. the official veterinarian of the suspect undertaking shall inform the competent authority of the designated establishment of this intention of sending eggs to it;
3. the competent authority responsible for the designated establishment shall ensure that:
   (a) eggs referred to in 1 (b) will be kept isolated from other eggs from the time they arrive until they are processed;
   (b) the shells of such eggs shall be regarded as high-risk material in accordance with Article 2 (2) of Directive 90/667/EEC [1] and shall be dealt with in accordance with the requirements of Chapter II of that Directive;
   (c) the packaging material, the vehicles used to transport eggs referred to in 1 (b) and all premises with which the eggs come into contact are cleaned and disinfected in such a way as to destroy all avian influenza virus;
   (d) the official veterinarian of the suspect holding shall be informed of all consignments of processed eggs.

[1] OJ No L 363, 27. 12. 1990, p. 51.

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ANNEX II

PROCEDURE FOR CLEANING AND DISINFECTING AN INFECTED HOLDING

I. **Preliminary cleaning and disinfecting**

(a) As soon as the carcases of the poultry have been removed for disposal, those parts of the premises in which the poultry was housed and any parts of other buildings, yards etc. contaminated during slaughter or post-mortem examination should be sprayed with disinfectants approved for use in accordance with Article 11 of this Directive.

(b) Any tissue of poultry or eggs which could have contaminated buildings, yards, utensils etc. should be carefully collected and disposed of with the carcases.

(c) The used disinfectant must remain on the surface for at least 24 hours.

II. **Final cleaning and disinfection**

(a) Grease and dirt should be removed from all surfaces by the application of a degreasing agent and washed with water,

(b) After washing with water as described in (a), further spraying with disinfectant should be applied,

(c) After seven days the premises should be treated with a degreasing agent, rinsed with cold water, sprayed with disinfectant and rinsed again with water.

(d) Used litter and manure must be treated by a method capable of killing the virus. This method must comprise one of the following practices:

(i) incineration or steam treatment at a temperature of 70º C;

(ii) burying deep enough to prevent access by vermin and wild birds;

(iii) stacking and dampening (if necessary to facilitate fermentation), covering to keep in the heat so that a temperature of 20º C is attained and leaving covered for 42 days so as to prevent access by vermin and wild birds.

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ANNEX III

DIAGNOSTIC PROCEDURES FOR THE CONFIRMATION AND DIFFERENTIAL DIAGNOSIS OF AVIAN INFLUENZA (AI)

The following procedures for the isolation and characterization of avian influenza viruses should be regarded as guidelines and the minima to be applied in the diagnosis of the disease.

For the purpose of the diagnostic procedures for the confirmation and differential diagnosis of avian influenza the following definition shall apply.

'Avian influenza' means an infection of poultry caused by any influenza A virus which has an intravenous pathogenicity index in six-week-old chickens greater than 1.2 or any infection with influenza A viruses of H5 or H7 subtype for which nucleotide sequencing has demonstrated the presence of multiple basic amino acids at the cleavage site of the haemagglutinin.

CHAPTER I

Sampling and treatment of samples

1. *Samples*

Cloacal swabs (or faeces) and tracheal swabs from sick birds; faeces or intestinal contents, brain tissue, trachea, lungs, liver, spleen and other obviously affected organs from recently dead birds.

2. *Treatment of samples*

The organs and tissues listed in paragraph 1 may be pooled, but separate treatment of faecal material is essential. Swabs should be placed in sufficient antibiotic medium to ensure full immersion. Faeces samples and organs should be homogenized (in an enclosed blender or using a pestle and mortar and sterile sand) in antibiotic medium and made to 10—20 % w/v suspensions in the medium. The suspensions should be left for about two hours at ambient temperature (or longer periods at 4º C) and then clarified by centrifugation (e.g. 800 to 1 000 × g for 10 minutes).

3. *Antibiotic medium*

Different laboratories have used various formulations of antibiotic medium with success and National Laboratories will be able to offer advice for a particular country. High concentrations of antibiotics are required for faeces samples and a typical mixture is: 10 000 units/ml penicillin, 10 mg/ml streptomycin, 0,25 mg/ml gentamycin and 5 000 units/ml mycostatin in phosphate buffered saline. These levels can be reduced up to five-fold for tissues and tracheal swabs. For control of Chlamydia organisms 50 mg/ml oxytetracycline may be added. It is imperative when making the medium that the pH is checked after the addition of the antibiotics and readjusted to pH 7,0—7,4.

