L.N. 367 of 2018

VETERINARY SERVICES ACT
(CAP. 437)

Community Measures for the Control of Newcastle Disease Regulations, 2018

IN EXERCISE of the powers conferred by article 5(1) and (2) of the Veterinary Services Act, the Minister for the Environment, Sustainable Development and Climate Change, in conjunction with the Parliamentary Secretary for Agriculture, Fisheries and Animal Rights has made the following regulations:

Citation and scope.

1. (1) The title of these regulations is the Community Measures for the Control of Newcastle Disease Regulations, 2018.

(2) These regulations shall come into force on such date as the Minister responsible for the Environment, Sustainable Development and Climate Change may by notice in the Gazette appoint, and different dates may be so appointed for different provisions and different purposes of these regulations.

(3) The scope of these regulations is to transpose Council Directive 92/66/EEC introducing Community measures for the control of Newcastle disease as amended by Directive (EU) 2018/597 of the European Parliament and of the Council of 18thApril 2018.

(4) These regulations establish control measures to be taken in the event of an outbreak of Newcastle disease in poultry, racing pigeons and other birds kept in captivity.

Interpretation.

2. (1) In these regulations unless the context otherwise requires, the definitions found in Article 2 of Council Directive 2009/158/EC of 30th November 2009 on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs shall apply as appropriate.

(2) The following definitions shall also apply:

"the Act" means the Veterinary Services Act; Cap. 437.

"The Commission" means the European Commission;

"competent authority" means the Department of Veterinary Services as established by article 2 of the Act;

"Crisis Unit" means the Animal Health Unit within the competent authority;

"the Directive" means Council Directive 92/66/EEC as amended by Directive (EU) 2018/597;

"European Union reference laboratory" means the laboratory referred to in regulation 15;

"infected poultry" means any poultry in which the presence of Newcastle disease has been officially confirmed following an examination by an approved laboratory or in the case of second and subsequent outbreaks in which clinical symptoms or post-mortem lesions consistent with Newcastle disease are present;

"official veterinarian" means a warranted veterinarian working for the competent authority;

"pigeon house" means any installation used for keeping or breeding racing pigeons.

"poultry suspected of being contaminated" means any poultry which may have been directly or indirectly exposed to the Newcastle-disease virus;

"poultry suspected of being infected" means any poultry showing clinical signs or post-mortem lesions which are such that the presence of Newcastle disease may reasonably be suspected;

"racing pigeon" means any pigeon transported or intended for transport from its pigeon house to any other destination;

"Standing Committee on the Food Chain and Animal Health" means the committee referred to in Article 25 of the Directive; and

"swill" means waste from kitchens, restaurants or, where appropriate, from industries using meat;

Non-applicability.

3. These regulations do not apply where Newcastle disease is detected in wild birds living freely. In such cases the competent authority shall inform the Commission of any measure it takes.

Notification requirements.

4. (1) When poultry in a holding are suspected of being infected or contaminated with Newcastle disease, the official veterinarian shall immediately activate official investigation arrangements to confirm or rule out the presence of the disease and shall take necessary samples for laboratory examination.

(2) As soon as the suspected infection is notified, the competent authority shall place the holding under official surveillance and shall have the power to order 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,

- of poultry meat or carcasses, or of animal feed, implements, waste, droppings, manure litter or anything liable to transmit Newcastle disease, be subject to authorisation by the competent authority;

(e) no 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 (according to lists drawn up by the competent authority) and transported under an authorisation which has been granted by the competent authority. Such authorisation must meet the requirements laid down in Schedule I;

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

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

(3) Until such time as the official measures laid down in sub-regulation (2) are enforced, the owner or keeper of any poultry in which disease is suspected shall take all reasonable action to ensure compliance with sub-regulation (2) except for the epizootiological inquiry;

(4) The competent authority may apply any of the measures provided for in sub-regulation (2) to other holdings should their location, their configuration or contacts with the holding where the disease is suspected give reason to suspect possible contamination;

(5) The measures referred to in sub-regulation (1) and sub-regulation (2) shall not be withdrawn until the suspicion of Newcastle disease has been ruled out by the official veterinarian.

Procedures.

