



TECHNICAL SPECIFICATIONS ON AVIAN INFLUENZA (AI)

Epidemiological status

- Country free of highly pathogenic Avian Influenza in commercial farmed poultry.
- Highly pathogenic influenza A virus infection in wild and backyard birds - disease limited to certain zones, as of 15 May 2023.
- Highly pathogenic Avian Influenza virus infection in wild birds - disease limited to certain zones.

Reference documents

- [SDA Normative Instruction no. 17, dated April 7, 2006 amended by SDA Ordinance \(Portaria\) no. 275, dated April 16, 2021;](#)
- [SDA Normative Instruction no. 32, dated May 13, 2002;](#)
- [Contingency Plan for Zoonosantary Emergencies - General Part - version 1.0-June -2023](#)
- Contingency plan for highly pathogenic Avian Influenza and Newcastle Disease - Specific part - version 1.0 June - 2023;
- [Manual for the Taking, Storing and Shipping Samples – PNSA – 1st Edition – 2020;](#)
- Letter (Ofício) no. 3/2021/DSA/DIPOA/SDA/MAPA;
- [Surveillance Plan for Avian Influenza and Newcastle Disease - Version of July 2022](#)

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AGENT

Avian Influenza Virus A

The subtypes of the Influenza A virus are identified based on the surface proteins, there being 16 hemagglutinin subtypes (H) and 9 neuraminidase (N) subtypes. According to the pathogenicity index, they are classified as Highly Pathogenic Avian Influenza (HPAI) or Low Pathogenic Avian Influenza (LPAI). Subtypes H5 and H7 have been identified so far as responsible for the infections of HPAI. Most isolates of H5 and H7 and all other subtypes are characterized as low pathogenicity

SUSCEPTIBLE SPECIES

Most poultry and wild birds, particularly the aquatic birds (major reservoirs)

CLINICAL SIGNS AND LESIONS

The signs and lesions may vary a great deal, depending on the susceptible species, the strain and pathogenicity of the virus, of the immune status of the birds, of the presence of secondary infections and of the environmental conditions.

Highly Pathogenic Avian Influenza (HPAI):

High and sudden mortality rate, without the manifestation of clinical signs; or severe disease, with intense depression and respiratory and neurologic signs; cyanosis and necrotic spots on the comb and wattles, drop in egg production, production of deformed eggs, with thin shells or no pigmentation. In the post-mortem examination, edemas, congestion, hemorrhages, and necrosis in several inner organs and skin.

Low Pathogenic Avian Influenza (LPAI)

Most LPAI viruses are kept asymptomatic in wild birds.

In domestic poultry, the signs of the IABP may be absent or mild, including respiratory signs (snicking, coughing, nasal and ocular discharges), diarrhea, lethargy, oedema of the face, and also drop in production, reduction in water and feed intake. In the post mortem examination, rhinitis, sinusitis, trachea congestion, hemorrhage in the reproductive tract of layers, airsacculitis, and peritonitis may be found.

SURVEILLANCE

The target diseases of the surveillance for the Respiratory and Nervous Syndrome in Poultry (NRS) are Avian Influenza (AI) and Newcastle Disease (ND).

Objectives:

- Early detection of outbreaks of AI and ND in the populations of domestic and wild birds
- Demonstration of the absence of AI and ND in industrial poultry production.
- To monitor the occurrence of viral strains of AI to underpin the strategies for public health and animal health.

Target population: commercial production poultry*, backyard poultry, exhibition, ornamental, companion, and wild birds (free living or captive).

**commercial production poultry: all birds farmed to produce any commercial products (meat, eggs, among others) or for reproduction for these purposes.*

TRANSMISSION

Direct contact between birds (nasal and ocular discharges, and feces of infected birds).

Indirect contact (water, food, fomites, transit of people, pieces of equipment, materials, vehicles, clothing, products, insects, rodents and other pests, litter, feces and contaminated carcasses).

Reservoirs: wild birds, mainly aquatic birds.

Incubation period: the period of incubation period for HPAI depends on the infectious dose, exposure pathway, affected species, and capacity for sign detection, and may vary from some hours to 14 days.

It is a zoonotic disease of great interest for public health, mainly transmitted by direct contact with infected birds. Most low pathogenicity strains cause mild manifestations in humans. However, since 2013, it was identified that a low pathogenicity lineage (H7N9) detected in China causes severe cases in humans.

NOTIFICATION CRITERION

Immediate notification to the Official Veterinary Service (OVS) of any suspected Case of AI (category 1 of the list of diseases in the Appendix of Normative Instruction no. 50/2013).

