2022

Avian Influenza and Newcastle Disease Surveillance Plan

Department of Animal Health

Agriculture and Livestock Protection Bureau

Ministry of Agriculture, Livestock, and Food Supply

Summary

LIST OF ABBREVIATIONS

NCD: Newcastle Disease

DSA: Department of Animal Health

ELISA: Enzyme-linked immunosorbent assay

e-Sisbravet: Electronic tool of the Brazilian Veterinary Surveillance and Emergency System

AI: Avian influenza

MAPA: Ministry of Agriculture, Livestock, and Food Supply

OV: Official Veterinarian

OESA: Farming Health Enforcement Authority

WOAH: World Organization for Animal Health

PCR: Polymerase chain reaction

PNSA: National Avian Health Program

SDA: Agriculture and Livestock Protection Bureau

SIGEP: Epidemiological Study Management System

SVE: State veterinary service for animal health

OVS: Official Veterinary Service

SRN: Respiratory Nervous Syndrome in Birds

AGID: agar gel immunodiffusion

IAV: influenza A virus

ELISA: enzyme-linked immunosorbent assay

HI: hemagglutination inhibition

ICPI: intracerebral pathogenicity index

IVPI: intravenous pathogenicity index

NDV: Newcastle disease virus

NDV-F: Newcastle disease virus F gene

NDV-M: Newcastle disease virus M gene

NI: neuraminidase inhibition

RT-qPCR: reverse transcriptase reaction followed by real-time polymerase chain reaction

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1. INTRODUCTION

Poultry farming in Brazil began to develop in the 1970s, when the market received companies that specialized in poultry production and processing. Technological transformations, intensive production techniques, and the development of genetic improvements have contributed toward great advances in this business. The birth of the vertical integration system in the country's South leveraged an extraordinary growth in meat production, turning Brazil into the third largest producer and largest exporter on the globe. In that more intensive and vertical partnership model, integrated poultry farmers began to have industry support with regard to the supply of the main inputs for the business, such as animal feed and medications, on top of technical support and replenishment of lots (young chicken). The production is sent to the manufacturing industry, which ensures farmers compensation and a stabilized supply of quality raw materials.

Egg production farming has also evolved largely, and it exceeded 50 billion eggs produced in 2020. The number of egg-laying birds housed in the countryside has been increasing in the last few years, boosted by an increase in the domestic per-capita consumption. The exports of shell eggs and processed byproducts, although still little representative, totaled 11.3 thousand tons in 2021, accounting for a 81% growth over 2020.

The sanitation condition of the national poultry farming is quite favorable, since it is free of Avian Influenza (IA) and the Newcastle Disease (NCD), which are very important diseases economically and are widely distributed around the world. Maintenance of this condition in Brazil provides higher food security to the Brazilian population and a competitive edge for an access to foreign markets. The growing international transit of people, the international trade of animals and products, the intensifying production, and the diversity of wild birds present on different migratory routes are contributing toward increasing risks of introduction and dissemination of these diseases, with social, economic, and environmental costs that can be extremely high. Thus, prevention and surveillance measures are becoming more and more important. In light of these growing risks, it is necessary to provide increasingly robust evidence to certify the health of the animals and products sold and secure a capacity to meet the domestic demand. Such evidence is grounded in the guidelines of the World Organization for Animal Health (WOAH) for the health security of international trade.

AI has an important zoonotic and pandemic potential, in case the infection of birds spills over into the human population and human-to-human transmission is sustained, which justifies surveillance efforts for early detection for the benefit not only of animals, but also of public health. Thus, in addition to the biosecurity procedures, surveillance accounts for one of the primary components of animal health systems and allows for early detection of emerging and re-emerging animal diseases, enabling efficient control and eradication, as well as certification of a disease-free condition, expanding the access of production systems to the domestic and foreign markets. This AI and NCD Surveillance Plan aims to enhance the country's surveillance system, incorporating the recent international concepts in the field, through risk-based surveillance so as to improve its efficacy and efficiency, and contributing toward the protection of public and animal health.

The AI and NCD Surveillance Plan has been developed under the coordination of the Department of Animal Health (DSA), reporting to the Agriculture and Livestock Protection Bureau (SDA) under Brazil's Ministry of Agriculture, Livestock, and Food Supply (MAPA), with cooperation from the Pan American Center for Foot-and-Mouth Disease and in consultation with the Federal Superintendent Offices for Agriculture, Livestock, and Food Supply (SFA), the state farming health authorities (OESA), the federal health authorities (Ministry of Health) and environmental authorities (Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio), and representatives from the concerned private sectors.

2. DESCRIPTION AND EPIDEMIOLOGICAL CONTEXT OF AI AND NCD

2.1. Avian influenza (AI)

AI is caused by the Influenza A virus from the family *Orthomyxoviridae* and genus *Alphainfluenzavirus*, which are the only influenza viruses to affect birds naturally.

The disease is highly contagious and affects several species of domesticated and wild birds and occasionally mammals such as rats, cats, dogs, horses, swine, and man. Water birds are the largest reservoirs of the virus, and most isolates are regarded as low pathogenicity for chicken and turkeys.

Influenza A Virus subtypes are identified based on surface proteins and comprise 18 subtypes of hemagglutinins (H) and 11 subtypes of neuraminidases (N). According to the pathogenicity index, they are classified as Highly Pathogenic Avian Influenza (HPAI) or Low Pathogenic Avian Influenza (LPAI). Only a few H5 and H7 subtypes have been identified as responsible for HPAI infections. Most H5 and H7 and all other subtypes are characterized as low pathogenicity.

The epidemiology of AI is complex, as the viruses are constantly evolving through mutation and rearrangements, giving birth to new subtypes and a possible adaptation to new hosts, causing an impact on animal health and production. On top of this, some AI subtypes have caused zoonotic infections (H5, H7, H9) in some parts of the world and exhibit a pandemic potential in case the mutations allow for sustained transmission among humans.

The signs and injuries in birds can be quite variable, depending on the susceptible species, the virus strain and pathogenicity, the immunization state of the birds, the presence of secondary infections, and the environmental conditions:

- Highly Pathogenic Avian Influenza (HPAI): High rate of sudden deaths without clinical signs; or a severe disease with intense depression and respiratory and neurological signs; cyanosis and necrotic foci on the comb and wattle, in addition to reduced egg laying and production of deformed eggs with a thin or colorless shell. A post-mortem examination can reveal edema, congestion, hemorrhage, and necrosis in several internal organs and skin.
- Low Pathogenic Avian Influenza (LPAI): The vast majority of LPAI viruses are kept asymptomatically in wild birds. In domesticated birds, the signs can be nonexistent or mild, including respiratory signs (sneezes, cough, and nasal and eye discharge), diarrhea, lethargy, and facial edema, on top of reduced production and water and food consumption. A post-mortem examination can reveal rhinitis, sinusitis, trachea congestion, hemorrhage in the reproductive tract of egg-laying hens, airsacculitis, and peritonitis.

In Brazil, any suspected AI case of either high or low pathogenicity is required to be immediately notified to the Official Veterinary Service (OVS) as defined in MAPA Executive Ruling (*Instrução Normativa)* No. 50, dated September 23, 2013. A confirmed focus requires the containment and eradication measures provided in the Contingency Plan for the disease.

The WOAH includes AI in its list of diseases, and all countries must notify an event of infection by the HPAI virus in any bird species and type, including wild birds, or even cases of LPAI in domesticated and captive-bred wild birds when there is a potential of natural transmission to humans associated with serious consequences for public health and animal health.

It is important to highlight that, as set out in the WOAH Terrestrial Animal Code, occurrence and notification of HPAI in subsistence farm birds and in wild birds, or notification of LPAI in domesticated or captive-bred wild birds, including ornamental, display, and companion, wild, synanthropic, or other birds, does not change the country's HPAI sanitation condition. No member country must impose bans on the international trade of poultry commodities in response to such notifications or other information about the presence of any Influenza A virus in birds for which notification is not legally required.

As standardized in the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Brazil's DSA/Mapa uses the following criteria for confirming an AI case, according to agent detection diagnostic tests:

- Highly pathogenic avian influenza (HPAI) - infection of poultry by any influenza A virus that exhibits: a HA0 cleavage site amino acid sequence similar to any of those that have been observed in high-pathogenicity viruses in hens; or an intravenous pathogenicity index (IVPI) higher than 1.2 in 10 hens (aged 4 to 8 weeks) inoculated intravenously; or mortality higher than 75% in 10 days in at least 8 hens (aged 4 to 8 weeks) inoculated intravenously.
- Low pathogenic avian influenza (LPAI) - infection of poultry by any influenza A virus that exhibits: a HA0 cleavage site amino acid sequence that is different from those that have been observed in high-pathogenicity viruses or lower mortality rate and IVPI than the values observed in HPAI.

Currently, the primary factors contributing to avian influenza transmission are the following:

- Migratory/wild birds – Direct exposure to infected wild birds is the primary AI transmission risk factor for domestic poultry, in either commercial or subsistence production. These birds act as a natural host and reservoir of the AI viruses, playing an important role in their evolution, maintenance, and dissemination. These birds can have an infection without falling ill or often recover and keep infecting others, which allows them to carry the virus over long distances across their migration routes. The main wild species involved are usually migratory seabirds, especially from the Anseriformes and Charadriiformes orders.
- Globalization and international trade – The intense flow of people around the world, as well as of goods, considerably increases the risk of disease dissemination, including AI.
- Markets/fairs selling live poultry - They can facilitate a close contact between different bird species and other animals, as well as man, which not only favors transmission but also increases a possibility of genetic recombinations between different Influenza virus subtypes.

Therefore, applying biosecurity measures at poultry establishments aiming to limit the exposure of domestic poultry to wild birds, especially migratory seabirds, is the main risk mitigation measure against the introduction of the AI virus into the national poultry pool and, as a result, to reduce the risk of a mutation to highly pathogenic forms and recombination with components of other influenza viruses to form viruses that may not only infect birds and humans, but also be transmitted among humans.

Although never detected in Brazil, HPAI is a globally distributed disease with pandemic cycles and a steady growth in the number of affected countries and circulating subtypes in the last few years, with serious consequences for the international trade of poultry products. From 2005 to 2022, 76 countries have notified HPAI.

The global situation of AI is continuously monitored by the WOAH (https://www.WOAH.int/en/disease/avian-influenza/) and by FAO (https://empres-i.apps.fao.org/diseases).

Figure 1. World areas with high-pathogenicity avian influenza occurrences (red) from 2005 to 2022, according to WAHIS/WOAH.

Figure 2. Global distribution of high-pathogenicity avian influenza as observed from July 1, 2021 to July 4, 2022, according to EMPRES/FAO (https://empres-i.apps.fao.org/diseases).

2.2. Newcastle Disease (NCD)

NCD is a highly contagious, frequently serious disease caused by virulent strains of a virus of the Paramyxovirus family (APMV-1), found all over the world and affecting more than 200 bird species. Its onset may take different forms of severity according to the virus strain and the host species:

- Viscerotropic velogenic - highly pathogenic with a frequent onset of hemorrhagic intestine injuries; with a severe disease and high mortality in chicken, with sudden death, apathy, inappetence, conjunctival hyperemia, respiratory signs, cyanosis, greenish disease, reduced egg laying, and anomalous eggs.
- Neutrotropic velogenic - causes high mortality and is usually associated with respiratory and neurological signs, such as: sneezes, nasal discharge, noisy breathing, swollen head and face, weakness, torticollis, leg palsy, and muscle tremors, with elevated mortality (as high as 100% of non-vaccinated birds). Birds with sudden death or neurological signs exhibit little or no macroscopic injury. Characteristics of the injuries caused by velogenic strains that occur mostly in chicken/hens: Edema in the head and periorbital area and neck; congestion and hemorrhages in the tracheal mucosa and pharynx; diphtheric membranes in the oropharynx, trachea, and esophagus; petechiae and ecchymoses in the proventriculus, hemorrhagic injuries, ulcers, and/or necrosis in the cecal tonsils and lymphoid tissues of the intestinal wall (Peyer's patches); enlarged friable spleen; pancreatic necrosis and lung edema; swollen or reduced and hemorrhagic ovaries.
- Mesogenic - usually causes low mortality (< 10%); more common in young birds; associated with mild respiratory signs, reduced egg laying, and occasionally the onset of neurological signs. Signs are more severe when co-infections are present.
- Lentogenic or respiratory - respiratory infection with mild or subclinical signs in young birds;
- Subclinical or enteric - enteric infection, which is usually subclinical.

The velogenic strains of APMV-1 are endemic to a large part of Asia, Africa, Middle East, and some Central America and South America countries. Lentogenic isolates occur in domestic and wild birds all over the world, causing however few outbreaks. A large deal of the infected birds exhibit few clinical signs or are even asymptomatic, which helps the virus be maintained and released into the environment. In addition, these strains can go through mutations and become highly virulent.

Although rare, the infection of humans is possible and usually mild, causing conjunctivitis in people in direct contact with infected birds. There have been no reports of infection due to the consumption of poultry products.

APMV-1 has one variant, which is the Pigeon Paramyxovirus - serotype 1 (PPMV-1), the natural hosts of which are the Columbiformes and may occasionally infect domestic and wild birds. In pigeons, PPMV- 1 causes mortality ranging from 10 to 100% with clinical signs of depression, diarrhea, torticollis, ataxia, and neurological signs. Domestic and wild birds may exhibit clinical signs compatible with an APMV-1 infection.

The NCD virus infection is part of the WOAH's list of diseases and any suspected NCD is required to be immediately notified in Brazil, as set forth in MAPA Executive Ruling No. 50/2013. To the WOAH, NCD foci in domestic poultry need to be notified. A confirmed focus in Brazil requires the containment and eradication measures provided in the Contingency Plan for the disease.

As standardized in the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Brazil's DSA/Mapa employs as an NCD case confirmation criterion the isolation and identification of the agent or detection of the specific APMV-1 viral RNA that is characterized as high pathogenicity (intracerebral pathogenicity index 9 – ICPI in one-day-old chicks higher than or equal to 0.7 or with a pattern typical of residues of multiple basic cleavage site amino acids, as demonstrated through molecular sequencing) in domestic birds. A confirmed infection with the same virus in other types of birds is called an APMV-1 infection.

NCD is regarded as one of the most important poultry diseases in the world. Outbreaks can have a considerable impact on developing countries, where these birds are a significant source of protein. In developed countries where highly virulent APMV-1 strains have been eradicated, outbreaks cause serious economic losses due to trade bans and restrictions, on top of the costs with containing and eliminating the foci.