5. (1) Once the presence of Newcastle disease in poultry has been officially confirmed on a holding, the competent authority shall have the power to order that the following measures be undertaken:

(a) all poultry on the holding shall without delay be killed on the spot and the poultry which have died or been killed and all eggs shall be destroyed. These operations shall be carried out in a way which minimises 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 in accordance with instructions given by the official veterinarian in order to ensure the destruction of any Newcastle-disease 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 wherever possible be traced and destroyed, unless they have previously been properly disinfected;

(e) after carrying out operations listed under paragraphs (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 disinfected in accordance with the provisions of regulation 11;

(f) no poultry shall be introduced to the holding until at least twenty-one (21) days after completion of operations provided for under (e);

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

(2) The competent authority may extend the measures provided for in sub-regulation (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.

(3) Where a strain of Newcastle-disease virus with an ICPI (intracerebral pathogenicity index) greater than 0,7 and lower than 1,2 is isolated in a flock of poultry that presents no clinical signs of Newcastle disease and it has been demonstrated by the European Union reference laboratory referred to in regulation 15 that the virus isolate in question derives from an attenuated live Newcastle-disease vaccine, the competent authority may grant a derogation from the requirements under paragraphs (a) to (f) of sub-regulation (1), provided that the holding concerned is placed under official surveillance for thirty (30) days and must require in particular that:

- the provisions of regulation 4(2) (a), (b), (d), (e), and (f) are applied;

- no poultry may leave the holding except to be taken directly to a slaughterhouse designated by the competent authority.

(4) The competent authority responsible for this slaughterhouse must be informed of the intention to send poultry there for slaughter and as soon as the poultry arrive at the slaughterhouse they shall be kept and slaughtered separately from other poultry.

(5) Fresh meat from the poultry referred to in sub-regulation (3) of this regulation must carry the identification mark applied in accordance with Schedule II, Section I of Regulation (EC) 853/2004.

(6) The provisions laid down in sub-regulation (3) shall be subject to a review taking into account developments in scientific research with a view to adopting harmonised rules for the use of Newcastle-disease vaccines in the Member States.

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 5 of Regulation (EU) No 182/2011, grant a derogation from the requirements of regulation 5 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. Derogation.

Procedure for the epizootiological inquiry.

7. The epizootiological inquiry shall deal with:

(a) the length of time during which Newcastle disease may have existed on the holding or in the pigeon house;

(b) the possible origin of the Newcastle disease on the holding or in the pigeon house and the identification of other holdings or pigeon houses in which there are poultry, pigeons or other birds kept in captivity which may have become infected or contaminated from the same source;

(c) the movement of persons, poultry, pigeons or other birds kept in captivity or other animals, vehicles, eggs, meat and carcases and any implement or substance likely to have carried Newcastle-disease virus to or from the holding or pigeon house in question.

The crisis unit.

8. The crisis unit shall provide full coordination of all measures necessary to ensure the eradication of Newcastle disease as quickly as possible and to carry out the epizootiological inquiry.

Holdings placed under official control.

9. (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 sub-regulation (2).

(2) The purpose of the official control shall be to detect immediately any suspicion of Newcastle disease, count the poultry and monitor their movements and, where appropriate, to take the action provided for in sub-regulation (3).

(3) When a holding is subject to official control under sub-regulations (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 authorisation, the official veterinarian must have carried out a clinical examination of all the poultry to exclude presence of Newcastle disease on the holding. The movement restrictions referred to in this regulation shall be imposed for a period of twenty-one (21) days from the latest date of potential contamination; however, such restrictions must apply for a period of at least seven (7) days.

(4) Where it considers that conditions permit, the competent authority may limit the measures provided for in this regulation 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.

(5) Where the official veterinarian has reason to suspect racing pigeons or any pigeon house of being contaminated by the Newcastle-disease virus, he shall take all appropriate steps to ensure that the pigeon house is subject to restrictions, including a ban on all movement of racing pigeons outside the pigeon house for twenty-one (21) days.

Measures to be taken.

10. (1) Once the diagnosis of Newcastle disease has been officially confirmed in poultry, the competent authority shall establish 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 ten kilometres. The establishment of zones must take account of geographical, administrative, ecological and epizootiological factors relating to Newcastle disease, and of monitoring facilities.

(2) The measures applied in the protection zone shall include:

(a) the identification of all holdings having poultry within 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 or persons handling poultry, poultry carcasses and eggs and vehicles carrying poultry, carcasses and eggs within the zone; in general, transport of poultry shall be prohibited.