CHAPTER 2

Virus isolation

Virus isolation in embryonated fowls' eggs

The clarified supernatant fluid should be inoculated in 0,1—0,2 ml amounts into the allantoic cavity of each of a minimum of four embryonated fowls?A eggs which have been incubated for eight to 10 days. Ideally, these eggs should be obtained from a specific pathogen free flock, but when this is impracticable it is acceptable to use eggs obtained from a flock shown to be free of antibodies to avian influenza. The inoculated eggs are held at 37º C and candled daily. Eggs with dead or dying embryos as they arise, and all remaining eggs six days after inoculation should be chilled to 4º C and the allantoic-amniotic fluids tested for haemagglutination activity. If no haemagglutination is detected, the above procedure is repeated using undiluted allantoic/amniotic fluid as inoculum.

When haemagglutination is detected the presence of bacteria should be excluded by culture. If bacteria are present the fluids may be passed through a 450 nm membrane filter, further antibiotics added and inoculated into embryonated eggs as above.

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CHAPTER 3

Differential diagnosis

1. *Preliminary differentiation*

   Because it is important that control measures aimed at limiting the spread of virus should be implemented as soon as possible, each regional laboratory should be in a position to identify any isolated haemagglutinating virus as influenza viruses of H5 or H7 subtype in addition to Newcastle disease virus. The haemagglutinating fluids should be used in a haemagglutination inhibition test as described in Chapters 5 and 6. Positive inhibition i.e. 2^4, or more, with polyclonal antisera specific for H5 or H7 subtypes of influenza A and of a titre of at least 2^9 would serve as preliminary identification enabling the imposition of interim control measures.

2. *Confirmatory identification*

   Since there are 13 haemagglutinin subtypes and nine neuraminidase subtypes of influenza viruses and variations occur within each of these it is not practicable nor cost effective for each national laboratory to hold antisera which will allow full antigenic characterization of influenza isolates. However, each national laboratory should:

   (i) confirm that the isolate is an influenza A virus using an immunodouble-diffusion test to detect group antigens as described in Chapter 9 (immunofluorescence or ELISA techniques to detect group antigens may be used if preferred by the national laboratory);

   (ii) determine whether or not the isolate is of H5 or H7 subtype;

   (iii) carry out an intravenous pathogenicity index test in six-week-old chickens as described in Chapter 7. Intravenous pathogenicity indices of greater than 1.2 indicate the presence of virus requiring a full implementation of control measures (it would be a useful exercise if national Laboratories also carried out tests to determine the capacity of an isolate to produce plaques in cell cultures as specified in Chapter 8).

   National laboratories should immediately submit all avian influenza and all H5 and H7 isolates to the Community Reference laboratory for full characterization.

3. *Further typing and characterization of isolates*

   The Community Reference Laboratory should receive all haemagglutinating viruses from the national laboratories for further antigenic and genetic studies to enable a greater understanding of the epizootiology of the disease(s) within the European Community in keeping with the functions and duties of the reference laboratory.

   In addition to these duties the Community Reference Laboratory shall carry out full antigenic typing for all influenza viruses received. For H5 and H7 viruses which do not have intravenous pathogenicity indices greater than 1.2, nucleotide sequencing of the haemagglutinin gene to determine whether or not there are multiple basic amino acids at the cleavage site of the haemagglutinin protein should also be carried out.

CHAPTER 4

Seriological tests for avian influenza virus antibodies

1. During eradication programmes where the H subtype of the virus responsible is already known, or by using the homologous virus as antigen, serological monitoring for evidence of infection may be done using haemagglutination inhibition tests as described in Chapters 5 and 6.

   If the haemagglutinin subtype is not known, evidence for infection with influenza A viruses may be obtained by detecting antibodies directed to the group specific antigens.

   For this purpose either an immunodoublediffusion test (as described in Chapter 9) or an ELISA test may be used (a problem with ELISA is the host specificity of the test since it is dependent on the detection of host immunoglobulins). Waterfowl rarely give positive results in immunodouble-diffusion tests and, unless the subtype is known, it is probably only practicable to examine such birds for the presence of antibodies to H5 and H7 subtypes.

2. (a) *Samples*

   Blood samples should be taken from all birds if the flock size is less than 20 and from 20 birds from larger flocks (this will give a 99 % probability

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of detecting at least one positive serum if 25 % or more of the flock is positive, regardless of flock size). The blood should be allowed to clot and serum removed for testing.