DIFFERENTIAL DIAGNOSIS

Compatible clinical signs may also be present in other diseases such as Newcastle Disease (ND), Avian Infectious Laryngotracheitis (ILT), infectious bronchitis, encephalomyelitis, Gumboro disease, poisoning, duck viral hepatitis, fowl cholera (acute form).

LABORATORY DIAGNOSIS

- Isolation and identification of the virus and subtype of AI.
- Detection of the antigen to, or of specific ribonucleic acid (RNA) for, AI.
- Determination of the intravenous pathogenicity index (IVPI).
- Genetic sequencing (characterization of multiple cleavage site base amino acids).

DEFINITION OF CASE

In Brazil, **all types of birds described in the target population** (commercial broilers, backyard poultry, exhibition, ornamental, companion and wild birds) are part of the surveillance for NRS. Therefore, the suspect cases shall be immediately notified to the Official Veterinary Service, and the probable cases shall be subjected to laboratory diagnosis, according to the criteria defined below.

Suspected case of SRN: identification of at least one of the following criteria:

1. mortality greater than or equal to 10% within 72 hours in any breeding establishments of farmed poultry or in a single poultry house of the poultry commercial or breeding establishments; or
2. exceptional mortality (sudden and high) in populations of backyard, exhibition, ornamental, companion or wild birds; or
3. abnormal behaviors in wild bird populations, mainly in migratory waterfowl;
4. presence of clinical signs or lesions** (neurological, respiratory or digestive) compatible with ARNS in any types of birds; or
5. sudden drop equal to or greater than 10% in egg production and an increase in malformed eggs in breeding or hatching birds; or
6. positive result in serological test or nucleic acid detection (PCR) in any types of birds.

Suspected cases of respiratory and nervous syndromes in slaughterhouses: identify birds showing clinical signs or lesions (neurological, respiratory or digestive), or the presence of dying or dead birds at the live-bird receiving platform, compatible with RNS. Other criteria for notifying suspected cases (1 to 6) do not apply to slaughterhouses

Probable case of RNS: any suspected case that meets at least one of the following criteria:

1. Increased rates of mortality without proof of the occurrence of a non-infectious insult***; or
2. Presence of birds showing neurological signs compatible with RNS; or
3. The association of two or more criteria of suspected cases; or
4. a positive test result for the detection of nucleic acid (PCR) of the agent in approved laboratories; or
5. an epidemiological link to a confirmed case or signs of probable exposure to the agent.

****lesions:** to identify the presence of lesions of RNS, and the Official Veterinarian must necropsy the birds with clinical signs or recently dead.

*****Non-infectious issues:** this involves external factors such as an electrical blackout, equipment failure, weather-related events, damage to facilities, handling error, or others.

Confirmed case of HPAI: Isolation and identification of the agent, or detection of the specific viral RNA of any Influenza A virus characterized as being highly pathogenic****, according to [Chapter 3.3.4 of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Animal Health Organization \(WOAH\)](#).

NOTE: considering the detection of confirmed cases of HPAI in wild birds in the country, as an alternative to sequencing that determines the pathogenicity of the virus, a specific molecular test can be performed (RT-qPCR for detection of the H5 subtype clade 2.3.4.4) for the simultaneous detection of virus and inference of its pathogenicity.

Confirmed case of LPAI: Isolation and identification of the agent, or detection of the specific viral RNA of any Influenza A virus not characterized as being highly pathogenic****.

Outbreak of HPAI/LPAI: epidemiological unit where at least one outbreak of HPAI or LPAI was confirmed, according to the criteria of definition of established outbreaks..

NOTE: in an HPAI outbreak, all birds with compatible clinical signs will be considered confirmed cases.

Ruled-out suspected case: a suspected case notified to the Official Veterinary Service that was not classified by the Official Veterinarian as a probable case of RNS.

Ruled out case of HPAI or LPAI: a probable case investigated by the Official Veterinary Service, whose laboratory test results do not fit the criteria for definition of a confirmed case of HPAI or LPAI.

SAMPLING

Appropriate Personal Protective Equipment must be used.

For laboratory investigation of probable cases, collect swabs from 30 live birds and organ samples from 5 necropsied birds (with clinical signs or lesions compatible with AI and NCD or from birds that have recently died - without evidence of organ autolysis), as detailed below:

- 30 individual trachea swabs divided into 6 pools, each pool with 5 swabs;
- 30 individual vent swabs divided into 6 pools, each pool with 5 swabs;
- 5 samples of digestive system organs (pool of small intestine with pancreas and cecum with cecal tonsils) packed in individual tubes, 1 sample for each bird;
- 5 samples of respiratory system organs (pool of lung and trachea) packed in individual tubes, 1 sample for each bird; and
- 5 samples of nervous system organs (pool of brain and cerebellum) packed in individual tubes, 1 sample for each bird.