In Brazil, the country's industrial poultry farming is regarded as NCD-free. The last confirmed cases took place in 2006 in subsistence farming poultry in the states of Amazonas, Mato Grosso, and Rio Grande do Sul. NCD vaccination is mandatory for poultry establishments engaged in commercial reproduction and egg laying.

Figure 3. World areas with Newcastle Disease (NCD) occurrences from 2005 to 2021, according to WAHIS/WOAH.

3. JUSTIFICATIONS

AI and NCD surveillance aims to prevent infection and maintain the disease-free situation both for purposes of poultry production and trade security and for protection of public health and food security. The high costs to control the disease foci and especially the trade restrictions as a consequence of both diseases lead to several economic losses, both on a local level, with poultry production pools being destroyed and an interruption of several production stages, and on a national level, with losses in markets, revenues, and jobs, and a potential risk for human health, in the case of AI.

A quick detection of AI or NCD cases is key to successful actions for a response to emergencies, focus control and eradication, and a quick recovery of the public health condition, and demonstrating a disease-free condition is an important requisite for a guaranteed access to and maintenance of the international trade of poultry products.

In addition, AI has a potential to cause a serious disease in humans, and the direct exposure to infected domestic poultry is the main transmission pathway, so AI surveillance plays a relevant role in the context of the “One Health” approach. In this regard, implementing an efficient surveillance system is essential to support risk analysis and for an assessment and review of the prevention and eradication strategies against diseases subject to control by the country’s official animal health service.

Passive surveillance is the most adequate strategy for an early detection and must be based on mandatory and immediate notification of suspected cases for an investigation by the Official Animal Health Service, with the necessary actions being taken to confirm the focus and apply the measures provided in the Contingency Plan to contain and eradicate the disease and restore the disease-free condition.

Active surveillance is an important strategy to prove the absence of viral HPAI and NCD circulation in domestic production poultry, apply for international trade certification of poultry products and genetic material, or identify LPAI circulation, with aims to contain its spread and prevent evolution into high-pathogenicity strains. Moreover, active surveillance is important to monitoring infection in migratory wild birds, with aims to direct the risk mitigation actions and prevent its introduction into domestic birds.

Thus, the DSA has reviewed the current components of the AI and NCD surveillance system and developed a Surveillance Plan aiming to strengthen prevention and response to the emergencies for these diseases, on top of optimizing the use of employed resources, for the primary purpose of protecting the domestic poultry production and economy against said diseases and their economic, social, and public health impacts, as well as ensuring certification for market accesses.

This Plan is considering a scenario where there are no cases in Brazil as well as the disease characteristics to demonstrate the disease-free condition. If there is any relevant change to the epidemiological situation of these diseases in the country or area, the sample components and design must be adjusted to the new reality.

4. OBJECTIVES OF THE SURVEILLANCE PLAN

AI and NCD surveillance in Brazil has the following objectives:

Objective 1: early detection of AI and NCD cases in the populations of domestic and wild birds

It is the primary objective of surveillance for absent (eradicated or exotic) diseases in the country and allows for immediate reaction, eradication, and restoration of the disease-free condition. Early detection of suspected AI and NCD cases is only possible by strengthening passive surveillance, which is based on mandatory immediate notification of suspected cases for a quick investigation by the official animal health service, which must coordinate the application of the diagnosis, containment, and eradication actions provided in the Contingency Plans.

Objective 2: demonstrating the absence of AI and NCD in industrial poultry farming according to international trade-related surveillance guidelines.

Demonstrating the absence of AI and NCD through an active surveillance system provides support on and allows for certification of a disease-free health condition of the populations targeted by surveillance toward the WOAH and business partners.

This objective is met especially through the components of active surveillance, with epidemiological studies being conducted on the target populations selected according to risk and production and trade impact criteria.

The proposed sampling design allows us to identify whether or not there has been a prior exposure to LPAI or a viral transmission of lentogenic NCD or LPAI strains. These data, associated with the results from the suspected case investigation, allow us to provide assurances of certification for the international trade of poultry and poultry products.

Objective 3: monitoring the occurrence of viral AI strains to support public health and animal health strategies

Surveillance directed at certain AI target populations allows for an identification and monitoring of the circulation of virus strains that can be introduced in the country by moving migratory birds, making it possible to contain the dissemination among production poultry, prevent LPAI virus mutations to HPAI, and protect public health with the current “One Health” focus.

5. EXPECTED RESULTS AND PRODUCTS

The actions provided for in this Plan are contingent upon satisfaction of the objectives described in item 4.

All the collected data must be recorded in the specific systems for passive and active surveillance (e-SISBRAVET e SIGEP or another system defined by MAPA).

The results will be presented in the form of:

- Records and databases of the corresponding information systems used for surveillance management;
- Annual report of the surveillance system; and
- Analyses of the system components and evaluation of surveillance indicators.

The resulting information will be used by the system managers to make decisions and for specific actions such as:

- Immediately responding to and investigating within a maximum of 12 hours any suspected cases of SRN notified to the official animal health service in order to either rule out or confirm the target diseases;
- Evaluating the execution performance of passive surveillance to instruct the procedures during the investigations;
- Activating the quick-response systems in case a disease focus is confirmed;
- Adjusting the detection and response capabilities based on the results of surveillance indicators in view of an indicated potential increase in the risk to the susceptible population;
- Supporting the assurances of certifications and trade negotiations requiring evidence of the disease-free condition; and
- Reviewing strategies and procedures upon their detection.

The AI and NCD surveillance plan must be assessed in terms of parameters and structure following the first year, and once every 3 years thereafter. Updates may be done following changes to risk assessments or when the DSA deems them necessary.

Significant changes to risk factors that increase the probability that AI or NCD will be introduced into the country must lead to a review of this Plan, even when outside the established frequency.

6. STAKEHOLDERS

All those involved in bird breeding, handling, transportation, production, surveillance, inspection, diagnosis, teaching, research, and care, among other activities, are regarded as stakeholders in the AI and NCD Surveillance Plan and have responsibilities for performing parts of the surveillance tasks.

The main stakeholders of the Plan are the members of the OVS, which in Brazil is comprised of the departments of the government institutions responsible for regulating, planning, coordinating, executing, and assessing the procedures relating to animal health surveillance, animal product inspection, analysis at federal farming protection laboratories, and international farming surveillance, with MAPA representing the central and top level of the Universal Agriculture and Livestock Health Care System - SUASA and the OESA representing the intermediate and local levels of the official veterinary service in the states.

Farmers and players from the agricultural industries also have an important participation, and so do service providers and input suppliers, professionals, and institutions involved with wild birds. Table 1 shows the responsibilities of the segments involved or interested in the Plan.

Table 1. Responsibilities of stakeholder segments of the AI and NCD Surveillance Plan.

Stakeholders	Description	Responsibility
Official Animal Health Service	Veterinarians and assistant staff under the authority of the Official Animal Health Service of MAPA and SVE	Standardizing, managing, maintaining the database, performing analyses and disclosing information, investigating suspected cases, collecting samples, promoting awareness, training, education, communication, and funding
Farmers/Companies/Cooperatives	Owners of production poultry	Notifying suspected cases; employing good production practices with a commitment to being able to recognize the diseases in question, taking biosecurity measures, and recording the activities; funding
LFDA	Federal Farming Protection Laboratories	Acting as a reference for diagnostic screening and confirmation testing
Embrapa and other research institutions	Veterinarians, biologists, animal science technicians, agronomists, and assistants involved in research	Conducting research; notifying suspected cases; spreading information; training
Environment authorities	Veterinarians, biologists, and assistants involved in environmental management and conservation	Conducting research; notifying suspected cases; providing information; spreading information; collecting samples
Industry	Agro-industrial segment of poultry products and suppliers of farming inputs	Notifying suspected cases; spreading information; providing direct surveillance information; funding, biosecurity
Accredited laboratories	Public or private laboratories accredited by MAPA to conduct screening tests	Notifying suspected cases; spreading information; conducting screening tests
Private laboratories	Private laboratories that conduct diagnostic testing for production system diseases	Notifying suspected cases; spreading information; sending samples received from suspected or probable cases
Authorized Veterinarians	Private veterinarians with an assignment from the Official Animal Health Service to carry out a specific action	Notifying suspected cases; collecting samples; generating relevant information (reports); biosecurity; spreading information; awareness-training-education

Service providers	One-time or permanent services: advice, clinical care, farming product dealers, vaccination professionals, dead bird collection	Notifying suspected cases; spreading information; biosecurity
Veterinarians and professionals of the environmental services	Veterinarians, biologists, animal science technicians, and other professionals engaged in the field of environmental conservation and management of preservation areas	Conducting research; notifying suspected cases; collecting samples; spreading information; biosecurity
ABPA	Brazilian Animal Protein Association	Spreading information; funding
CNA	Brazilian Confederation for Agriculture and Livestock Farming	Spreading information
AVAL	Brazilian Alternative Poultry Production Association	Spreading information
Animal health protection funds	Private funds established for the purpose of raising funds for indemnities in support of farming protection actions	Spreading information; funding
Rural extension	Veterinarians and assistants	Notifying suspected cases; spreading information; biosecurity; animal health education
Education institutions	Veterinarians, animal science technicians, agronomists and assistants	Notifying suspected cases; spreading information; conducting research and training
Poultry transporters	Professionals who transport poultry between properties and up to the slaughterhouse	Notifying suspected cases; spreading information; biosecurity
International farming surveillance	Veterinarians and assistant staff associated with surveillance for the international transit of animals and goods	Notifying suspected cases; spreading information; biosecurity

7. TARGET POPULATION DESCRIPTION

The AI and NCD Surveillance Plan covers the populations of the various poultry production systems in the country, including wild birds, in which the disease impacts would be more significant and for which the risks of occurrence of the target diseases are higher. Described below are the main categories or target subpopulations in the AI and NCD Surveillance Plan.

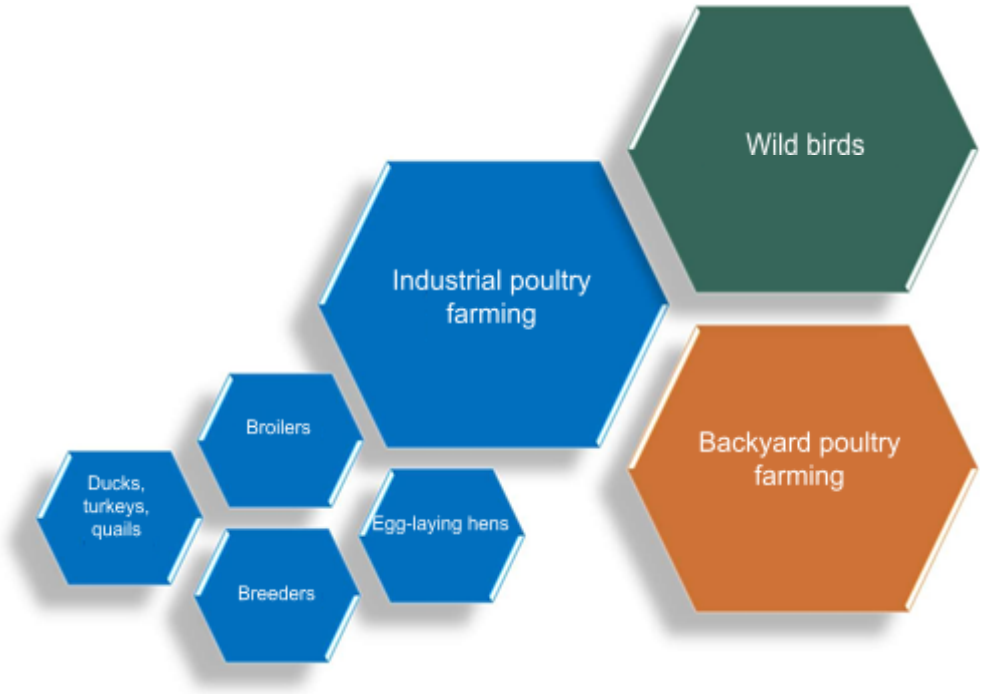

Figure 4. Categories comprising the target population of the AI and NCD Surveillance Plan

7.1. Industrial poultry farming

According to data from MAPA, in Brazil, 53.7% of the poultry establishments are for meat poultry (chicken and turkeys), 39.8% for breeder birds, 3.4% for commercial egg-laying birds, 0.1% for ornamental birds, and 2.9% for other birds.

7.1.1. Poultry breeding

Poultry breeding encompasses the genetic material of the whole production system and consists in pure-line birds or cross-bred birds for meat or egg production. The increase in lineage performance has been sharp in the last decades due to genetic improvement programs, which has provided expressive productivity gains.

In these bird pools, genetic selection is performed, which is a highly technological process limited to few multinational companies, located mainly in the USA, Canada, France, United Kingdom, the Netherlands, and Germany. These poultry farms breed the pure lines and great grandparents whose eggs and day-old chicks are exported to Brazil for production of great grandparent and grandparent birds, respectively.

Great grandparent chicken farms produce grandparent birds. Grandparent chicken farms produce parent stock. Parent stock farms, in their turn, have as their product the hybrids obtained from crossbreeding between parents, which are sold as day-old chicks and will become broilers or egg-laying hens. Thus, broilers and egg-laying hens are hybrids produced from the crossbreeding of three to four lines. Therefore, protecting this part of the production chain is strategic and key to domestic production. In Brazil, breeding farms have high levels of biosecurity and health monitoring, precisely to avoid the introduction and dissemination of diseases into the production systems. Currently, Brazil is a large exporter of bird genetics, especially due to the efficient production systems established and our sanitation condition, in particular relatively to AI and NCD.

Of all registered breeder bird establishments, 71 are great grandparent, grandparent, specific pathogen-free (SPF) birds, and controlled-egg production facilities and 1,439 are parent stock facilities that produce fertile eggs. In the year 2020, 55.3 million parent stock birds for meat were housed. In spite of the good biosecurity levels, breeder birds are regarded as categories with a higher risk of exposure to the AI and NCD viruses because they have long life and production cycles. Thus, the Surveillance Plan includes breeding farms among the priority risk categories, although with a weight that modulates its relative risk.