(b) *Examination for antibodies*

Individual serum samples should be tested for their ability to inhibit influenza virus haemagglutinating antigen in standard haemagglutination inhibition tests as defined in Chapter 6.

There is some debate as to whether 4 or 8 haemagglutinin units should be used for the H1 tests. It would appear that either is valid and the choice should be left to the discretion of the national laboratories.

However, the antigen used will affect the level at which a serum is considered positive; — for 4 HAU a positive serum is any showing a titre of 2^4 or greater, for 8 HAU a positive serum is any showing a titre of 2^3 or greater.

CHAPTER 5

Haemagglutination (HA) test

Reagents

1. Isotonic saline buffered with phosphate (0,05M) to pH 7,0—7,4.
2. Red blood cells (RBC) taken and pooled from a minimum of three specific pathogen free chickens (if not available blood may be taken from birds regularly monitored and shown to be free of Avian influenza antibodies) into an equal volume of Alsever?As solution. Cells should be washed three times in PBS before use. For the other test a 1 % suspension (packed cell v/v) in PBS is recommended.
3. The Community Reference Laboratory will supply or recommend H5 and H7 viruses of low virulence for use as standard antigens.

Procedure

1. Dispense 0,025 ml PBS into each well of a plastic microtitre plate (V-bottomed wells should be used).
2. Place 0,025 ml of virus suspension (i.e. allantoic fluid) in the first well.
3. Use a microtitration diluter to make two-fold dilutions (1:2 to 1:4 096) of virus across the plate.
4. Dispense a further 0,025 ml of PBS to each well.
5. Add 0,025 ml of 1 % red blood cells to each well.
6. Mix by tapping gently and place at 4º C.
7. Plates are read 30—40 minutes later when Red Blood Cells control are settled. Reading is done by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. Wells with no HA should flow at the same rate as the control cells with no virus.
8. The HA titre is the highest dilution that causes agglutination of the RBCs. That dilution may be regarded as containing one HA unit (HAU). A more accurate method for determining the HA titre is to do HA tests on virus from a close range of initial dilutions i.e. 1:3, 1:4, 1:5, 1:6, etc. This is recommended for the accurate preparation of antigen for haemagglutination inhibition tests (Chapter 6).

CHAPTER 6

Haemagglutination inhibition (Hl) test

Reagents

1. Phosphate buffer solution (PBS).
2. Virus containing allantoic fluid diluted with PBS to contain 4 or 8 HAU per 0,025 ml.
3. 1 % chicken RBCs.
4. Negative control chicken serum.
5. Positive control serum.

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Procedure

1. Dispense 0,025 ml PBS into all wells of a plastic microtitre plate (with V-bottomed wells).
2. Place 0,025 ml of serum into first well of plate.
3. Use microtitration diluter to make two-fold dilutions of serum across plate.
4. Add 0,025 ml of diluted allantoic fluid containing 4 or 8 HAU.
5. Mix by tapping and place plate at 4º C for a minimum of 60 minutes or room temperature for a minimum of 30 minutes.
6. Add 0,025 ml 1 % RBCs to all wells.
7. Mix by gentle tapping and place at 4º C.
8. Plates are read after 30—40 minutes when control RBCs are settled. This is done by tilting and observing the presence or absence of tear-shaped streaming at the same rate as control wells containing RBCs (0,025 ml and PBS (0,05 ml) only.
9. The Hl titre is the highest dilution of antiserum causing complete inhibition of four or eight units of virus (an HA titration to confirm the presence of the required HAU should be included in each test).
10. The validity of the results is dependent on obtaining a titre of less than 2^3 for 4 HAU or 2^2 for 8 HAU with a negative control serum and a titre of within one dilution of the known titre of the positive control serum.

CHAPTER 7

Intravenous Pathogenicity Index (IVPI)

1. Infective allantoic fluid from the lowest passage level available, preferably from the initial isolation without any selection, is diluted 10^1 in sterile isotonic saline.
2. 0,1 ml diluted virus is injected intravenously into each of 10 six-week-old chickens (specific pathogen free birds should be used).
3. Birds are examined at 24 hour intervals for 10 days.
4. At each observation each bird is recorded normal (0), sick (1), severely sick (2) or dead (3).
5. Record results and calculate index as shown in this example:

Clinical Signs	Day after inoculation										Total Score
	1	2	3	4	5	6	7	8	9	10	
Normal	10	2	0	0	0	0	0	0	0	0	12 × 0 = 0
Sick	0	4	2	0	0	0	0	0	0	0	6 × 1 = 6
Severely sick (*)	0	2	2	2	0	0	0	0	0	0	6 × 2 = 12
Dead	0	2	6	8	10	10	10	10	10	10	76 × 3 = 228
											Total = 246

$$\text{Index} = \text{mean score per bird per observation} = \frac{246}{100} = 2,46$$

(*) This has to be a subjective clinical judgment but normally this would involve birds showing more than one of the following signs: respiratory involvement, depression, diarrhoea, cyanosis of exposed skin or wattles, oedema of face and/or head, nervous signs.