The pool must only consist of swabs collected from birds of the same species.

When there are not enough birds for the set of samples determined above, collect the swabs from all existing birds and the organs only from birds with clinical signs, without mixing samples from different species.

Samples intended for virological diagnosis are to be kept refrigerated (at 2 to 8°C) for up to 96h (taking into account the transportation time to the laboratory) or frozen at -80°C or lower temperatures if there is a need to store them for longer than 72h. It is not recommended to keep swabs and organs at -20°C (common/domestic freezer), since the ND and AI viruses are sensitive to this temperature.

Not using natural cotton swabs with wooden rods, that may interfere with the performance of the laboratory tests, and that, for this reason, are ruled out at the laboratory. We recommend the use of swabs with plastic rods, in the following order of performance: flocked nylon, polyurethane, non-flocked polyester. In the event none of these swabs can be used, rayon swabs may be used.

NOTE: once an outbreak of HPAI has been confirmed in wild waterfowl in a municipality, the collection of samples from new probable cases, epidemiologically linked to an existing outbreak, can be disregarded for species that have already had laboratory diagnosis confirmation of the disease. These new cases must be considered by clinical-epidemiological criteria as confirmed cases in an existing outbreak, within 30 days of the last confirmed case.

Means of preservation/transportation:

- Minimum Essential Medium (MEM) , Brain Heart Infusion Broth (BHI) or Buffered Tryptose Phosphate Broth (TPB) containing antibiotics and formulated according to [Manual for the Taking, Storing and Shipping Samples – PNSA](#);
- *UTM – Universal Transport Medium or VTM – Viral Transport Medium.*

For further details, check the following documents: [Manual for the Taking, Storing and Shipping Samples – PNSA – 1st Edition – 2020.](#)

LABORATORY

The Federal Laboratory of Animal and Plant Health LFDA-SP (in the city of Campinas) is the official laboratory for diagnosing probable cases of Respiratory and Nervous Syndrome in poultry (RNS).

APPLICABLE MEASURES

The measures are detailed in the [Contingency Plan for AI and ND.](#)

Applicable measures in the investigation of probable cases of RNS: sampling for laboratory diagnosis, isolating the lots/animals, blocking of the epidemiological unit, tracing of entry and exit, investigation of epidemiological links. Contingent upon OVS evaluation and approval, the lots may be immediately eliminated after samples have been taken for diagnosis, as a preventive measure, and to avoid a possible spread of the agent.

Measures applicable to AI outbreaks: elimination of all susceptible animals in the epidemiological unit, destruction of the carcasses and all products and by-products, and waste from the production system, disinfection, standstill, applying strict biosecurity measures, using sentinel animals, and proving the absence of viral circulation, surveillance within the protection zone and the surveillance zone.

Applicable measures in suspicions found in poultry Slaughterhouses: as laid down in [Joint Circular Letter \(Ofício-Circular Conjunto\) no. 3/2021/DSA/DIPOA/SDA/MAPA.](#)

CONCLUSION OF THE INVESTIGATION

If the suspicions for SRN have been ruled out, the investigation may be closed immediately.

For probable cases of RNS, the investigation may be closed after the final negative diagnosis for AI and ND.

An AI outbreak will only be completed after the susceptible animals in the epidemiological unit have been eliminated, proof of absence of viral transmission, and conclusion of the surveillance procedures in the sanitary emergency zones, according to the [Contingency Plan for AI and ND](#).

Outbreaks with isolated cases of HPAI in wild waterfowl may be closed after eliminating the cases and destroying the carcasses. **In the outbreaks in areas of wild bird flocking, new confirmed cases may be included according to clinical-epidemiological criteria, within 30 days of the last confirmed case.**

NOTIFICATION TO WORLD ORGANISATION FOR ANIMAL HEALTH (WOAH)

The Brazilian Delegate at the WOAH (the Director of the Department of Animal Health) shall immediately notify the WOAH on the infection by the high pathogenicity virus in any type of bird, and also the infection of low pathogenicity avian influenza virus in domestic poultry or wild birds in captivity, when natural transmission to humans associated to severe consequences is proved. However, the occurrence of an infection by highly pathogenic avian influenza A in non-commercial birds, including wild birds or the infection of the low pathogenicity avian influenza virus do not affect the epidemiological status of the highly pathogenic avian influenza in the country