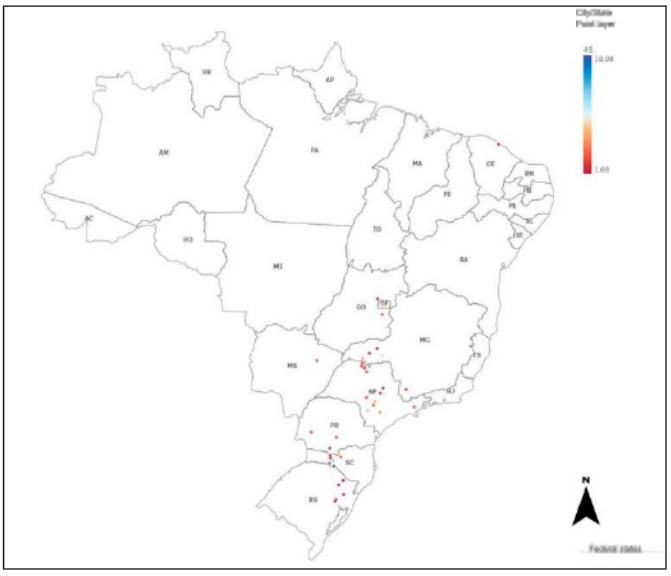

Figure 5. Distribution of poultry establishments engaged in grandparent and great grandparent poultry breeding

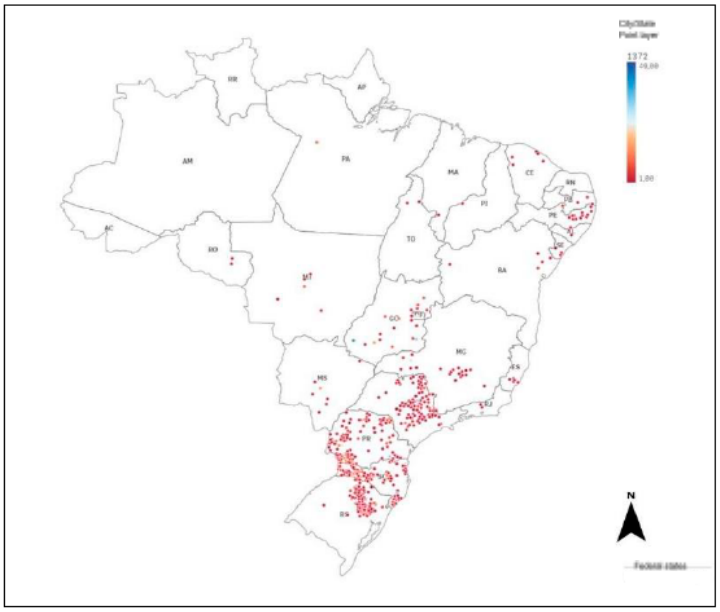

Figure 6. Distribution of poultry establishments engaged in parent stock breeding

7.1.2. Broiler production

The Brazilian production of chicken meat was 14.3 million tons in 2021, with over 6 billion birds slaughtered, which makes Brazil the world's 3rd largest chicken producer and largest exporter with 4.6 million tons exported.

Production is distributed across the country with 35.54% in Paraná; 14.89% in Santa Catarina; 13.65% in Rio Grande do Sul; 8.32% in São Paulo; 8.27% in Goiás; 7.44% in Minas Gerais; 3.76% in Mato Grosso; 2.75% in Mato Grosso do Sul; 1.10% in Pernambuco, and the remainder in other states (ABPA, 2022).

Broiler production in Brazil has competitive advantages due to the low production costs, quick production cycle, and a prevalence of integration systems in which the integrating company supplies the chicks, ration, products, logistic support, and veterinary care and the poultry farmer is responsible for the facilities and equipment and the labor, animal science management, sanitation precautions, and poultry well-being. In this chain, agro-industry plays several roles and is primarily responsible for coordinating the production, raw material, industrialization, and product distribution links. This integration system has allowed the employment of modern planning, organization, coordination, and management systems and the incorporation of new technologies that have resulted in production and productivity increases, reduced costs, and a diversity of products offered.

Currently, on top of genetic quality, broiler production employs modern techniques for nutrition, environmental comfort, management, health controls, and biosecurity, allowing for increasingly shorter and efficient production cycles and making it possible to achieve slaughter weights with shorter and shorter life times.

Although it is the most numerous portion in Brazil's bird population, that population has the lowest risk in the surveillance system based on AI and NCD risk due to good system biosecurity and especially the very short life cycle of the birds, which reduces the possibility of infection and detection of an immune response to AI. Thus, this category has been regarded as the lowest risk on in the AI and NCD Surveillance Plan.

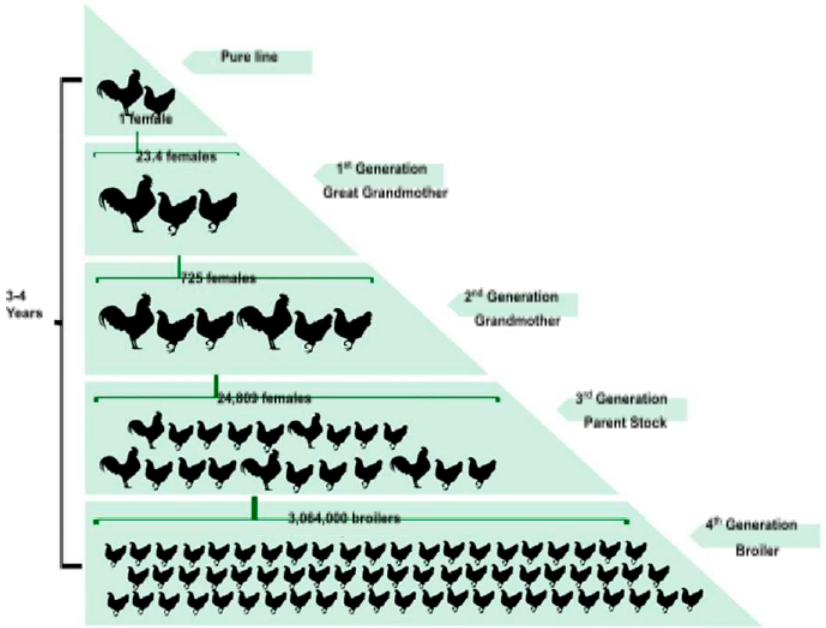

Figure 7. Meat poultry breeding flow chart. Adapted from Shaoting Li et al., 2021.

7.1.3. Commercial egg production

The housing of commercial egg-laying poultry was 114,637,958 hens in 2021, with a production of 54.9 billion eggs. Production is distributed across the country with 29.63% in São Paulo; 10.54% in Minas Gerais; 9.17% in Espírito Santo; % 8.19% in Pernambuco; 5.82% Rio Grande do Sul; 5.59% in Mato Grosso; 5.26% in Ceará; 4.75% in Paraná; 4.63% in Goiás; 3.15% in Santa Catarina; 1.71% in Mato Grosso do Sul, and 1.67% in Bahia (ABPA, 2022).

The egg-laying hen production and management systems can be classified as: intensive (in cages or over the floor, in open sheds with covering screens or closed sheds), with the conventional one being the most common one; and extensive or alternative (cage-free and/or organic), with outdoor access being the main risk advantage relatively to the conventional system.

In the conventional system, raising is done in sheds using conventional cages, with sections of overlaid cages. Raising can be done in open sheds with covering screens, which are less expensive and use natural ventilation, whether or not aided by fans.

This form of hen raising is more common in mild-weather areas and poses a higher risk of exposure to infection agents and sources, whether due to the wind or on direct or indirect contacts with wild birds. Due to the lower biosecurity levels, higher exposure risks, and the long life cycle of the birds, these systems are regarded as higher risk and must be prioritized for surveillance.

Closed-shed raising systems, on the other hand, require forced ventilation and evaporative cooling, as well as good sealing. It comprises more complex and expensive facilities, which offer better biosecurity conditions with reduced chances of exposure to infectious agents due to the wind or wild birds, and are less prioritized for surveillance when compared to open sheds.

Alternative production systems are growing with aims to meet the market demands for offering hens an increased well-being. This type of production is done without cages, with the poultry kept free for some part of the day or all day long with an access to pasture paddocks. That form of raising also provides a minimum number of nestles, roosts, and bed and pasture area per bird, among other specifications. That system is however disadvantageous from a sanitation standpoint relatively to the cage system as it significantly increases poultry and egg exposure to infectious agents, as well as makes it harder to clean and disinfect the facilities. Therefore, these systems have a higher risk that diseases may be introduced when compared to conventional systems and must be prioritized for surveillance.

7.1.4. Turkey, quail, and duck farming

Turkeys: The Brazilian production of turkey meat is small and has been decreasing in the last few years, dropping from 442.2 thousand tons in 2012 to 157.05 in 2021, and is concentrated 56.54% in Rio Grande do Sul, 39.45% in Santa Catarina, and 3.99% in Paraná (ABPA, 2022).

In commercial turkey production, day-old birds are sent from the hatchery plant to the poultry farms for the starting stage, which spans from their arrival to 35-38 days. The termination stage for females covers a period of 78-80 for slaughtering at around 6.5 to 6.7 kilograms; or as long as 90 days to reach 8 kilograms of live bird weight. For males, the goal is to reach 18-20 kilograms at the age of 128-140 days. These long production cycles increase the production costs relatively to chicken and the risk of the poultry's exposure to AI, NCD, and other diseases, and therefore these categories are regarded as a priority for surveillance in this plan.

Quails: The quail pool in Brazil has been growing and has reached a total of 16.5 million birds, concentrating mainly in the country's Southeast (IBGE, 2021). The production system is similar to the egg-laying hen system, with raising done in cages inside open sheds with screens or closed sheds, using poultry production technologies and controls. Also due to the long life cycle and production system, quail farms are regarded as relevant for AI and NCD surveillance.

Ducks: The Brazilian production of duck meat is very small and was 5,083 tons in 2021, with a concentration of 99.75% in Santa Catarina; 0.09% in São Paulo; 0.05% in Paraná, and 0.03% in Rio Grande do Sul; and 68% of which sent for exports especially to the Middle East (ABPA, 2022).

Because it is a waterfowl bird from the Anatidae family, duck raising is regarded as highly relevant for AI surveillance, due to that family's special resistance to the disease and the fact that the poultry can become reservoirs for the virus.

7.2. Subsistence poultry farming

All over the Brazilian territory, there are traditional raising systems for production of eggs and loosely raised chicken intended for consumption by farmer families. According to the WOAH, these establishments have a low risk for AI and NCD dissemination because they are limited to a household environment and do not include a direct or indirect contact with farming poultry or other establishments. However, their importance is clear when it comes to detecting diseases that circulate in natural populations of wild birds, considering a higher contact probability.

The local trade of surplus animals raised in a non-technical manner for additional household income at small rural properties takes place irregularly and, for that reason, it requires specific actions to inspect and even collect samples from probable cases. In general, hens, helmeted guineafowl, turkeys, and ducks are raised loose or semiconfined, feeding from grazing and food leftovers and possibly some commercial ration or corn.

8. DATA SOURCES AND USE

Table 2 shows the main sources of data of interest to the AI and NCD surveillance system.

Table 2. Main sources of data of interest to the AI and NCD surveillance system.

Type of data	Data source	Place recorded and accessed	Description
Records of notifications and investigations of suspected cases of poultry diseases with a mandatory notification requirement	MAPA and OESA	e-Sisbravet	Data controlled by the OESA and managed by both the OESA and MAPA. PNSA uses them to monitor responses to events of probable cases of AI and NCD and assess passive surveillance
Business and poultry pool registries with geolocation	OESA	OESA' computerized system	Registries in the OESA's computerized systems and databases, updated by rural farmers and used to design surveillance.
Records of animal transportation from Animal Transportation Forms (GTA) issued	OESA	OESA' computerized system	Data on transportations registered by farmers and OESA-authorized veterinarians and used by PNSA to structure animal health management actions
Registries of slaughtering facilities and information on slaughtered poultry and official inspection	MAPA and OESA	SIGSIF, SIGPOA, and the OESA's computerized system	Data consolidated by MAPA (facilities under federal surveillance or registered with the SISBI-POA) and by the OESA (state slaughtering facilities) used to assess surveillance for slaughterhouses.
Records of business, vaccination, and inspection records used in PNSA management	MAPA and OESA	Half-yearly spreadsheets of program management	Data consolidated by the OESA and sent to MAPA for an assessment of the surveillance system for poultry diseases with a mandatory notification requirement.
Data on the human, financial, and structural resources of MAPA, the OESA, and emergency funds	MAPA and OESA	Yearly report spreadsheets	Data updated and consolidated on an annual basis by OESA, SFA, and DSA serving as a supplement to conduct analyses under PNSA.
Record of international surveillance data	MAPA/VIGIAGRO	SIGVIG and spreadsheets	Data obtained from Vigiagro at points of entry of people, animals, and various goods
Record of data from official and accredited laboratories	LFDA and accredited laboratories	SIGEP or another system defined by MAPA and spreadsheets	Data on analyses regarding surveillance and monitoring used by PNSA
Record on active surveillance data for domestic birds	MAPA	SIGEP or another system defined by MAPA	Data registered by the OESA and managed by DSA.
Information on free-living wild birds, screening and rehabilitation centers for wild animals, and conservation breeding centers	IBAMA, ICMBio, state and city environmental authorities	Publications, spreadsheets, and messages from the authority	Data registered by IBAMA, ICMBio, state and city environmental authorities, and population management agents
Information on aggregation or important sites for migratory wild birds and frequently occurring species	ICMBio	Publications and archives of the authority	Registered and compiled by ICMBio from different sources
Data on relevant epidemiological events and presence or absence of diseases	WOAH and international bodies	WAHIS/WOAH system and others	Data used by DSA for a risk assessment, reports, and specific analyses

Data from the Cananeia Quarantine Station - EQC and authorized quarantine facilities for wild bird imports	EQC and companies	Spreadsheets and bulletins	Data used by PNSA for a risk assessment, reports, and specific analyses
Records of data from laboratory tests of target businesses for international trade certification	Companies and accredited laboratories	Spreadsheets and bulletins	Data used by PNSA for a risk assessment, reports, and specific analyses

9. SURVEILLANCE SYSTEM COMPONENTS

Each component of the surveillance system covers an activity used to investigate one or more hazards in the target population. The set of surveillance components or activities capable of producing data on the condition of the particular disease or on the condition of a specific population constitutes a surveillance system.

This Plan is grounded in the guidelines proposed by the WOAH and the United Nations Food and Agriculture Organization (FAO) in its documents, notably the "Terrestrial Animal Health Code" and the WOAH "Manual of Diagnostic Tests and Vaccines", and the FAO "Manual for Risk-Based Disease Surveillance".

Taking regional diversities and the production systems into consideration, the AI and NCD Surveillance Plan seeks to establish a program with better efficacy and cost-effectiveness, in particular with regard to the existence of special risks of diseases in the various locations and types of production premises across the country.