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

(ii) of day-old chicks or ready-to-lay pullets to a

holding within the surveillance zone at which there are no other poultry. However, the competent authority, may give authorisation for the said day-old chicks and pullets to be transported to a holding outside the surveillance zone. The holdings referred to above shall be placed under official control in accordance with regulation 9(2);

(iii) of hatching eggs to a hatchery designated by the competent authority; before dispatch, eggs and their packing must be disinfected:

Provided that movements allowed under sub-paragraphs (i), (ii) and (iii) shall be directly executed, under official control. They shall be authorised only after the official veterinarian has carried out a health inspection before and after use;

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

(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 twenty-one (21) days after the carrying out of preliminary cleaning and disinfection operations on the infected holding in accordance with regulation 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 fifteen (15) days, except for movement directly to a slaughterhouse outside the surveillance zone designated by the competent authority.

(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 paragraphs (a) and (b), the prohibition of transport of poultry.

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

(6) Where the epizootiological enquiry referred to in regulation 7 confirms that the outbreak is due to an infection where there is no evidence of lateral spread, the size and duration of the protection and surveillance zones may be reduced under the procedure laid down in Article 5 of Regulation (EU) No 182/2011.

11. The competent authority has the power to; Powers of the competent authority.

(i) determine the arrangements allowing it to trace the movement of eggs, poultry and birds kept in captivity;

(ii) request the owner or keeper of poultry and/or racing pigeons and/or birds kept in captivity to supply information concerning poultry and eggs entering or leaving his holding, as well as information on the races or shows in which the racing pigeons have taken part;

(iii) request all persons engaged in the transport or marketing of poultry, eggs, racing pigeons and birds kept in captivity to supply information concerning the movements of poultry, eggs, racing pigeons and birds kept in captivity which they have transported or marketed and to furnish all the details concerning such information.;

(iv) approve the disinfectants to be used and their concentrations;

(v) ensure that the cleaning and disinfection operations are carried out under official supervision in accordance with instructions given by the official veterinarian and the procedure for cleaning and disinfecting an infected holding, as laid down in Schedule II.

12. The collection of samples and laboratory testing to detect the presence of Newcastle disease virus shall be carried out in accordance Samples and laboratory testing.

with Schedule III.

Information requirements.

13. The competent authority shall take all the necessary measures for persons established in the protection and surveillance zones to be informed of the restrictions in force and shall make all necessary arrangements for the implementation of the measures in question.

National Laboratory.

14. (1) The National Veterinary Laboratory (NVL) in Malta shall maintain facilities and expert personnel to permit full antigenic and biological typing of Newcastle-disease virus at all times;

(2) Vaccines allowed for prophylactic use or stock-piled for emergency use may be tested for efficacy, potency and purity at the national veterinary laboratory.

(3) The national laboratories listed in Schedule IV shall be responsible for coordinating standards and methods of diagnosis, use of reagents and testing of vaccines.

(4) The national laboratories listed in Schedule IV shall be responsible for co-ordinating the standards and diagnostic methods laid down in each Newcastle-disease diagnostic laboratory within Malta and Gozo. To this end:

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

(b) they shall control the quality of all diagnostic reagents used;

(c) they shall arrange comparative tests periodically;

(d) they shall hold isolates of Newcastle-disease virus from cases confirmed;

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

(5) The national laboratories listed in Schedule IV shall liaise with the European Union reference laboratory referred to in regulation 15.

The European Union reference laboratory.

15. (1) The European Union reference laboratory referred to in sub-regulation (5) of regulation 14 shall have the following functions and duties:

(i) to coordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing

Newcastle disease, specifically by:

(a) typing, storing and supplying strains of Newcastle disease 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 standardise the tests and reagents used in the Member States;

(c) building up and retaining a collection of Newcastle disease virus strains and isolates;

(d) organising periodical comparative tests of diagnostic procedures at Union level;

(e) collecting and collating data and information on the methods of diagnosis used and the results of tests carried out in the Union;

(f) characterising isolates of Newcastle disease viruses by the most up-to-date methods available to promote a greater understanding of the epidemiology of Newcastle disease;

(g) keeping abreast of developments in Newcastle disease surveillance, epidemiology and prevention throughout the world;

(h) retaining expertise on Newcastle disease virus and other pertinent viruses to enable a rapid differential diagnosis;

(i) acquiring a thorough knowledge of the preparation and use of the products of veterinary immunology used to eradicate and control Newcastle disease;

(ii) to actively assist in the diagnosis of outbreaks of Newcastle disease in Member States by receiving virus isolates for confirmatory diagnosis, characterisation and epidemiology studies;

(iii) to facilitate the training or retraining of experts in laboratory diagnosis with a view to the harmonisation of techniques throughout the Union.