CHAPTER 8

Assessment of plaque-forming ability

1. It is usually best to use a dilution range of virus to ensure that an optimum number of plaques are present on the plate. Ten-fold dilutions up to 10^{-7} in PBS should be sufficient.

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2. Confluent monolayers of chick embryo cells or a suitable cell line (Madin-Darby bovine kidney for example) are prepared in 5 cm diameter Petri dishes.

3. 0,2 ml of each virus dilution is added to each of two Petri dishes and the virus allowed to absorb for 30 minutes.

4. After washing three times with PBS the infected cells are overlaid with the relevant medium containing 1 % w/v agar and either 0,01 mg/ml trypsin or no trypsin. It is important that no serum is added to the overlay medium.

5. After 72 hours incubation at 37º C the plaques should be of sufficient size. They are best seen by removing the agar overlay and staining the cell monolayer with crystal violet (0,5 % w/v) in 25 % v/v ethanol.

6. All viruses should give clear plaques when incubated in the presence of trypsin in the overlay. When trypsin is absent from the overlay only viruses virulent for chickens will produce plaques.

CHAPTER 9

Immunodoublediffusion

The preferred method to show the presence of influenza A virus is to demonstrate the possession of the nucleocapsid or matrix antigens which are shared by all influenza A virus. This is generally done in immunodoublediffusion tests involving either concentrated virus preparations or extracts from infected chorioallantoic membranes.

Suitable preparations of concentrated virus may be made by simple high speed centrifugation of infectious allantoic fluid and disruption of virus to release the internal nucleocapsid and matrix antigens by treatment with the detergent sodium lauroyl sarcosinate. Acid precipatation may also be used by adding 1N HCl to infective allantoic fluid to give a final pH of 3,5—4,0, chilling for at least one hour at 0º C and low speed centrifugation at 1 000 g for 10 minutes.

The supernatant may be discarded and the virus-containing precipitate resuspended in a minimum volume of glycine-sarkosyl buffer (1 % sodium lauroyl sarcosinate buffered to pH 9,0 with a 0,5M glycine). These preparations possess both nucleocapsid and matrix antigens.

Beard (1970) described the preparation of nucleocapsid-rich antigen from chorioallantoic membranes removed from infected eggs. This method involves: removal of the chorioallantoic membranes from infected haemagglutinin positive eggs, grinding or homogenising the membranes, freezing and thawing three times followed by centrifugation at 1 000 g for 10 minutes. The pellet is discarded and the supernatant treated with 0,1 % formalin for use as antigen.

Either of these two antigens may be used in immunodoublediffusion tests using 1 % agarose, or agar, gels containing 8,0 % sodium chloride made up to 0.1M phosphate buffer pH 7,2. Influenza A virus is confirmed by precipitin lines formed by test antigen and known positive antigen against a known positive antiserum coalescing to give a line of identity.

