ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLE VENEZIE

EUROPEAN UNION REFERENCE LABORATORY FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE

Istituto Zooprofilattico Sperimentale delle Venezie

GUIDELINES AND MINIMUM REQUIREMENTS FOR DIAGNOSIS OF AVIAN INFLUENZA AND NEWCASTLE DISEASE

1. Clinical signs in birds infected with High Pathogenic Avian Influenza (HPAI) and Velogenic Newcastle disease (vND) virus

Clinical signs are highly variable and influenced by factors such as the virulence of the infecting virus, the species affected, age, sex, concurrent diseases, environment and the immune status of the host against the virus (i.e. efficacy of vaccination, if used, in particular for ND).

Early signs can include inappetence, reduction in water intake and relatively low mortality. However, alternatively, the disease may suddenly appear in a flock and several birds may die either without premonitory signs or with minimal signs of depression, inappetence, ruffled feathers and fever. Generally, the longer the birds survive the more marked are the clinical signs. The timeline for the development of signs depends on the virus, the host, the initial infecting dose and the husbandry system. The virus spreads more slowly in caged layers or outdoor birds rather than in broiler houses.

Unvaccinated hens infected with the HPAI or vND virus may at first lay soft-shelled eggs, but soon stop laying. Sick birds often sit or stand in a semi-comatose state with their heads touching the ground. Combs and wattles are cyanotic and oedematous, and may have petechial or ecchymotic haemorrhages at their tips. Profuse diarrhoea is frequently present and birds are excessively thirsty. Respiration may be laboured and excessive lacrimation may be seen. Haemorrhages may be seen on unfeathered areas of skin. The flock mortality rates vary from 5 to 100%.

In gallinaceous birds, the signs of HPAI and vND usually include severe depression, inappetence, and a very marked increase in mortality may be the first abnormality observed. Oedema of the face and neck and neurological signs such as torticollis and ataxia may also be seen.

Ducks and geese may show no clinical signs when infected with HPAI or vND viruses, but some strains have been reported to induce signs in geese infected with the HPAI virus including the signs of depression, inappetence and diarrhoea. Younger birds may exhibit neurological signs.

Generally, infection spreads more slowly in a collection of captive birds due to the variety of different species kept, with different susceptibilities, inconsistent levels of virus shedding and often relatively slow transmission due to a low contact rate and relatively low stocking densities.

2. Post-mortem lesions in birds infected with HPAI and vND virus

Birds that die peracutely may show minimal gross lesions, consisting of dehydration and congestion of viscera and muscles.

In birds that die after a prolonged clinical course, petechial and ecchymotic haemorrhages occur throughout the body, particularly in the larynx, trachea, proventriculus, cecal tonsils and epicardial fat, and on serosal surfaces adjacent to the sternum. There is extensive subcutaneous oedema, particularly around the head and hocks. The carcase may be dehydrated. Yellow or grey necrotic foci may be present in the spleen, liver, kidneys and lungs. The air sac may contain an exudate. The spleen may be enlarged and haemorrhagic.

AI is characterised histologically by vascular disturbances leading to oedema, haemorrhages and perivascular cuffing, especially in the myocardium, spleen, lungs, brain, pancreas and wattles. Necrotic foci are present in the lungs, liver and kidneys. Gliosis, vascular proliferation and neuronal degeneration may be present in the brain.

3. Differential diagnosis

HPAI and ND caused by vND viruses may often have a similar clinical picture. The following diseases, in particular, must be considered in the differential diagnosis:

(a) other diseases causing sudden high mortality such as:
  (i) infectious laryngotracheitis;
  (ii) duck plague;
  (iii) acute poisonings;

(b) other diseases causing swelling of the combs and wattles, such as:
  (i) acute fowl cholera and other septicaemic diseases;
  (ii) bacterial cellulitis of the comb and wattles.

Clinical signs in birds infected with Low Pathogenic Avian Influenza (LPAI) viruses or mesogenic Avian Orthoavulaviruses (mAOAVs 1)

The severity of the disease produced by LPAI viruses or mAOAVs 1 is greatly influenced by:

(a) the strain of the virus;
(b) the species and age of the host;
(c) the immune status of the host against the virus
(d) the presence of other infectious agents, such as:
  (i) Pasteurella spp.;
  (ii) live vaccine strains;
  (iii) avian pneumovirus, infectious bronchitis virus;
  (iv) E. coli;
  (v) Mycoplasma spp.;
(e) immunodeficiency conditions;
(f) environmental factors (such as excess ammonia, dust, hot or cold temperatures).

If on one hand the clinical signs of the disease may be inapparent or slight, producing only mild respiratory signs or egg production problems in laying birds, on the other infections with LPAI or mAOAVs 1 viruses may be associated with severe clinical signs of the disease, especially in gallinaceous birds, usually with rales, coughing, swelling of the infraorbital sinuses, diarrhoea and a febrile condition associated with loss of

appetite and high mortality. Pigeon variant of NDv causes severe disease and high mortality in Columbiformes.

LPAI and ND caused by mAOAVs 1 may be confused with, or complicated by, many of the diseases with respiratory or enteric signs. AI and ND must be suspected in any disease outbreak in poultry that persists despite the application of preventive and therapeutic measures for other diseases.

4. Clinical signs in wild birds

LPAIv and NDv infections are generally subclinical in wild birds. H5 HPAIVs belonging to the Gs/Gd (i.e. Goose/Guangdong/1/96) lineage can induce a range of clinical outcomes from inapparent to severe/fatal disease resulting in high mortality; infection in susceptible wild bird species causes prominent respiratory and neurological signs, including circling, ataxia and torticollis. The pigeon variant of NDV can cause severe disease in feral pigeons and doves characterized by nervous signs, persistent diarrhea and mortality.