It is important to highlight that a satisfactory performance of all components, according to the provisions of this Plan, is key so that the surveillance system can achieve the expected objectives and coverage of the bird population.

The AI and NCD Surveillance Plan is made up of the five components below, illustrated in Figure 8:

1. **PASSIVE SURVEILLANCE: INVESTIGATIONS OF SUSPECTED SRN CASES**
2. **PASSIVE SURVEILLANCE: INVESTIGATION OF EXCEPTIONAL MORTALITY OF WILD BIRDS**
3. **ACTIVE SURVEILLANCE FOR INDUSTRIAL POULTRY FARMING**
4. **ACTIVE SURVEILLANCE FOR SUBSISTENCE FARMING BIRDS IN AREAS WITH HIGHER RISK OF AI INTRODUCTION**
5. **ACTIVE SURVEILLANCE FOR AI- AND NCD-FREE COMPARTMENTS**

Figure 8. Components of the AI and NCD Surveillance.

9.1. COMPONENT 1 – PASSIVE SURVEILLANCE: INVESTIGATIONS OF SUSPECTED SRN CASES

Passive surveillance is an important component for maintaining the capacity of the animal health information system, the flow of samples sent for diagnosis, and laboratory and emergency management capacity, which are essential conditions for keeping the readiness for a quick response. In addition, the data resulting from passive surveillance contribute toward demonstrating the absence of diseases in the production system.

MAPA executive ruling No. 50/2013 orders mandatory immediate notification of any suspected case of AI and NCD, which are the diseases targeted by the surveillance for the Respiratory and Nervous Syndrome (SRN) in birds. This is most important surveillance component for an early detection of HPAI and NCD.

The importance of the network of local veterinary units and offices for supporting the community existing in the country is worth highlighting, as it enables an easy access by communities and a quick response to notified suspected cases.

Clinical and epidemiological investigation of suspected cases must be carried out within a maximum of 12 hours by the veterinarian of the official animal health service, whose assessment must determine a need for collecting samples for a laboratory diagnosis at the national reference laboratory, LFDA/SP, according to the case definitions described in the corresponding Technical Sheets.

http://sistemasweb.agricultura.gov.br/pages/fichas_tecnicas/ficha-tecnica-INFLUENZA-AVIARIA-maio-2021.pdf ;

http://sistemasweb.agricultura.gov.br/pages/fichas_tecnicas/ficha-tecnica-NEWCASTLE-maio-2021.pdf

9.1.1. Investigation of suspected cases in production and subsistence farming birds

Most of the passive surveillance efforts are directed at domestic birds, encompassing production farming and subsistence farming.

Those who keep daily contact with the poultry, such as treating personnel, farm managers, or local veterinarians, as well as the personnel involved in the field work, play a key role in early detection and must be capable of recognizing the signs of the disease and immediately notify them to the official animal health service, allowing for quick and effective containment of foci.

9.1.2. Investigation of suspected cases in slaughterhouses

Poultry slaughtering facilities constitute an important source of information to the surveillance system, as they act as a point of convergence of a large number of birds coming from various poultry farms and cities. Nonetheless, the birds sent to slaughterhouses tend to be young and healthy, which accounts for an important sampling bias. Moreover, according to data on AI foci in Europe, the probability that LPAI will be detected at the moment of inspection is very low, because the high mortality of the disease prevents the poultry from even getting to the slaughtering facility.

Yet, the inspections conducted in the ante- and post-mortem routine by the veterinarians of the official inspection services may from time to time detect clinical signs and injuries that are compatible with the AI and NCD. In case birds are detected with clinical signs and injuries that are compatible with the AI and NCD or if dying or dead birds are found at the reception platform, the official inspection service must immediately notify the nearest official animal health service to conduct the clinical and epidemiological investigation, as set out in Joint Circular Letter No. 3/2021/DSA/DIPOA/SDA/MAPA
https://www.gov.br/agricultura/pt-br/assuntos/sanidade-animal-e-vegetal/saude-animal/programas-de-saude-animal/pnsa/2021_03.DSA.DIPOA.SEI_MAPA15035292OfcioCircularConjunto.pdf

9.1.3. The role of education or research institutions and private diagnostic laboratories

The admission of education or research institutions and diagnostic laboratories in the OVS notification system has particular importance for an early detection of suspected cases. At these laboratories, professionals from the field and university professors are routinely called by integrating companies, farmers, and veterinarians in charge of breeding facilities for diagnoses of diseases other than those subject to an official control.

In the event of AI or NCD at industrial poultry farms, these laboratories may receive samples of suspected cases even before notification to or a response from the OVS. Since the samples come from clinically ill birds, which may include AI or NCD cases, these account for a high surveillance value for early detection. Therefore, under conditions characterizing suspected cases of these diseases, these laboratories must immediately notify the official animal health service for investigations, as shown in the technical sheets for the diseases. Any AI or NCD detection in domestic or wild birds, both in high- and in low-pathogenicity subtypes, must be immediately notified to the official animal health service so that adequate actions can be taken.

The OVS in each federal state must keep up-to-date contact information of the private diagnostic laboratories, universities, or research institutions and conduct periodical contact to exchange information, promote awareness, and provide clarification regarding the disease notification obligations.

9.2. COMPONENT 2 – PASSIVE SURVEILLANCE: INVESTIGATION OF EXCEPTIONAL MORTALITY OF WILD BIRDS

Infection by an Influenza A virus of both high and low pathogenicity and infection by a type-1 avian paramyxovirus (APMV-1) can cause mortality in a large number of wild birds, especially in migratory waterbirds, which account for the highest risks of AI introduction into the country.

A systematic investigation of events of exceptional mortality of these birds in nature, particularly in concentration areas for migratory waterbirds, water stopover sites, and other bodies of water, aiming to determine whether HPAI or NCD is causing the events, provides a great opportunity to detect the viruses in the country, learn about the health situation of wild populations, and allow the production pool protection actions to be intensified, thus mitigating the introduction risks.

Events of exceptional mortality of wild birds are regarded as situations in which dead or sick birds are found in a number above what is commonly observed and due to an unknown cause, excluding for example anthropic actions (poisoning, chemical accidents, death by guns, bombs, traps, etc.) and natural phenomena (storms, earthquakes, droughts, floods, hurricanes, and harmful algal blooms, etc.).

The birds of interest to this surveillance component are primarily migratory waterbirds, that is, those that, at least in some of their population, undertake cyclic and seasonal moves with high loyalty to their breeding sites, associated with aquatic environments, such as the Anseriformes (ducks, geese, and dabbling ducks) and the Charadriiformes (seagulls, wattled jacana, sandpipers, and terns).

Authorities, agencies, and government and non-government organizations engaged in environmental management and preservation of natural resources are key players in detecting suspected cases of AI and NCD based on events of exceptional mortality involving the relevant wild birds. Events of exceptional mortality involving wildlife are frequently observed by different players, though their characterization as suspected cases of diseases subject to official control requires an accurate observation of clinical signs and criteria that justify considering an abnormality as worthy of clinical, epidemiological, and laboratory investigation by the OVS, failing which, the veterinary services and laboratories may be overloaded with groundless and epidemiologically irrelevant events. The training of agents at environmental authorities on the various levels (federal, state, and municipal) may minimize interpretation mistakes in cases of field mortality, since they are the primary parties called to act upon these events and usually have a higher understanding of the species involved and their ecology.

Thus, defining criteria as to what must be investigated by the OVS upon events of exceptional mortality of wild birds is hard, but must be sought and continuously enhanced by the OVS jointly with the institutions and players engaged in environmental management and conservation and society as a whole.

To optimize surveillance and elevate the chances of detection through a rational use of resources, the priority locations for an investigation of exceptional events, as part of the AI and NCD surveillance, are the concentration areas for migratory water birds or sites close to them, water stopover sites, and other bodies of water. These sites have the highest risk of infections by the Influenza A virus and type-1 avian paramyxovirus in wild birds due to the presence of the main susceptible taxonomic groups and the crowding and flow of animals of different origins that may carry the viruses. In this regard, it is expected that viewing routine for these locations enables the knowledge of a minimum track record to help characterize the exceptional nature of the observed mortality, both by environmental technicians and the other members of society, such as ornithologists, tourists, rural farmers, and local communities.

This approach may be strengthened through actions of animal health education and communication to be spread on different communication channels according to each target audience.

Listed below are a few criteria that must be used to characterize the exceptional events of wild bird mortality that are relevant to an official investigation, which aim to instruct the notifying parties and the OVS, considering the current scenario of the diseases in Brazil. Such parameters are very useful, especially in situations of an absent track record of the location being notified.

- Verify whether the species involved correspond to the profile of highest interest, that is, whether they are migratory water birds: with a gregarious behavior (forming kettles or colonies), feeding, resting, nestling, or breeding associated with water environments, anatomy adapted to water environments (interdigital webbing for swimming or thin, elongated legs for walking in wetland areas), spatula-shaped beak for catching water plants or thin, elongated beaks for fishing. Their classification under the Anseriformes (ducks, geese, and dabbling ducks) or Charadriiformes (seagulls, wattled jacana, sandpipers, and terns) orders is a good indicator;

- Verify whether the location is a water environment or close to the following: swamp, lake, pond, mangrove swamp, estuary, river, creek, dams, sea, *restinga*, and others. Particular attention must be given to locations recognized by institutions as sites or migratory water birds or stopover water points;

- Verify whether there has been mortality of at least one group of birds or whether they are in a dying condition (signs of imminent death). One or few birds found dead or dying, individually, do not constitute an event of exceptional mortality;

- Verify, if available, the track records of previous investigations and the epidemiological situation of the area in order to identify whether the notifier's report corresponds to a situation that is different from what could be regarded as normal for a certain location or another cause that has already before;

- Rule out, if possible, anthropic causes of mortality, such as poisoning, chemical accidents, death by guns, bombs or traps, checking detailed information provided by the notifier, rumors, news broadcast in the media, and reports from other visitors and residents of the area; and

- Rule out, if possible, causes from natural phenomena, such as storms, earthquakes, droughts, floods, hurricanes, and harmful algal blooms, checking information provided by the notifier, rumors, news broadcast in the media, and reports from other visitors of the area.

State and federal authorities and entities must develop cooperation actions to effectively implement this surveillance component, with participation from all relevant players, so that the suspected situations can be correctly characterized and adequate sampling and laboratory diagnoses can be done timely and accurately to confirm cases or rule out suspected infections by Influenza A and APMV-1 viruses in wild birds. Monitoring of the Influenza A viruses must also be conducted to determine whether new viral rearrangements or mutations are occurring, which may give birth to new agents to affect human or bird health.

The institutions involved must follow the information flows and the Communication Plan established by MAPA and state veterinary services, from case notification to confirmation, with aims to avoid rumors and undue news that may cause overreactions by the communities and the markets that import poultry products. In this regard, the "national network for notification of wild animal diseases" is being structure, for which the defined flows and communications are being addressed with the participating institutions.

In order that surveillance for the relevant wild bird diseases can be really effective, the institutions on all levels must facilitate communication and sample flows between the federal and state institutions and organizations involved in the surveillance, as well as establish in advance the actions to be taken in cases of confirmed infection by an Influenza A virus in wild birds.

The records of investigations on events of exceptional wild bird mortality must be part of an assessment of the surveillance sensitivity.

Upon the occurrence of probable or confirmed cases in wild birds, there will be no shutting down of nearby businesses or the area of occurrence or any other restrictive measure. The OVS will conduct the investigation at the premises close to the area of occurrence and take contingency measures. The procedures and instructions from the Technical Sheets and the DSA's manual for sample collection, storage, and submission must be followed, with biosecurity reinforcement measures being put in place at the production poultry farms, aiming to avoid transmission into these premises.

Interaction with the institutions engaged in environmental management and wildlife preservation constitutes a key mechanism for an exchange of information, submission of samples for laboratory diagnosis, and sharing of actions and resources, securing surveillance for the diseases. In Brazil, the Brazilian Institute for Environment and Renewable Natural Resources (Ibama) and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) are the primary players in environmental management and preservation and are greatly relevant to surveillance for wildlife diseases.

9.3. COMPONENT 3 – ACTIVE SURVEILLANCE FOR INDUSTRIAL POULTRY FARMING

This surveillance system component aims, through robust and representative sampling, to detect NCD and AI if present in industrial poultry farming in Brazil. When the target diseases are not detected through this active surveillance effort, it means secured certification of their absence in industrial poultry farming.

In case the official animal health services of the states develop knowledge and propose more specific studies to identify areas or properties with a higher risk of AI introduction that are different from those proposed in this plan, they may present them to the DSA for an assessment of and change in the sampling strategies for the next surveillance cycles.

A cross-sectional study has been planned with a two-stage planning strategy, the first stage referring to selection of poultry farms according to the risks attributed to the various activities performed at the poultry establishments (European Food Safety Authority, 2012; Stärk et al., 2006) and the second one directed at defining the number of animals to be sampled inside the selected establishments (Cameron and Baldock, 1998; Humphry et al., 2004).

In order to achieve the objectives of this study, the industrial poultry farming population has been defined as the set of establishments that raise hens, turkeys, ducks, and quails with a housing capacity superior to 1000 birds. The group excludes farmers for subsistence, ornamental, or other purposes that do not belong in the food production chain (meat and eggs).

Since Brazil shelters several species of migratory water birds, the first sampling stage has been added with a criterion for selecting properties in cities where migratory birds that are epidemiologically important to the diseases targeted in this surveillance plan have already been identified.

The use of additional risk factors for AI detection is due to the fact that this disease has never been detected in Brazil, the vast availability of references in the literature, and its high relevance to the country. Considering the similarities of the acute clinical conditions in these SRN diseases, the strategy is advantageous including for detecting NCD.