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ANNEX IV

LIST OF NATIONAL AVIAN INFLUENZA LABORATORIES

Belgium	Institut National de Recherches Vétérinaires, Groeselenberg 99, B-1180 Brussels
Denmark	National Veterinary Laboratory, Poultry Disease Division, Hangøvej 2, DK-8200 Aarhus N
Germany	Institut für Kleintierzucht der Bundesforschungsanstalt für Landwirtschaft, Braunschweig-Völkenrode, Postfach 280, D-3100 Celle
France	Centre National d'Etudes Vétérinaires et Alimentaires — Laboratoire Central de Recherches Avicoles et Porcines, B.P. 53, F-22440 Ploufragan
Greece	Ινστιτούτο Λοιμωδών και Παρασιτικών Νοσημάτων 66, 26ης Οκτωβρίου, GR-54627 Θεσσαλονίκη Institute of Infections and Parasitological Diseases, 66, 26th October Street, 546 27 Thessaloniki
Ireland	Veterinary Research Laboratory, Abbotstown, Castleknock, Dublin 15
Italy	Istituto Patologie Aviaire, Facoltà di Medicina Veterinaria, Università di Napoli, via Aniezzo, Falcone 394, I-80127 Napoli F Delpino 1
Luxembourg	Institut National de Recherches Vétérinaires, Groeselenberg 99, B 1180 Brussels
Netherlands	Centraal Diergeneeskundig Instituut, Vestiging Virologie, Houtribweg 39, NL-8221 RA Lelystad
Portugal	Laboratório Nacional de Investigação Veterinária (LNIV), Estrada de Benfica 701, P-1500 Lisbon
Spain	Centro Nacional de Referencia para la Peste Aviar es el Laboratorio Nacional de Sanidad y Producción Animal de Barcelona, Zona Franca Circunvalación-Tramo 6, Esquina Calle 3, Barcelona
United Kingdom	Central Veterinary Laboratory, New Haw, UK-Weybridge, Surrey KT15 3NB
▼A1 Austria	Bundesanstalt für Virusseuchenbekämpfung bei Haustieren, Wien-Hetzendorf
Finland	Eläinlääkintä- ja elintarvikelaitos, Helsinki — Anstalten för veterinärmedicin och livsmedel, Helsingfors
Sweden	Statens veterinärmedicinska anstalt, Uppsala

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ANNEX V

COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA

Name of Laboratory

Central Veterinary Laboratory,
New Haw,
UK-Weybridge,
Surrey KT 15 3NB,
United Kingdom.

The functions and duties of the EC reference laboratory for avian influenza shall be:

1. to coordinate, in consultation with the EC Commission, the methods employed in the Member States for diagnosing avian influenza. Specifically by:
   (a) typing, storing and supplying strains of avian influenza virus for serological tests and the preparation of antisera;
   (b) supplying standard sera and other reference reagents to the National Reference Laboratories in order to standardize the tests and reagents used in the Member States;
   (c) building up and retaining a collection of avian influenza virus strains and isolates;
   (d) organizing periodical comparative tests of diagnostic procedures at Community level;
   (e) collecting and collating data and information on the methods of diagnosis used and the results of tests carried out in the Community;
   (f) characterizing isolates of avian influenza viruses by the most up-to-date methods available to allow greater understanding of the epizootiology of avian influenza and to gain an insight into the epizootiology of the virus and the emergence of highly pathogenic and potentially pathogenic strains;
   (g) keeping abreast of developments in avian influenza surveillance, epizootiology and prevention throughout the world;
   (h) retaining expertise on avian influenza virus and other pertinent viruses to enable rapid differential diagnosis;
   (i) acquiring a thorough knowledge of the preparation and use of the products of veterinary immunology used to eradicate and control avian influenza;
2. to actively assist in the diagnosis of avian influenza outbreaks in Member States by receiving virus isolates for confirmatory diagnosis, characterization and epizootiological studies. In particular, the laboratory should be able to carry out nucleotide sequencing analysis to allow determination of the deduced amino acid sequence at the cleavage site of the haemagglutin molecule of avian influenza viruses of H5 or H7 subtpye,
3. to facilitate the training or retraining of experts in laboratory diagnosis with a view to the harmonization of techniques throughout the Community.

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ANNEX VI

CRITERIA FOR CONTINGENCY PLANS

Contingency plans shall meet at least the following criteria:

1. the establishment of a crisis centre on a national level, which shall coordinate all control measures in the Member State concerned;
2. a list shall be provided of local disease control centres with adequate facilities to coordinate the disease control measures at a local level;
3. detailed information shall be given about the staff involved in control measures, their skills and their responsibilities;
4. each local disease control centre must be able to contact rapidly persons/organizations which are directly or indirectly involved in an outbreak;
5. equipment and materials shall be available to carry out the disease control measures properly;
6. detailed instructions shall be provided on action to be taken on suspicion and confirmation of infection or contamination, including proposed means of disposal of carcases;
7. training programmes shall be established to maintain and develop skills in field and administrative procedures;
8. diagnostic laboratories must have facilities for post-mortem examination, the necessary capacity for serology, histology etc. and must maintain the skills for rapid diagnosis. Arrangements must be made for rapid transportation of samples;
9. details shall be provided of the quantity of avian influenza vaccine estimated to be required in the event of a reinstatement of emergency vaccination;
10. provisions shall be made to ensure the legal powers necessary for the implementation of the contingency plans.