(2) The designation of the European Union reference laboratory

for Newcastle disease referred to in Schedule V to Directive 92/66/EEC, shall remain effective until a European Union reference laboratory for Newcastle disease has been duly designated in accordance with Article 15 of Directive 92/66/EEC.

Vaccination requirements.

16. (1) Vaccination against Newcastle disease authorised by the competent authority may be used for a prophylactic purpose or to supplement the control measures carried out when the disease appears.

(2) The only vaccines allowed are those which have received a marketing authorisation from the competent authority.

(3) Further criteria for using vaccines against Newcastle disease may be established in accordance with the procedure laid down in Article 5 of Regulation (EU) No 182/2011.

(4) The competent authority shall inform the Commission when voluntary or compulsory prophylactic vaccination against Newcastle disease is carried out.

(5) The information given in accordance with sub-regulation (4) must specify:

(a) the characteristics and composition of the vaccine to be used;

(b) the procedures for supervision of the distribution, storage and use of vaccines;

(c) the species and categories of poultry which may or shall be subject to vaccination;

(d) the areas in which vaccination may or shall be carried out;

(e) the reasons for which vaccination is carried out.

(6) The competent authority may provide for the establishment of a vaccination programme for racing pigeons. Where this is the case, the competent authority shall inform the Commission. Without prejudice to such a programme, the competent authority shall ensure that the organisers of races and shows take the necessary steps so that only racing pigeons which have been vaccinated against Newcastle disease may be entered in races and shows.

Vaccination of designated species of poultry.

17. (1) When Newcastle disease has been confirmed, the competent authority, in order to supplement the other control measures provided for in these Regulations has the power to specify a territorial

area and period in which the prompt and systematic vaccination (emergency vaccination) of designated species of poultry shall be carried out under official control. The competent authority shall inform the Commission and other Member States within the framework of the Standing Committee on Plants, Animals, Food and Feed (PAFF Committee), established by Article 58(1) of Regulation (EC) No 178/2002, about the Newcastle-disease situation and emergency vaccination programme.

(2) In the case referred to in sub-regulation (1), the vaccination or re-vaccination of poultry on the holding subject to the restrictions referred to in Article 5 is prohibited.

(3) In the case referred to in sub-regulation (1):

(a) the designated species of poultry shall be vaccinated as soon as possible;

(b) all poultry of designated species born on or transferred to a holding inside the vaccination area must be or have been vaccinated;

(c) throughout the vaccination operation provided for in sub-regulation (1) all poultry of designated species kept on holdings inside the vaccination area must remain there except:

(i) day-old chicks moved to a holding inside the vaccination area where they shall be vaccinated;

(ii) poultry moved direct to a slaughterhouse for immediate slaughter. If the slaughterhouse is located outside the vaccination area the movement of poultry shall be permitted only after the official veterinarian has carried out a health inspection of the holding;

(d) when the vaccination operations provided for in paragraph (a) have been completed movements out of the vaccination area may be authorised for:

(i) day-old chicks intended for meat production may be moved to a holding where they shall be vaccinated; the holding in question must be kept under surveillance until the poultry which have been moved have been slaughtered;

(ii) poultry vaccinated more than twenty-one (21) days previously and intended for immediate slaughter;

(iii) hatching eggs which originate from breeding

poultry vaccinated at least twenty-one (21) days previously; the eggs and their packing must be disinfected before movement.

(4) The measures provided for in paragraphs (b) and (d) of sub-regulation (3) shall be applied for a period of three (3) months following completion of vaccination operations provided for in sub-regulation (1); they may be extended for one or more additional periods of three (3) months.

(5) By way of derogation from paragraphs (a) and (b) of sub-regulation (3), the competent authorities may exempt certain flocks of particular scientific value from systematic vaccination, provided that all necessary steps are taken by the competent authority to ensure protection of their health and to subject them to periodic serological checks.

Investigation Procedures.

18. (1) Where carrier pigeons or birds kept in captivity are suspected of being infected with Newcastle disease, the official veterinarian will immediately start the official investigation procedures for confirming or notifying the presence of the disease; in particular he shall take adequate samples for laboratory examination or see that they are taken.