Several sampling design strategies for studies on avian influenza detection in birds have been verified, including, but not limited to, the programs of European Union countries and the United States. (Castellan, 2012; EUROPEAN COMMISSION, 2010; USDA APHIS VS, 2013)

The reference population, on which the sampling will be conducted, will be obtained from the databases of rural establishment registries, as supplied by the State Veterinary Services to MAPA. The design of this component has gone through the stages described below:

a) Definition of sampling areas
b) Sample size calculation
c) Definition of risk categories for poultry establishments
d) Sampling period

a. Definition of sampling areas

Active surveillance is focused on the whole national territory and, for that purpose, the poultry population has been divided into seven subpopulations according to their geographic and productive characteristics and taking into account the practical aspects of management and logistics of the states' administrative divisions. Each of these subpopulations or surveillance areas is the subject of an independent sampling. The areas defined have been:

- Area 1 (Rio Grande do Sul)
- Area 2 (Santa Catarina)
- Area 3 (Paraná)
- Area 4 (Southeast)
- Area 5 (Northeast)
- Area 6 (North), and
- Area 7 (Central-West)

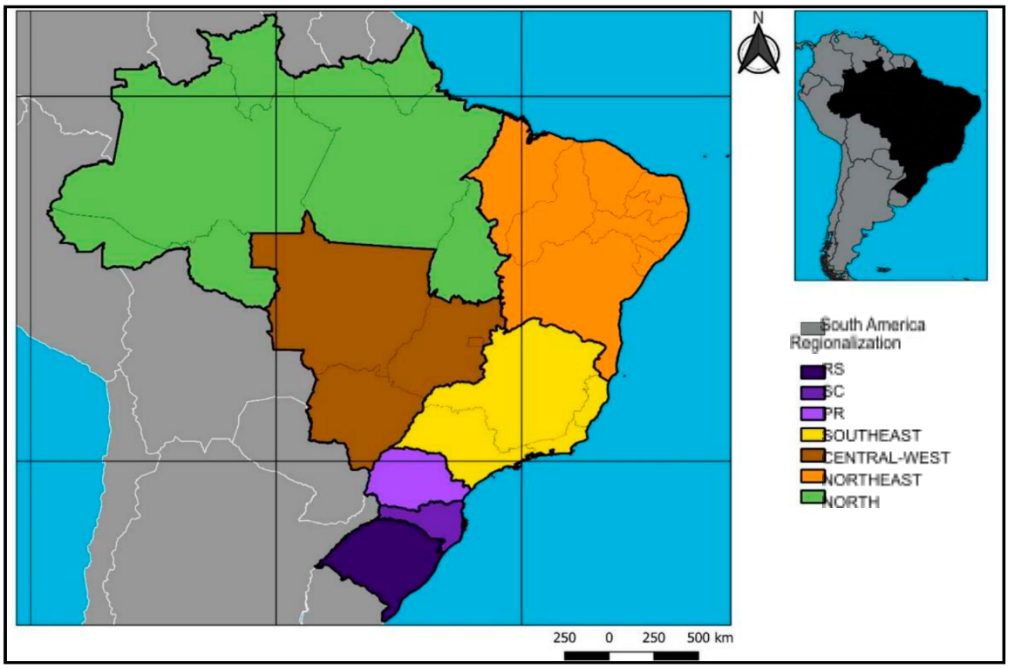

Figure 9. Sampling areas of the industrial poultry farming active surveillance component

b. Sample size calculation

The target population considered for the sampling design of this component is comprised of the establishments raising hens, turkeys, ducks, and quails totaling more than 1000 animals. Inside each sampling area, the study population is distributed according to the risk associated with the type of poultry activity performed and its proportion in the population.

The sample size has been calculated at two stages. Relatively to the first stage (number of poultry farms), a prevalence of 1% among poultry farms and a confidence level of 95% are presumed, with an expectation that, in case AI or NCD were present in these areas, at least 1% of the poultry farms would be infected.

With regard to the second stage (number of birds per poultry farm), the sample size will be determined based on an estimated prevalence at poultry farms of 30% and a confidence level of 95%, with an expectation that, in case AI or NCD were present at a poultry farm, at least 30% of the birds would be infected.

By following these parameters, **11 will be randomly sampled in each farm core**. If there are several sheds in a core or farm, the samples must be distributed as maximum as possible across the sheds, considering the premises that, on any outbreak of AI, at least 30% of the sheds will be affected and there will not be more than 50 sheds per establishment (EUROPEAN COMMISSION, 2010; USDA APHIS VS, 2013).

The diagnosis parameters considered for preparing the presented design are 95% and 95% sensitivity for the tests “ELISA for AI” and “PCR for NCD”, respectively. The specificity has been considered at 100% for the diagnosis protocol, associated with the clinical and epidemiological investigation procedures and the supplementary tests provided for in this Plan.

The types of establishments and their risk categories have been defined considering an absence of AI and NCD in Brazil, their track record in other countries (European Food Safety Authority, 2017; WAHIS, WOAH), surveillance plans prepared by other animal health entities, and environmental and production conditions in the Brazilian territory. The most relevant aspects for this category definition have been, in this order of importance: susceptibility of the species present, duration of the breeding cycle of animals, and the impact of the management, health, and biosecurity practices.

Avian influenza and Newcastle disease surveillance plan

- VERY LOW RISK → Establishments raising broilers
- LOW RISK → Establishment raising breeding hens (parent stock, grandparent, great grandparents, or pure-line animal facilities)
- MODERATE RISK → Establishments raising egg-laying hens
- HIGH RISK → Establishments raising turkeys, ducks, and quails

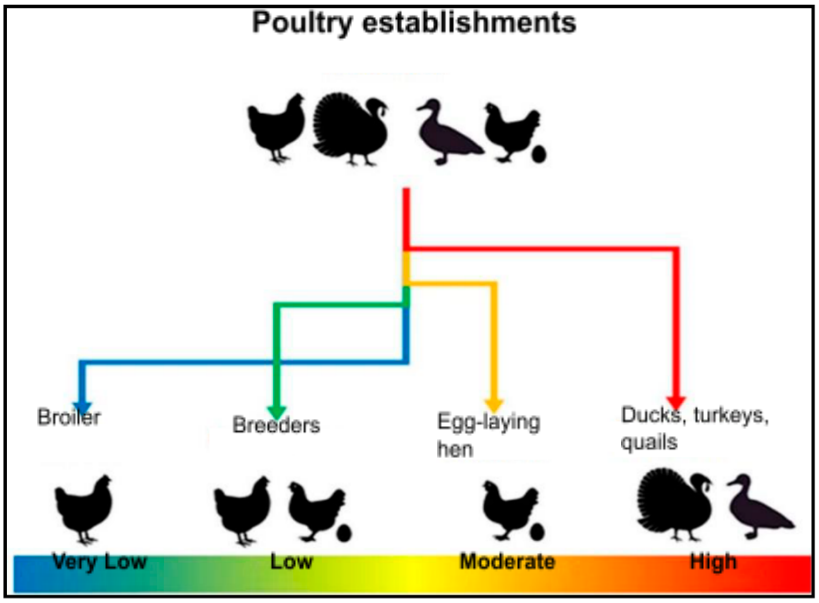

Figure 10. Types of establishments and risk categories in active surveillance for industrial poultry farming.

C. Incremental sampling

Incremental sampling aims to increase the sensitivity of the component by considering the presence of migratory bird sites in the cities (Stärk et al., 2006). Thus, more establishments were selected, equivalent to 10% of the initial sampling, exclusively in those cities where sites of nearctic migratory birds from the groups of Charadriiformes and Anseriformes species were found (Liang et al., 2020; Velkers et al., 2021).

The other criteria and strategies follow the same design as used for the initial sampling (a minimum of 1,000 birds at the establishment, categorization by type of activity, risk level applied, and sampling design).

The list of establishments to be sampled will be prepared annually by the DSA jointly with the OESA of each state. However, when evaluating the selected establishment, the OESA technician in charge of the collection must certify whether it meets the necessary characteristics to fall under its assigned category, as described in this Plan.

Table 3 shows an example of the composition of the process of sample stratification across the poultry farm categories according to AI risk and its frequency and sample distribution in the epidemiological area.

Table 3. Example of sampling stratification across poultry farm categories according to AI risk, frequency, and proportion in the epidemiological area.

Items	Chicken farms	Egg production farms	Breeding farms	Farms for ducks, turkeys, quails	Total
1. Risk assigned to the categories (Weight)	0,04	0,30	0,18	0,47	1
2. Category proportion in the population of the epidemiological area (example)	0,4	0,35	0,2	0,05	**1**
3. Combined weight (1x2)	0,02	0,11	0,04	0,02	**0,19**
4. Category weight in the sample distribution	0,09	0,58	0,20	0,13	**1**
5. Distribution of poultry farms in the epidemiological area	30	182	63	41	**315**
6. Distribution of poultry farms in the area of highest risk	3	18	6	4	**32**
7. Total poultry farms to be sampled in the epidemiological area	**33**	**200**	**69**	**45**	**347**

All proposed calculations are simulated in the “epiR” and “base” packages of the software program “R”. (Nunes et al., 2020; R Core Team, 2019).

d. Sampling period

Taking into account the seasonality of nearctic bird migrations relatively to South America, which has historically taken place starting in September/October and ending in April/May, and that the goal of this component is to detect the presence of AI and NCD in industrial poultry farming, **the months of June through November** must be prioritized for the collection.

Said period is immediately after the return of the nearctic birds to their breeding sites. Thus, the birds will have already gone through the whole period of higher probability of contact with migratory birds and it will be more likely that an infection can be detected if present in the industrial poultry population.

9.4.COMPONENT 4 – ACTIVE SURVEILLANCE FOR SUBSISTENCE FARMING BIRDS IN AREAS WITH HIGHER RISK OF AI INTRODUCTION

Subsistence bird farms can be regarded as sentinels for the surveillance for avian diseases of interest to national poultry production, especially those located in areas with a higher risk of contact with migratory water birds. Prioritizing sampling from subsistence farms where there may be some contact with migratory water birds is an important strategy to be employed for AI and NCD detection, since most cases of introduction of the viruses and outbreaks in other countries bore that relationship.

Given Brazil's condition that it is a habitat for a vast population of migratory birds, who have even cross-hemisphere migration routes, this component is key to an active search for diseases in birds.

Active AI and NCD surveillance at farms close to sites of migratory water birds has been conducted for many years now in Brazil, following technical criteria for defining the sampling plan of sample collection that has led to the detection of AI antibodies.

Moreover, the OVS present in these locations is a great opportunities for actions of risk communication and education aiming at higher engagement of farmers in notifying suspected cases of diseases and bird mortality, taking into consideration that the experience of other countries has demonstrated that HPAI introduction in certain areas by wild birds is usually signaled by the mortality of backyard birds, on top of the mortality of wild birds in their natural environment.

Taking the opportunity of the surveillance activities at backyard establishments, the OVS teams must also inspect the migratory bird sites to check for dead or dying birds. In these cases, samples must be taken from those birds. For still living sick birds that can be captured, blood samples must be collected to obtain serum, as well as tracheal and cloacal swabs. For recently deceased birds, organs must be collected, as well as tracheal and cloacal swabs, when possible.

This component has been reshaped to provide a better design, expand the coverage, sensitivity, and specificity of the sampling, and enhance the criteria of regularity, frequency, and selection of places of interest to surveillance.

It is worth highlighting that the sampling strategy in this component aims to detect AI and NCD in subsistence poultry populations located in risky areas, due to a more probable exposure to migratory birds, and that have a higher density of birds, or industrial poultry establishments, aiming not only to research virus occurrence, but also to provide alerts of occurrence in place with an impact on the country's production systems, allowing for actions for biosecurity reinforcement and protection of industrial poultry production.

For that purpose, a long database has been used that was given by CEMAVE/ICMBio on places with records of present migratory birds detected through sightings and field research. Due to the large quantity of species and sighting places existing in Brazil, four criteria have been employed to select the places for surveillance:

1. **existing migratory birds** from the families with higher epidemiological importance for HPAI transmission, represented by the Anseriformes (Anatidae) and Charadriiformes (Charadriidae);

2. **species whose migration pattern follows the routes coming from the Northern Hemisphere** (nearctic), since they are those posing a higher risk of AI introduction in the country, considering that South America remains HPAI-free.

For **Brazil's South**, the specific routes in the South American continent have also been considered, establishing the inclusion of cities in that region where migratory birds are present;

3. bird farm concentration in the area; and

4. existing industrial poultry farming (establishments with over a thousand birds) in the cities where the sites are located. With a view to optimizing resources, an association with the early detection of any introduction of HPAI virus strains in backyard farms in cities where there might be impacts on industrial systems allows for quick sanitation and biosecurity actions to avoid introduction into these poultry farms.

The list of locations selected for sampling must be evaluated by the OESA, which will enable them to propose inclusions of replacements of locations, taking into account the following criteria to justify the changes:
1. cities with settlement sites of migratory birds of interest to them; 2. significant existence of subsistence poultry, even with deficient records; and 3. an important existing amount of bird transportation to other areas with industrial poultry farming. Accordingly, the OESA must request an exclusion of selected cities in case they justify that the selection done does not meet said criteria.

Sampling period and surveillance schedule

The surveillance activities must take place once a year at the selected risk locations, during the time when migratory birds are in larger settlements with mixed species and nestling. As this country includes different migratory routes comprising different species, the best time for surveillance varies from region to region. Generally speaking, nearctic wild birds migrate in early winter in the Northern Hemisphere and arrive in Brazil from September to December and remain until March-April. Thus, the best time for the sampling in this component is **December to April**.

Nonetheless, as there are much variation inside the country, the establishment of more specific sampling periods for each selected risk location can be better done upon the OVS' interaction with the environment management and conservation institutions and organizations present in the federal states and the communities surrounding the sites.

Annex 1 shows the details of the sampling design for this component.

9.5. COMPONENT 5 - ACTIVE SURVEILLANCE FOR AI- AND NCD-FREE COMPARTMENTS

Active surveillance for AI- and NCD-free compartments is necessary for maintenance of the health certification by evidencing the disease-free condition.

Executive Ruling 21, dated October 21, 2014, as amended by Executive Ruling 18, dated June 9, 2017, lays down the primary procedures that must be carried out:

At **breeding farms,** the sampling must be performed **every six months** and, at **broiler farms**, surveillance based on poultry farm sampling must be done every six months.

The activities must involve clinical evaluations of poultry and collecting samples for a laboratory diagnosis of AI and NCD, performed by veterinarians in charge in the company or authorized veterinarians, under the OVS' coordination. The costs with collecting, submitting, and processing samples, whether regular or random, are to be borne by the company of the compartment.

It is worth noting that, on top of regular, active surveillance collections, the OVS may order other sampling activities at its own discretion, at any time. Furthermore, the protocols may be more intensive than as recommended in this Plan when aiming to meet certification demands for specific commercial partners.

The laboratory testing for AI and NCD must be conducted at MAPA-accredited public laboratories, and the test reports must contain all required information.

Test reports with negative results will be sent by the laboratory to the SFA, to the SVE, and to the person in charge of the management team of the compartment. Any test reports the results of which are not negative, in any of the tests, will be immediately sent by the laboratory only to the DSA, SFA, and SVE.