5. Guidelines to be considered in case of suspicion of AI and ND on a holding

The variability of clinical signs for both AI and ND means that clear-cut guidance for a suspected outbreak is not possible. A sudden, high mortality in poultry with or without any of the associated clinical signs earlier described must be investigated by submitting samples for laboratory tests; however, in the absence of high mortality, it is more difficult to suspect or exclude the presence of AI or ND.

Since rapid diagnosis is of paramount importance in their early control and eradication, AI and ND must always be considered in the differential diagnosis of respiratory problems, egg production problems and elevated mortality in poultry. The appropriate samples must be submitted for laboratory investigation.

6. General procedures for the collection and transport of samples

Whenever the official veterinarian has a clinical suspicion of an outbreak of AI or ND or in case results of any laboratory test for one of these diseases are not negative, the competent authority must ensure that an investigation is performed and satisfactorily completed before the presence of the disease is excluded.

7. Laboratory protocol and interpretation of diagnostic testing

The competent authority may consider that the presence of AI and ND viruses can be excluded when an appropriate number of sick or dead birds and tracheal/oropharyngeal or cloacal swabs and/or sera have been submitted for laboratory tests, in accordance with the indication reported in this document, resulting negative by using one of the appropriate detection methods authorized by the competent authority and recommended by the EURL for AI and ND for the detection of that viruses, their genome or specific antibodies against AI or ND viruses.

8. Standard set of samples for virological or serological laboratory testing

Whenever an official veterinarian inspects a holding where an outbreak is suspected, the following measures must be carried out:

(a) A check of the production and health records of the holding, if such records exist. The daily mortality and egg-production data, as well as feed and/or water intake for the period preceding of one week the onset of clinical signs of AI until the date of the inspection of the holding by the official veterinarian, must be documented in the inspection-report by the official veterinarian.

(b) A clinical inspection in each production unit, including an evaluation of its clinical history and clinical examinations of poultry or other captive birds, in particular those that appear sick.

(c) Unless the competent authority is satisfied that a suspected outbreak may be excluded based on the clinical inspection in accordance with points (a) and (b), standard samples must be taken from each production unit.

(d) Regardless of the negative results to testing standard samples and subject to local factors, a clinical inspection of the poultry in each production unit must be carried out before the official surveillance may be lifted.

(e) Additional measures based on the epidemiological inquiry and standard samples must be taken from poultry or other captive birds that are killed in each production unit of holdings connected with the suspected outbreak.

To investigate a holding suspected of being infected with AI or ND virus, the standard set of samples for virological or serological testing as referred to in points (a) and (b) (the standard samples), must be taken from each production unit (e.g. each shed in a holding) and submitted directly for virological and serological laboratory tests.

(a) The standard set of samples for virological testing is:

(i) at least five sick/dead birds, if present; and/or
(ii) at least 20 tracheal/oropharyngeal and 20 cloacal swabs.

Carcases of recently dead birds or of severely sick or moribund individuals that are humanely killed must be collected.

Swabs must be taken from the number of birds referred to in point (a) or from all birds on the suspected holding where a smaller number of birds is present. Birds showing clinical signs must be targeted for sampling.

Cloacal swabs must be coated in faeces (optimum 1 g). If for any reason taking cloacal swabs from live birds is impracticable, carefully collected fresh faeces samples may serve as an alternative.

Frequently, it is most practical to collect tracheal/oropharyngeal swabs from the buccal cavity.

As soon as the growth characteristics of the virus are known, the competent authority may decide to choose either tracheal/oropharyngeal or cloacal swabs rather than to collect both, depending on whether the virus replicates better in the respiratory or gastrointestinal tract and also taking into account the species concerned.

(b) The standard set of samples for serological testing is a minimum of 20 blood samples

Samples must be taken from the number of birds referred to in point (b) or from all the birds in the holding, where a smaller number of birds are present. Birds appearing sick or that have apparently recovered must be targeted for sampling.

The competent authority may decide that the full range of standard samples do not need to be taken, but that a subset of the standard samples may be collected instead.

9. Sample processing

If submitted 'dry', swabs must be placed in sufficient medium to ensure full immersion. Samples may be pooled in batches of five when deriving from the same species, time and epidemiological unit. Pool of ten samples may be used in case a high number of samples must be examined over a restricted period of time (e.g. emergency surveillance in DPPA).

Carcases submitted to the laboratory must undergo post mortem examination and samples of the following organs must be taken: faeces or intestinal contents, brain tissue, trachea, lungs, liver, spleen and other obviously affected organs. Such organs and tissues may be pooled, but separate treatment of faecal material is essential.

Faeces samples and organs must be homogenised (in an enclosed blender or using a pestle and mortar and sterile sand) and made to 10-20 % w/v suspensions in the medium best suited to the test that needs to be performed.

Detailed procedures for sample preparation according to the diagnostic methods to be applied are extensively described in the SOPs available at https://www.izsvenezie.com/reference-laboratories/avian-influenza-newcastle-disease/diagnostic-protocols/

10. Transport of samples

Specific care must be taken for the storage and transport of samples to the laboratory for testing.

The swabs must be chilled immediately on ice or with frozen gel packs and submitted to the laboratory as quickly as possible. The samples must not be frozen unless absolutely necessary. If rapid transport within 24 hours to the laboratory is not guaranteed, the samples must be immediately frozen, stored and then transported on dry ice.

In addition and not as an alternative to chilling, the swabs must be placed and fully immersed in an antibiotic or specific virus transport medium at 4°C. In the absence of such medium, swabs must be returned to their casing and submitted dry to the laboratory for testing.

A variety of factors may affect the storage and transport of samples, so the method selected for transport has to be fit for purpose.