(2) As soon as it has been notified of the suspicion, the competent authority shall place the holding or pigeon house under official surveillance and order that no pigeon or bird kept in captivity, and nothing likely to transmit Newcastle disease, may leave the pigeon house or holding.

(3) The measures provided for in sub-regulations (1) and (2) shall not be withdrawn until the suspicion of Newcastle disease has been ruled out by the official veterinarian.

(4) As soon as the presence of Newcastle disease has been officially confirmed, the competent authority shall, inter alia, order:

(a) application of the control and eradication measures provided for in regulation 5(1)(a), (b), (e) and (f) to the carrier pigeons or birds kept in captivity and pigeon houses infected with Newcastle disease; or

(b) at least:

(i) a ban on movement of the pigeons or birds kept in captivity outside the pigeon house or holding for at least sixty (60) days after the clinical signs of Newcastle disease have disappeared;

(ii) destruction or treatment of any matter or waste likely to be contaminated. Treatment must guarantee the destruction of any Newcastle-disease virus present and all waste that has accumulated during the sixty-day(60) period referred to in sub-paragraph (i);

(c) an epizootiological inquiry in accordance with regulation 7.

(5) To the extent that it is required for the proper application of the measures laid down in this regulation, the competent authority shall submit to the Commission, within the Standing Committee on Plants, Animals, Food and Feed, information on the disease situation and the control measures applied;

The use of swill.

19. (1) The use of swill originating from means of international transport, such as ships, land vehicles or aircraft, shall be prohibited for the feeding of poultry; such swill shall be collected and destroyed under official supervision.

(2) The use of swill other than as defined in sub-regulation (1), or poultry scraps, may be authorised for the feeding of poultry only after a heat-treatment in appropriate facilities ensuring that the disease is not transmitted and the Newcastle-disease virus is destroyed.

Drawing up of a contingency plan.

20. (1) The competent authority shall draw up a contingency plan, specifying the national measures to be implemented in the event of an outbreak of Newcastle disease. The contingency plan shall be updated, as appropriate, to take account of developments in the situation.

(2) The contingency plan shall allow access to facilities, equipment, personnel and all other appropriate materials necessary for the rapid and efficient eradication of the outbreak of Newcastle disease. It shall give a precise indication of the vaccine requirements which the competent authority deems necessary for emergency vaccination.

(3) The contingency plans and any updates thereto shall be submitted to the Commission, which shall approve or if necessary amend them in accordance with the examination procedure referred to in Article 5 of Regulation (EU) No 182/2011.

On-the-spot checks.

21. Commission experts may, in collaboration with the competent authority, make on-the-spot checks. In order to do this, they may check a representative percentage of establishments to see whether the competent authority is checking that these establishments are fulfilling the requirements of the Directive. The Commission shall inform the competent authority of the result of the checks carried out. The competent authority shall give all the necessary assistance to the

experts in carrying out their duties.

The general provisions for implementing this regulation shall be determined in accordance with the procedure laid down in Article 5 of Regulation (EU) No 182/2011.

SCHEDULE I

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

The authorization issued by the competent authority to transport eggs from a suspect holding subject to the provisions of regulation 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 his 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 Category 3 Material in accordance with Article 3 Regulation (EC) No 1069/2009 and that shall be disposed of in accordance with the requirements Article 13 of that Regulation;

   (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 Newcastle disease virus;

   (d) the official veterinarian of the suspect holding shall be informed of all consignments of processed eggs.

SCHEDULE 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 regulation 11 of these regulations.

(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 at least one of the following practices:

(i) incineration or steam treatment at a temperature of 70 °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 20°C is attained and leaving covered for 42 days so as to prevent access by vermin and wild birds.

SCHEDULE III

DIAGNOSTIC PROCEDURES FOR THE CONFIRMATION AND DIFFERENTIAL DIAGNOSIS OF NEWCASTLE DISEASE

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

The virus responsible for Newcastle disease is the prototype virus of the Paramyxoviridae. At present, there are nine serologically distinguishable groups of Avian Paramyxoviruses, which have been designated PMV-1 to PMV-9. All Newcastle disease viruses are placed in the PMV-1 group. For the purpose of the diagnostic procedures for the confirmation and differential diagnosis of Newcastle disease the following definition shall apply:

'Newcastle disease' means an infection of poultry caused by any avian strain of the paramyxovirus 1 with an intracerebral pathogenicity index (ICPI) in day-old chicks greater than 0,7.