Accredited laboratories must send the samples to the LFDA in the following situations: positive ELISA test without the AGID test; or a positive AGID test; or a positive RT-PCR test for the M gene for AI and NCD.

The sampling plan for AI and NCD surveillance must follow the protocol below, but the criteria may be redefined by the DSA at any time considering epidemiological assessments.

Sampling protocol:

a) collect samples at **all farm cores** that have had birds **housed for at least thirty (30) days**;
b) cores of grown birds vaccinated with a live-virus vaccine for NCD must not be tested for this illness;
c) **distribute the sampling uniformly across the sheds** of each core collecting the following quantities of samples:
   - **ten (10) individual blood samples** to obtain blood serum;
   - **ten (10) tracheal swabs** divided into two pools, one of which including five swabs; and
   - **ten (10) cloacal swabs** divided into two pools, one of which including five swabs.

Collect the swab samples of the same **ten (10) birds** as the serum samples were collected.

The surveillance results and data for the international health certificate (IHCs, or CZI in Brazil) for exports of fertile eggs and life birds must also be computed and compiled, keeping a standardized flow of surveillance information on these establishments.

10. LABORATORY DIAGNOSIS

10.1. PASSIVE SURVEILLANCE:

For suspected cases of respiratory and nervous syndrome (SRN) in birds identified during passive surveillance, the procedures provided in the corresponding AI and NCD technical sheets must be followed.

Identification of a probable case of AI or NCD by the official animal health service requires the collection of samples for diagnostic testing at official MAPA Laboratories - Federal Farming Protection Laboratories (LFDA). Currently, passive surveillance samples are sent and analyzed at LFDA/SP, in Campinas-SP.

LFDA/SP, based in Campinas, has an NB3 biological safety laboratory and is accredited by the WOAH as a reference for AI and NCD diagnoses.

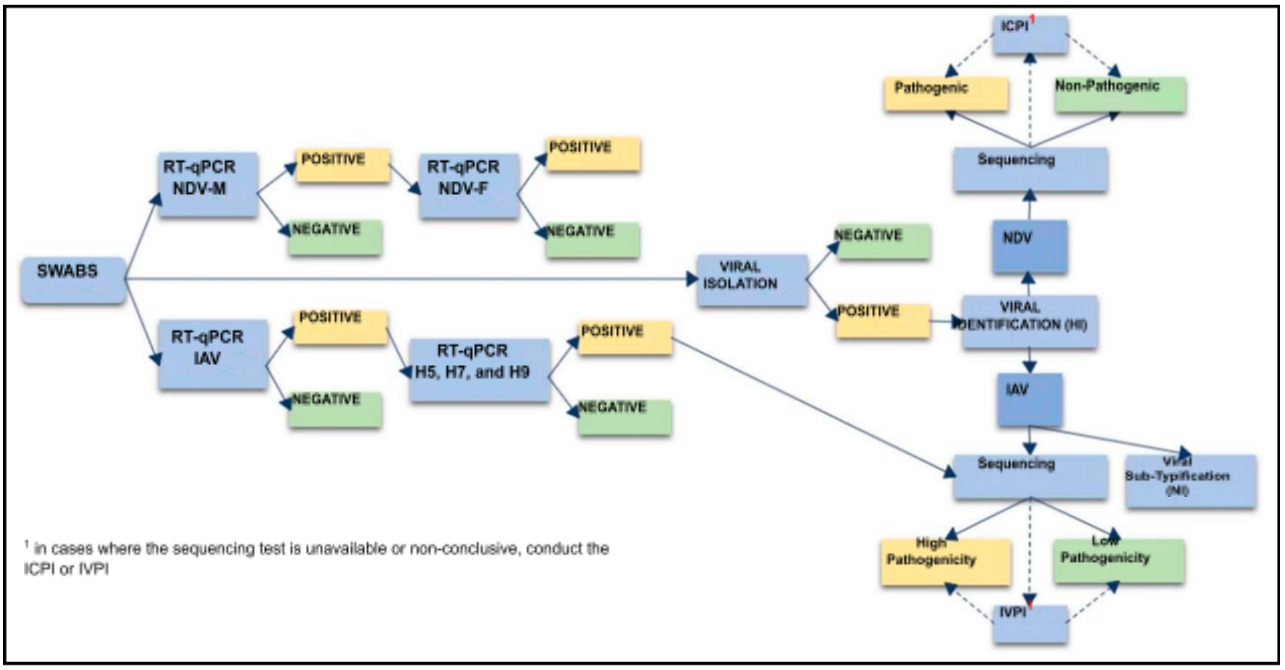

Figure 11. Laboratory diagnosis flow for samples of probable cases of SRN.

Keys:

- IAV: influenza A virus
- HI: hemagglutination inhibition
- ICPI: intracerebral pathogenicity index
- IVPI: intravenous pathogenicity index
- NDV: Newcastle disease virus
- NDV-F: Newcastle disease virus F gene
- NDV-M: Newcastle disease virus M gene
- NI: neuraminidase inhibition
- RT-qPCR: reverse transcriptase reaction followed by real-time polymerase chain reaction

10.2. ACTIVE SURVEILLANCE:

To conduct the tests of active AI and NCD surveillance, in this first cycle, the Federal Farming Protection Laboratories (LFDA) in Campinas-SP will be used.

With aims to expand the screening diagnosis network for active surveillance, official state laboratories that are properly accredited by MAPA may be included for the assays defined in the diagnosis protocols.

The simplified flow and laboratories of the active surveillance may be viewed in the figure below.

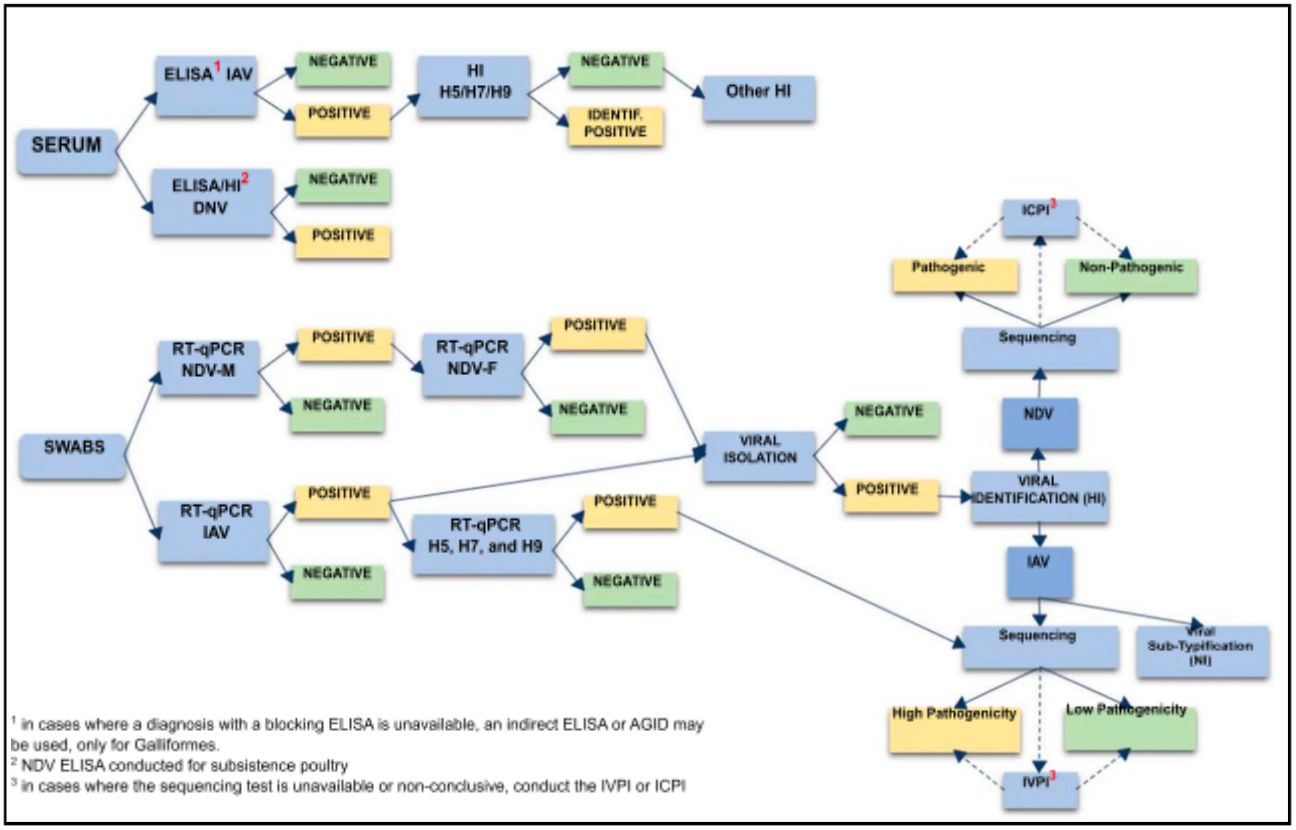

Figure 12. AI and NCD laboratory diagnosis flow for active surveillance samples.

Keys:

- AGID: agar gel immunodiffusion
- ELISA: enzyme-linked immunosorbent assay
- IAV: influenza A virus
- HI: hemagglutination inhibition
- ICPI: intracerebral pathogenicity index
- IVPI: intravenous pathogenicity index
- NDV: Newcastle disease virus
- NDV-F: Newcastle disease virus F gene
- NDV-M: Newcastle disease virus M gene
- NI: neuraminidase inhibition
- RT-qPCR: reverse transcriptase reaction followed by real-time polymerase chain reaction

Table 4. People or entities responsible, types of samples, laboratories, surveillance record systems, and frequency.

Type of Sampling	Person or entity responsible for collection	Type of sample	Laboratory	Data record system	Data entry
Investigations of suspected SRN cases in production birds	OESA	Tracheal and cloacal swabs and organs of choice	LFDA/SP	e-Sisbravet	Immediate and continuous
Investigation of events of exceptional mortality of wild birds	Environmental authorities jointly with the OESA	Tracheal and cloacal swabs and organs of choice	LFDA/SP	e-Sisbravet	Immediate and continuous
Active surveillance for industrial poultry farming	OESA	Blood serum, tracheal and cloacal swabs	LFDA/SP	SIGEP or another one	As activities progress
Active surveillance for subsistence farming birds in areas with a higher risk	OESA	Blood serum, tracheal and cloacal swabs	LFDA/SP	SIGEP or another one	As activities progress
Active surveillance for AI- and NCD-free compartments	Veterinarian in charge at the company	Blood serum, tracheal and cloacal swabs	Accredited public laboratory or LFDA/SP	Company reports submitted to the SFA	As activities progress

11. COMPONENT PERFORMANCE ASSESSMENT

The AI and NCD surveillance plan includes indicators and objective goals that allow for an assessment of system performance, aiming to adequately monitoring and correct deviations and deficiencies regarding the investigations and any actions taken.

An assessment of the performance indicators of the surveillance plan is part of the compilation and analysis of the results obtained from each of the components and will be performed by the PNSA coordinators with support from the focal points at the SFA and OESA in each federal state. They will be entered in the Half-Yearly Reports of each state involved with aims to assess surveillance goal achievement and will be sent to the Department of Animal Health. These data will be compiled by the DSA and will support the drafting of the annual report, as described in item 15.

For this plan, indicators have been proposed for an assessment of the following performance aspects, described in the tables below:

A. geographical representativeness;
B. representativeness of the types of production/age group;
C. representativeness over time;
D. consistency of results relatively to case definitions;
E. timeliness of surveillance.

Table 5. Components and indicators for an assessment of the geographical representativeness of the sampling.

Component	Characteristics assessed	Indicator	Goal
Investigations of suspected SRN cases in production birds	The geographical distribution of notifications and investigation of suspected cases must reflect the distribution of the bird population	NA	NA
Investigation of events of exceptional mortality of wild birds	The geographical distribution of notifications and investigation of suspected cases must reflect the distribution of the population of migratory water birds	NA	NA
Active surveillance for industrial poultry farming	The sampling in the federal states and cities must be close to the distribution of selected production plants, with a lower degree of substitution.	% selected establishments where collection is done	95%
Active surveillance for subsistence farming birds in areas with a higher risk	The sampling of subsistence farming poultry must be conducted in all higher-risk areas described in this Surveillance Plan.	% selected higher-risk areas with sampling	95%
Active surveillance for AI- and NCD-free compartments	The sampling will involve all compartments	% compartments with sampling	100%

Table 6. Components and indicators for an assessment of sample representativeness by types of production/age group

Component	Characteristics assessed	Indicator	Goal
Investigations of suspected SRN cases in production birds	The distribution of notifications and investigation of suspected cases must reflect the types of production existing in the corresponding surveillance area.	NA	NA
Investigation of events of exceptional mortality of wild birds	Not applicable	NA	NA
Active surveillance for industrial poultry farming	The number of establishments sampled in each state must reflect the proportion of establishments by type of production in the corresponding surveillance area. The distribution of collected samples must be close to the distribution of the various types of establishments selected. Sampling must be conducted with adult birds.	No. of correctly sampled establis. / No. of selected establis. % samples collected from adult birds.	95% 100%
Active surveillance for subsistence farming poultry in areas with a higher risk	The establishments sampled in each state must correspond to the type of subsistence farming meeting the criteria defined for the selection of establishments to be sampled. Sampling must be conducted with adult birds.	No. of correctly sampled establis. / No. of selected establis. % samples collected from adult birds.	95% 100%
Active surveillance for AI- and NCD-free compartments	The units sampled in the compartment must be representative of all cores in the compartment.	% sampled units that meet the defined criteria / total sampled units.	90%

Table 7. Components and indicators for an assessment of the representativeness over time of the sampling.