CHAPTER 1

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 4°C) and then clarified by centrifugation (e.g. 800 to 1,000g for 10 minutes).

3. Antibiotic medium

Different laboratories have used various formulations of antibiotic medium with success and laboratories referred to in Schedule II 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, 10mg/ml streptomycin, 0,25mg/ml gentamycin and 5 000 units/ml mycostatin in phosphate buffered saline (PBS) These levels can be reduced up to five-fold for tissues and tracheal swabs. For control of Chlamydia organisms, 50mg/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 supernatent fluid should be inoculated in 0,1-0,2ml amounts into the allantoic cavity of each of a minimum of four embryonated, fowls' eggs which have been incubated for 8 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 Newcastle-disease virus. The inoculated eggs are held at 37°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 4°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 hemagglutination 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.

CHAPTER 3

Differential diagnosis

1. Preliminary differentiation

It is intended that all hemagglutinating viruses should be submitted to the national laboratory referred to in Schedule II for full identification, characterization and pathogenicity tests. However, it is important that interim control measures for Newcastle disease aimed at limiting the spread of the virus should be implemented as soon as possible and regional laboratories should be able to identify the presence of Newcastle disease virus. The hemagglutinating fluids should, therefore, be used in an hemagglutination inhibition test as described in Chapters 5 and 6. Positive inhibition 1. e. 24, or more, with the Newcastle disease virus specific polyclonal antiserum of titre known to be at least 29 would serve as preliminary identification enabling the imposition of interim control measures.

2. Confirmatory identification

The national laboratory should undertake full differential diagnosis of any hemagglutinating agent. Confirmation of Newcastle-disease virus would again be by inhibition in hemagglutination inhibition tests with monospecific chicken antisera. Intracerebral pathogenicity index tests as described in Chapter 7 should be carried out on all positive isolates. Pathogenicity indices of greater than 0,7 indicate the presence of virus requiring the full implementation of control measures.

Recent developments in typing Newcastle-disease viruses, particularly monoclonal antibody techniques, has enabled grouping of the strains and isolates. In particular, some monoclonal antibodies are available which are specific for the vaccinal strains used on the territory of the Community and

can be employed in simple hemagglutination inhibition tests.

Since live vaccine strains may often be isolated from sampled poultry the advantage of their rapid identification at the national laboratory referred to in Schedule II is obvious. Such monoclonal antibodies should be obtained by the Community reference laboratory as referred to in regulation 14 and supplied to the national laboratories to enable confirmation of the isolation of vaccinal viruses.

The national laboratories should submit all hemagglutinating agents to the Community reference laboratory.

3. Further typing and characterization of isolates

The Community reference laboratory should receive all hemagglutinating 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 Community in keeping with the functions and duties of the reference laboratory.

CHAPTER 4

Rapid tests for detection of Newcastle-disease virus and antibodies

Rapid tests for the detection of Newcastle disease virus in vaccinated birds and the detection of antibodies in unvaccinated birds are outlined below:

1. Detection of Newcastle disease virus

Several rapid test that directly detect Newcastle-disease antigens have been employed in the diagnosis of infections in vaccinated birds. Those most commonly used to date are fluorescent antibody tests on longitudinal sections of the trachea and peroxidase antibody tests on the brain. There seems no reason to doubt that other direct antigen detection tests could be applied to Newcastle-disease virus infections.

The drawback to such tests is that it is impracticable to examine all the potential sites of replication of Newcastle-disease virus in the vaccinated birds. So that, for example, absence of evidence of virus in the trachea does not preclude virus replication in the gut. No direct detection method is recommended for routine use in the diagnosis of Newcastle disease, although in specific circumstances such tests may have a useful role.

2. Detection of antibodies in unvaccinated birds

The majority of laboratories involved in Newcastle disease diagnosis are familiar with the hemagglutination inhibition test and the recommendation described below relate to this test for the measurement of antibodies to the virus. However, enzyme-linked immunosorbent assays (Elisa) may be successfully used to detect antibodies to the virus. It is suggested that if there is a wish to employ an Elisa test at regional laboratory level the test should be monitored by the national laboratory referred to in Schedule II.

(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 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 Newcastle disease virus hemagglutinating antigen in standard hemagglutination inhibition tests as defined in Chapter 6.

There is some debate as to whether 4 or 8 haemagglutinin units should be used for the HI test. 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 24 or greater and for 8 HAU a positive serum is any showing a titre of 23 or greater.

CHAPTER 5

Hemagglutination (HA) test

Reagents

1. Isotonic saline buffered with phosphate (PBS) (0,05 M) 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 NDV antibodies) into an equal volume of Alsever's solution. Cells should be washed three times in PBS before use. For the test a 1% suspension (packed cell v/v) in PBS is recommended.

3. NDV strain Ulster 2C is recommended for use as standard antigen.

Procedure

(a) Dispense 0,025 ml PBS into each well of a plastic microtitre plate (V-bottomed wells should be used).

(b) Place 0,025ml of virus suspension (i.e. allantoic fluid) in the first well.

(c) Use a microtitration diluter to make two-fold dilutions (1:2 to 1:4096) of virus across the plate.

(d) Dispense a further 0,025 ml of PBS to each well.

(e) Add 0,025ml of 1% red blood cells to each well.

(f) Mix by tapping gently and place at 4°C.

(g) Plates are read 30 - 40 minutes later when controls 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.

(h) 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 hemagglutination inhibition tests (see Chapter 6).

CHAPTER 6

Hemagglutination inhibition (HI) test

Reagents (see Chapter 5)

(a) Phosphate buffered saline (PBS)

(b) Virus-containing allantoic fluid diluted with PBS to contain 4 or 8 HAU per 0,025ml.

(c) 1% chicken RBCs.

(d) Negative control chicken serum.

(e) Positive control serum.

Procedure

(a) Dispense 0,025ml PBS into all wells of a plastic microtitre plate (with V-bonomed wells).

(b) Place 0,025ml of serum into first well of plate.

(c) Use microtitration diluter to make two-fold dilutions of serum across plate.

(d) Add 0,025ml of diluted allantoic fluid containing 4 or 8 HAU.

(e) Mix by tapping and place plate at 4°C for a minimum of 60 minutes or room temperature for a minimum of 30 minutes.

(f) Add 0,025ml 1% RBCs to all wells.

(g) Mix by gentle tapping and place at 4°C.

(h) 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 PBCs (0,05 ml) only.

(i) The HI titre is the highest dilution of antiserum causing complete inhibition of 4 or 8 units of virus (an HA titration to confirm the presence of the required HAU should be included in each test).

(j) The validity of the results is dependent on obtaining a titre of less

than 23 for 4 HAU or 22 for 8 HAU with the negative control serum and a titre of within one dilution of the known titre of the positive control serum.

CHAPTER 7

Intracerebral pathogenicity index test

1. Infective freshly harvested allantoic fluid (HA titre must be greater than 24 is diluted 1:10 in sterile isotonic saline (anti-bodies must not be used).

2. 0,05 ml of the diluted virus is injected intracerebrally into each of 10 one-day old chicks (i.e. 24 hours; 40 hours after hatching). The chicks should be hatched from eggs obtained from a specific pathogen-free flock.

3. The birds are examined at intervals of 24 hours for eight days.

4. At each observation each bird is scored: 0 = normal; 1 = sick; 2 = dead.

5. The index is calculated as shown in the following example:

Clinical signs	**Day after inoculation (number of birds)**										
	1	2	3	4	5	6	7	8	**Total**		**Score**
normal	10	4	0	0	0	0	0	0	14 X 0	=	0
sick	0	6	10	4	0	0	0	0	20 X 1	=	20
dead	0	0	0	6	10	10	10	10	46 X 2	=	92
									Total	=	112

Index is a mean score per bird per observation = 112/80 = 1,4

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.

2. Confluent monolayers of chick embryo cells or a suitable cell line (Madin-Darby bovine kidney for example) are prepared in 5cm 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 0,01mg/ml trypsin or no trypsin. It is important that no serum is added to the overlay

medium.

5. After 72 hours, incubation at 37°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.

SCHEDULE IV

LIST OF NATIONAL NEWCASTLE DISEASE LABORATORIES

Malta	National Veterinary Laboratory Abattoir Square Albertown, Marsa Malta MRS 1123

Ippubblikat mid-Dipartiment tal-Informazzjoni (doi.gov.mt) — Valletta — *Published by the Department of Information (doi.gov.mt) — Valletta*
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