Component	Characteristics assessed	Indicator	Goal
Investigations of suspected SRN cases in production birds	The number of establishments sampled in each state must reflect the proportion of establishments by type of production in the corresponding surveillance area.	NA	NA
Investigation of events of exceptional mortality of wild birds	The distribution of the investigation of events of exceptional mortality of wild birds must more concentrated in the months of migrations of nearctic birds to Brazil.	No. of investigations within the defined period / total investigation	70%
Active surveillance for industrial poultry farming	The samplings in industrial poultry farming must be distributed according to the months defined in the Manual and are subject to small variations, but must not distributed over all months.	No. of establis. sampled within the defined period / total to be sampled	90%
Active surveillance for subsistence farming poultry in areas with a higher risk	The samplings in subsistence farming poultry must be distributed in the months defined in this Plan and are subject to small variations, but must not distributed over all months.	No. of establis. sampled within the defined period / total to be sampled	90%
Active surveillance for AI- and NCD-free compartments	The samplings in free compartments must be conducted every six months and are subject to small variations.	No. of cores sampled every 6 months / total existing cores	100%

Table 8. Components and indicators for an assessment of result consistency with case definitions

Component	Characteristic	Indicator	Goal
Investigations of suspected SRN cases in production birds	The notifications received must be classified by the OVS following the definition of a suspected case. The response and characterization of probable and confirmed cases must follow the provisions of the Technical Sheets.	No. of investigations correctly classified as probable cases / total probable cases	100%
Investigation of events of exceptional mortality of wild birds	The notifications received regarding events of exceptional mortality of wild birds must be classified by the OVS according to the definition of a probable case and abnormalities described in this Plan. The response must follow the procedures provided and the characterization of probable and confirmed cases as described in the corresponding Technical Sheets	No. of investigations correctly classified as probable cases / total probable cases	100%
Active surveillance for industrial poultry farming	The procedures of surveying characterization information on selected industrial poultry establishments, sample collection, and additional investigations must follow the provisions of the Manual. The flow and interpretations of laboratory diagnoses must follow what is described in the manual and be in accordance with the parameters laid down by the WOAH.	% establishments sampled as provided in the manual	95%
Active surveillance for subsistence farming poultry in areas with a higher risk	The procedures of surveying information on subsistence poultry breeders in areas with a higher risk, sample collection, and additional investigations for surveillance must follow the provisions of the Manual. The flow and interpretations of laboratory diagnoses must follow what is described in the manual and be in accordance with the parameters laid down by the WOAH.	% establishments sampled as provided in the manual	100%
Active surveillance for AI- and NCD-free compartments	The procedures of surveying information on the units where samples are collected, sample collection, and additional investigations for surveillance, as well as the flow and interpretations of laboratory diagnoses, must follow the provisions of this Plan.	% samples as established	100%

Table 9. Components and indicators for assessment of the timeliness of the surveillance plan

Component	Characteristic	Indicator	Goal
Investigations of suspected SRN cases in production birds	Responses to notifications of suspected cases must be conducted within 12 hours of the notification. The samples collected from probable cases must reach the LFDA within 48 hours of their collection. At the laboratory, the samples must be processed on an urgent basis and results made available **within 24 hours** following their receipt.	1. Response time. 2. Time between collection and arrival at the LFDA. 3. Time between sample receipt and result.	1. 12h 2. 48h 3. 24h
Investigation of events of exceptional mortality of wild birds	Responses to notifications of events of exceptional mortality of wild birds must be conducted within 12 hours of the notification. The samples collected from probable cases must reach the LFDA as quickly as possible, but no later than 48 hours of their collection. At the laboratory, the samples must be processed on an urgent basis and results made available within a maximum of 24 hours following their receipt.	1. Response time; 2. Time between collection and arrival at the LFDA. 3. Time between sample receipt and partial result	1. 12h 2. 48h 3. 24h
Active surveillance for industrial poultry farming	The samples from active surveillance for industrial poultry farming must be sent within a maximum of fifteen (15) days following collection. The samples must be stored in an ULT freezer at -80 ºC until dispatched. At the laboratory, the samples must be processed within seven (7) days following receipt and results made available immediately. Positive samples in screening testing must be processed in confirmation testing within a maximum of 48 hours following the screening results	1. Time between collection and sending of samples; 2. Time between receipt at the lab. and result 3. Time between the positive screening test result and beginning of the confirmation test	1. 15 days 2. 7 days 3. 2 days
Active surveillance for subsistence farming poultry in areas with a higher risk	The samples from surveillance for subsistence farming poultry must be sent within a maximum of fifteen (15) days following collection. The samples must be stored in an ULT freezer at -80 ºC until dispatched At the laboratory, the samples must be processed within seven (7) days following receipt and test results made available immediately. Positive samples in screening testing must be processed in confirmation testing within a maximum of 48 hours following the screening results	1. Time between collection and sending of samples; 2. Time between receipt at the lab. and result 3. Time between the positive screening test result and beginning of the confirmation test	1. 15 days 2. 7 days 3. 3 days
Active surveillance for AI- and NCD-free compartments	The samples collected for surveillance for free compartments must be sent as quickly as possible to the official accredited laboratory, never later than ten (10) days following collection. Upon arriving at the laboratory, the samples must be processed shortly, within seven (7) days following receipt, and the screening test results must be made available quickly after they are obtained. Positive samples in screening testing must be immediately sent to the LFDA/SP to be processed in confirmation testing within a maximum of 48 hours following the screening results.	1. Time between collection and sending of samples; 2. Time between receipt at the lab. and screening result 3. Time between the positive screening test result and beginning of the confirmation test	1. 10 days 2. 7 days 3. 3 days

12. ASSESSMENT OF SURVEILLANCE SYSTEM SENSITIVITY

The sensitivity of the surveillance system will be estimated at the end of a 12-month period after the plan was established. Performance of the established activities, such as investigating suspected cases and achieving the planned sampling goals, are determinant factors so that sensitivity can reach satisfactory levels. The calculations will be done independently for each of the seven defined sampling areas, and the relative contribution of each federal state will be assessed.

13. NOTIFICATION AND RECORD SYSTEMS

Notifications of suspected AI and NCD cases can be registered online by any type of user at the address of the e-Sisbravet system (http://sistemasweb.agricultura.gov.br/pages/SISBRAVET.html).

All registered notifications will be directed to the LVU in charge of the city where the suspected case is registered. Other forms of notification (in person, by telephone, electronic mail, etc.) must also be encouraged and continue to be used so that, accordingly, the emergency response by the official animal health service can be performed and recorded in e-Sisbravet.

All services provided within the “Component 1 – Passive surveillance - Investigations of Suspected SRN Cases” and “Component 2 - Passive surveillance - Investigation of events of exceptional mortality of wild birds” must be IMMEDIATELY registered in e-Sisbravet, the purpose of which is managing the procedures and data of responses in the official investigations conducted by the local, intermediate, and superior levels in connection with the passive surveillance.

All activities carried out for compliance with “component 3 - Active surveillance for industrial poultry farming” and “component 4 - Active surveillance for subsistence farming birds in areas with higher risk of AI introduction” must be registered in the Epidemiological Study Management System (SIGEP) or such other system as defined by MAPA, which aims to make it easier to monitor epidemiological studies and manage the data of active surveillance performed by the official animal health service.

For “component 5 - Active surveillance for AI- and NCD-free compartments”, the data on sample collections and laboratory results must be registered in systems owned by the companies and accredited laboratories and submitted to the SFA of the federal units where the poultry farms are located for compilations, analyses, and submission of alerts and annual reports to the PNSA coordinators.

14. RESULT REPORTING

The compilation and analysis of the results obtained from each of the components of this Plan will be performed by the PNSA coordinators with support from the focal points at the SFA and OESA in each federal state. Half-Yearly Reports will be prepared for each state involved with aims to assess surveillance goal achievement and will be sent to the Department of Animal Health. This information will be compiled by the DSA and will support the drafting of the annual report on AI and NCD surveillance actions.

The stakeholders must receive reports or bulletins with analyses and actions performed based on the Surveillance Plan data, and it is extremely important that all the links of this system are intertwined with them up to the local level. The DSA/MAPA will make the reports available to the SFA and the OESA through the Electronic Information System (SEI) and to the population on Mapa's website.

Information feedback is one of the characteristics of the surveillance system and is important to keep an active communication chain between stakeholders, adequately informing and encouraging cooperation through a perception of their contribution to the process. It thus secures their actual insertion and the quality of the data obtained.

15. PLAN RESOURCES AND FUNDING

The AI and NCD surveillance plan must be funded in a shared manner by different players interested in the benefits created. A cooperation agreement must be entered into between the parties involved in the country's poultry farming chain so that the funding is secured, with supplementation from either private sectors or MAPA, to support each component of the surveillance system in all federal states involved.

The costs entail human, physical, and financial resources, both government and private, which are key to the success of each of the components of the Plan. In each state, the involved players must hold meetings to define the distribution of the surveillance system costs and assess the application of funds and performance of the scheduled activities.

Table 10 illustrates an example of cost distribution across the main players involved, but, as expressed before, the arrangement to be established between government and private players must be built in accordance with how the production chain is organized in each state.

It is important to highlight that the surveillance system presented aims at an early detection of the diseases, mitigating economic and social impacts from any events, and generates data for evidencing the disease-free condition in industrial poultry farming, providing security to current markets and enabling new markets for avian products and genetic material from Brazil.

It can thus be noticed that the costs of the surveillance system are quite inferior to the benefits generated for the production chain and, therefore, a shared funding between government and private sectors is key in how human, physical, and financial resources are applied. Yet, future cost-effective analyses of the AI and NCD Surveillance Plan must be conducted with a view identifying more efficient actions and reporting to society.

Table 10. Proposed distribution of costs with human, physical, and financial surveillance resources between stakeholders

Component	Field activity and sample collection			Sample submission	Laboratory testing			Systems, analyses, and reports
	Human	Material	Financial		Human	Material	Financial	
Investigations of suspected SRN cases in production birds	OESA	OESA with support from funds and associations	OESA with support from funds and associations	OESA with support from funds and associations	LFDA/ MAPA	LFDA/ MAPA	LFDA/ MAPA	DSA/ MAPA
Investigation of events of exceptional mortality of wild birds	Environmental authorities or OESA	OESA with support from funds and associations	OESA with support from funds and associations	OESA or environmental authorities or funds/ associations	LFDA/ MAPA	LFDA/ MAPA	LFDA/ MAPA	DSA/ MAPA
Active surveillance for industrial poultry farming	OESA	OESA with support from funds and associations /Company	OESA with support from funds and associations /Company	Funds/ associations	LFDA/ MAPA	LFDA/ MAPA	Funds/ associations LFDA/ MAPA*	DSA/ MAPA
Active surveillance for subsistence farming poultry in areas with a higher risk	OESA	OESA with support from funds and associations	OESA with support from funds and associations	Funds/ associations	LFDA/ MAPA	LFDA/ MAPA	LFDA/ MAPA	DSA/ MAPA
Active surveillance for AI- and NCD-free compartments	Professionals in charge at the Company	Company	Company	Company	Accredited Laboratory LFDA/ MAPA**	Accredited Laboratory LFDA/ MAPA**	Funds/ associations LFDA/ MAPA*	DSA/ MAPA

Note: * Samples are tested and tests are conducted only in probable cases

** LFDA/MAPA will conduct the confirmation testing

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ANNEX 1 - SAMPLING PLAN FOR AI AND NCD DETECTION IN SUBSISTENCE FARMING OPERATIONS AND AREAS WITH A HIGHER RISK OF AI INTRODUCTION – COMPONENT 4

1. OBJECTIVE

This document describes active surveillance activities to detect AI and NCD viruses from a risk-based surveillance perspective, so that a situation of no detection helps evidence zero virus exposure in small-scale commercial production poultry (housing capacity up to 1000 birds) in Brazil.

2. STUDY DESIGN

2.1. METHODOLOGICAL STRATEGY

The study design incorporates the concept of risk-based surveillance, whereby the samples will be directed at the properties and birds with the highest risks of AI exposure across the various sampling areas in Brazil. This is a cross-sectional study conducted in two stages, with the first stage doing the selection of farms to be sampled and the second one defining the number of animals inside the breeding premises. It is important to bear in mind that each sampling area represents an independent study.

2.2. METHODOLOGY

The study design consists in five phases, as listed below:

I. Selecting cities with a higher risk considering the density of industrial poultry farming establishments and density of the main sites and routes of migratory water birds

II. Calculating the size of the sample of epidemiological units

III. Defining epidemiological unit

IV. Calculating the number of animals to be sampled and number of sampling pools to be analyzed per epidemiological unit

V. Structuring for sampling by dividing the cities into quadrants

I. Selecting cities with a higher risk

The active surveillance studies are established to represent all of Brazil's territory and will target the bird populations on subsistence or local commerce operations. However, by using a risk-based strategy, the sampling will be directed at specific areas inside the country.

For the selection of cities with a higher risk, the five main routes of nearctic migratory birds described in Brazil have been considered (Figure 1) (Antas & Antas, 1983; ICMBio, 2016, 2019), using as a reference the mapping of sightings of these animals (Figure 2) provided by ICMBio as well as the distribution and location of commercial poultry establishments around the country as provided by MAPA. The steps followed are presented below. For **Brazil's South**, the OESA may include cities with existing migratory birds from the specific routes of the South American continent, according to the criteria described in this plan.

a. Identifying and categorizing the primary migratory bird routes

First, the country was divided into three large regions relating to the main routes of migratory birds from the Northern Hemisphere, with one influential region defined as “Amazon Route”, another one as “Central Brazil Route”, and the third one as “Atlantic Northeast Route” (Figure 1). It is worth highlighting that, generally speaking, these routes follow some of the major water streams in Brazil.

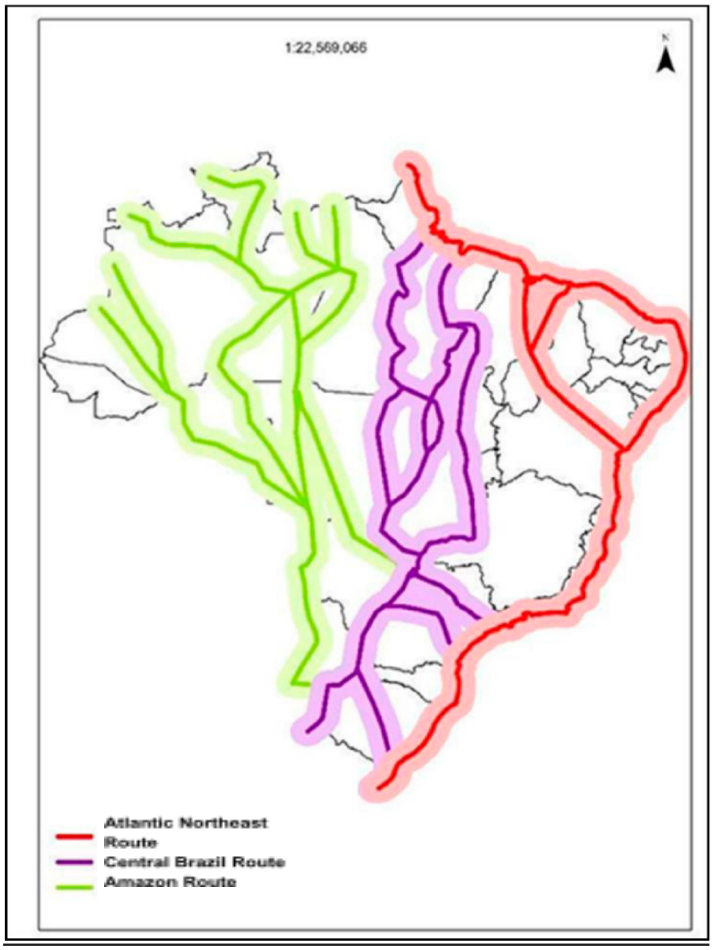

Figure 1: Primary migratory bird routes in Brazil and the three regions selected for surveillance for subsistence farming poultry.

b. Criteria for selecting cities

The cities with a higher risk in each of all three regions have been selected using the following criteria:

1) Cities located in a strip of one hundred (100) kilometers around the migration routes, which contain **sites of migratory birds sighted** and include **establishments for commercial poultry breeding**.
2) Cities located in a strip of one hundred (100) kilometers around the migration bird routes, which contain **sites of migratory birds sighted** located in areas with a **higher density of poultry breeders**.

The OESA may propose an inclusion or replacement of cities with settlement sites of migratory birds of interest to them, or a significant existence of subsistence farming poultry, even with deficient records, or the **trade of birds for exposure and/or ornamentation**, but with an important existing amount of bird transportation to other areas with industrial poultry farming.

With the evolving registration of subsistence farming properties and knowledge of migratory bird settlement sites, the list of selected cities may be updated.

Methodological development

Criterion 1 – Selection based on routes and establishments for commercial poultry breeding. A 100-kilometer range of influence (buffer) was drawn around and along the routes (Figure 2).

Criterion 2 - Then, cities were selected that contain sites of migratory birds sighted, have commercial poultry farms (starting at 1000 animals), and are inside the area of influence of the routes of migratory birds.

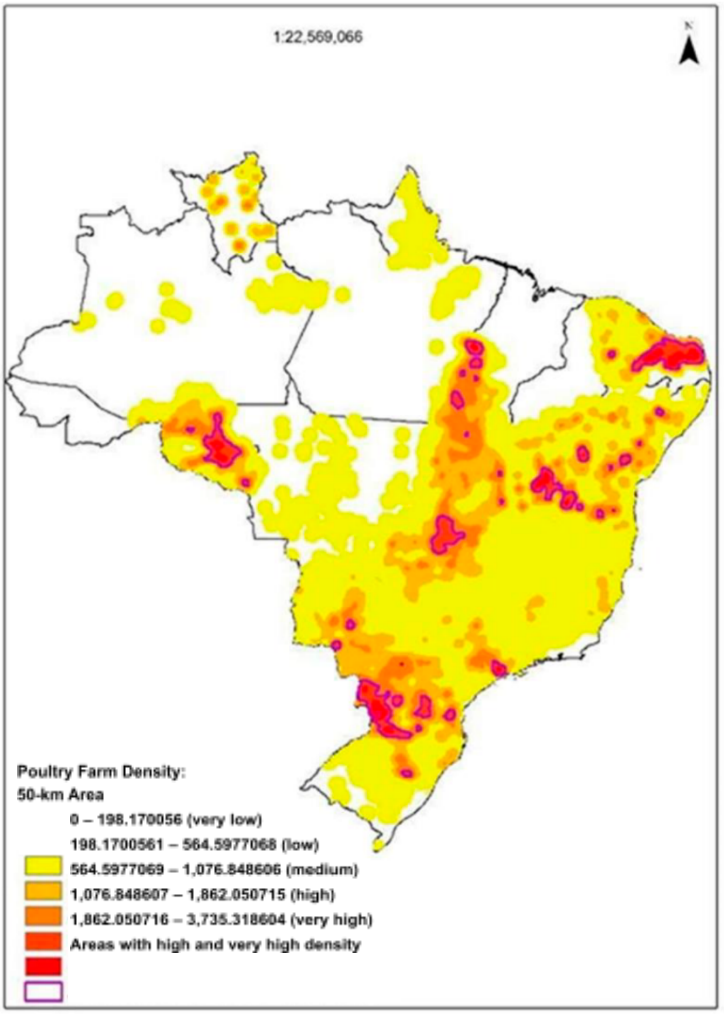

Figure 2: Criteria for selection of cities per 100-km areas of influence of the migratory bird routes (criterion 1) and based on the density of establishments (criterion 2)

From the registries of poultry farms and poultry breeders in Brazil, a density map (kernel) was generated using 50 km as radius. The kernel density estimate is a weighted distance smoothening technique in which a bivariate probability density function is applied to determine the intensity of a spatial point process (Bailey & Gatrell, 1995). The calculated intensity, in this case, was the concentration of poultry farms. The generated density of poultry farms was classified into 4 categories grouped by natural breaks (Armstrong et al., 2003): “Very high”, “high”, “medium”, and “low”.

The areas classified as “very high” and “high” density were chosen to represent the areas with a higher concentration of poultry establishments (Figure 2). These higher-density areas, along with the indicated risk criteria (100-kilometer strip around the migration routes and containing sites of migratory birds sighted), were added to the areas identified as risky.

II. Calculating the size of the sample of epidemiological units

The sample estimate was done based on a probabilistic strategy applied to the whole population of small-scale production poultry in the area at risk.

a. Parameters used for the probabilistic investigation

The sample size was calculated considering a two-stage strategy. For these calculations, we assumed 1% prevalence across epidemiological units and a 95% confidence level. That being so, it is expected that, if AI were present in these sub-populations, it would affect 1% of the poultry farms.

Intra-herd sensitivity is calculated taking into account the laboratory test used (ELISA). Estimates with 95% sensitivity and 100% specificity were considered.

b. Number of epidemiological units to be sampled per region of influence of migratory bird routes

For each region of influence, it is necessary to sample 322 epidemiological units with small-scale and/or subsistence commercial poultry breeding. This calculation takes into account the existence of a large number of commercial poultry breeding establishments, for which reason, the population was deemed to be infinite.

III. Defining epidemiological unit

At this first sampling stage, a comprehensive approach must be taken to defining the epidemiological unit, since we need to adapt to the reality of a population of transnational birds, for which reason, the epidemiological units have been defined according to two types of poultry property:

- In some cases, the epidemiological unit is a poultry farm with limited local trade (for example, small breeders of up to 1,000 birds). In these cases, it is likely that these places are well identified and have good definitions so that they can be regarded as an epidemiological unit itself.

- In other cases, epidemiological units will be subsistence breeders, who have a very small number of birds. In these cases, it is likely that there is no good record of the operation or a very clear definition of the area. Thus, the local grouping of this type of operation should be used, that is, regarding it as a cluster defined by a geographical point and the operations surrounding it.

IV. Estimating the number of animals to be sampled per epidemiological unit

a. Parameters used

The number of birds to be sampled per operation or cluster must be determined based on an estimated intra-herd prevalence of 30% with a 95% confidence level. That is, it is assumed that, if AI is present on a farm, 30% of the birds would be infected. In addition, it is assumed that the sensitivity of the laboratory diagnostic test is 95% and specificity is 100%.

When the property has hens, turkeys, or quails and Anseriformes, the birds to be sampled must preferably be the Anseriformes and adult birds, especially if dead or dying. Birds with clinical signs suggesting AI and NCD should also be prioritized in the sampling.

b. Number of birds to be sampled per epidemiological unit

The number of birds to be sampled per operation or cluster **must be equal to 11 birds**, regardless of the size of the epidemiological unit. For each bird selected for blood collection, tracheal and cloacal swabs must also be collected.

All proposed calculations have been done in the “epiR” and “base” packages of the software program “R”. (Nunes et al., 2020; R Core Team, 2019).

V. Structuring for sampling by dividing the cities into quadrants

This sampling proposes to provided flexibility to local veterinarians in choosing the epidemiological units to be sampled.

The cities selected in each region will be divided into 10x10-km quadrants, for which the quadrants may be randomly chosen to make it easier to sample the epidemiological units of small-scale commercial poultry breeders. Thus, the random choice will indicate a geographic location (such as the centroid of a 10-km square) and then the local services may choose the epidemiological units that are closest to that location, using the criteria below.

Criteria for selecting establishments to be sampled: based on a risk assessment, the selection of epidemiological units must prioritize those where the following situations exist:

a) existing points of attraction for wild birds (such as lakes, dams, etc.);
b) existing Anseriformes birds;
c) evidence of close contact between migratory birds and commercial domestic poultry;
d) poultry bred in liberty (not locked in cages);
e) surface water used as drinking water for birds; and
f) existence of more than one bird species living in the same operation.

In cases where no epidemiological units are found that meet one or more of the above criteria, sampling must be conducted in the neighboring quadrant that meets such conditions.

Study scheduling: Prior to sending the samples, the laboratory must be notified about the place of collection, expected date for the activities, expected quantity of samples to be collected, and expected date of arrival of the samples at the laboratory, **at least 15 days in advance**, in order that the submission can be authorized, subject to any demands by the Official Laboratory.

ANNEX 2. GENERAL PROCEDURES FOR ACTIVE SURVEILLANCE

1. Study planning and organization on a state level

State coordinator → Each federal state will be directed by a State Coordinator for the study, who will set up their own team work with field teams and, if necessary, may establish appoint regional coordinators. The field teams will be under the supervision of an official veterinarian. The total number of teams must be defined by the veterinary service of each state based on the number and geographical distribution of the selected poultry farms and study schedule.

Collection planning → The DSA will provide a list with the poultry farms and places selected for collection. The veterinarians in charge of the field teams must, with that list in hand, jointly with coordinators, indicate for each region the minimum structure to carry out the field activities within the established schedule. Among the initial activities, the state coordinators must distribute the collection forms to the field teams. Planning includes handing out the necessary materials for recording information, collecting the samples, and the biosecurity procedures, as well as training of all professionals involved.

Performance of field team activities → The veterinarian in charge of the field team will make sure that the forms and application for registering activities are correctly used, including their legibility, as well as that the samples collected are correctly identified, stored, and conserved until received by the central unit of the SVE or Regional Coordination, when used. The same professional is also responsible for securing the availability of personal protective equipment (PPE) and safety procedures for the whole team.

Central receiving unit → Each SVE will define a central unit for receiving and verifying the forms and samples, securing their conservation until they are sent to MAPA's laboratory network.

Central data entering team → Each SVE will have a central team for entering the data and information in SIGEP, used for controlling the study database. The LFDA that will receive the samples from each state will be informed by the state coordination of the study, with knowledge to the DISAV/DSA team.

Establishment identification → Each establishment sampled will have a unique identification, which will be the MAPA Code and will be correlated to the establishment code in the OESA's registry. This code will be generated according to the number of breeding establishments to be sampled, as provided by the DSA.

2. Criteria for selecting cores and sheds at the poultry farms sampled in industrial poultry farming

Broiler farms: due to the short life cycles, which leads to a very short period for detection of AI antibodies, the sampled cores and sheds must be those with animals nearing slaughter age, **above the age of thirty (30) days**.

Commercial egg production farms: sample sheds.

Commercial egg production farms: the sampling must prioritize egg-laying hens bred in **uncovered shed systems** or in **alternative (cage-free) commercial systems**, which pose higher risks of AI and NCD exposure.

Breeder bird farms: the sampling must prioritize **parent stock farms**.

Establishments raising ducks, turkeys, and quails (others): the sampling must prioritize these establishments, as they are regarded as a higher risk among industrial breeders. Therefore, replacing them with establishments from another category must be avoided.

The quantities of industrial poultry establishments for the sampling purposes in each state are classified as per **Annex 3**.

For active surveillance for subsistence breeders, see the instructions in **Annex 1**.

3. Criteria for selecting birds

3.1. **Bird age**: at the time of collection, it is necessary to take into account the bird housing period to ensure higher probability that the agent will be detected, in view of the incubation period for AI and NCD viruses. It is thus reasonable to check the age of the birds with the farmer before moving up to the poultry farm.

The birds for sample collection must have been housed at the core for at least **thirty (30) days**, subject to the following possibilities when scheduling collections:

3.1.1. **establishments with only one core**:

3.1.1.1. core with thirty (30) or more days' housing: conduct the investigation and collect the samples;

3.1.1.2. less than thirty (30) days' housing: wait for the minimum period of thirty (30) days and reschedule the investigation to collect the samples; and

3.1.1.3. core without living animals: replace the farm with another one belonging in the same sub-population as the selected poultry farm.

3.1.2. **establishments with more than one core**:

At least one core must be attended to within thirty (30) more days of housing.

3.1.2.1. In this case, collect a sample of cores with over 30 days' housing;

3.1.2.2. cores with no living animals and cores with birds housed for less than thirty (30) days must be ruled out; and

3.1.2.3. if there is no core with at least thirty (30) or more days of housing, reschedule the investigation to satisfy the minimum period in at least one core.

Notes:

a. Specifically for quails, consider a minimum housing period of **fifteen (15) days**.

b. In case one or more poultry farms chosen randomly for sampling have been shut down or depopulated or the bird species is different from the expectations, a new poultry farm must be chosen in lieu of the one that was chosen. For that purpose, the following criteria must be followed: poultry farms under the same risk category; poultry farm with birds of the same species as the selected farm; and preferably located in the same city or, if none, in a neighboring city.

3.2. Samples to be collected in each core:

All cores with birds at the minimum selected age at the randomly chosen poultry farm, up to a **maximum of five (5) cores**.

3.2.1. Type and quantity of samples per core

- **eleven (11)** individual blood samples to obtain blood serum;

- **eleven (11)** tracheal swabs divided into two pols, one with 5 swabs and another one with 6 tracheal swabs;
- **eleven (11)** cloacal swabs divided into two pols, one with 5 swabs and another one with 6 cloacal

swabs.

Notes:

a. Collect the swabs from the same birds that the blood samples will be collected from.

b. The veterinarian in charge of collecting the samples must conduct a general inspection and clinical examination of the birds at the poultry farm, writing their notes down on the corresponding sample collection form.

4. Pre-collection active surveillance checks

- Collection material received;
- Forms with identification of the selected poultry farms, as well as blank forms for cases of farm replacements;
- Age of the birds at the selected poultry farms;
- Form completion with identification of each selected poultry farm;
- Information from the identification tags;
- Investigation scheduling with some farm representative; and
- Attention to the biosecurity procedures applied to each type of bird production, preferably sending different teams for each type of category.

5. Collection and storage of active surveillance samples

Material collections will be carried out during the surveillance activity at the selected poultry establishments. At the time of collection at the poultry farm, the veterinarian must conduct a clinical assessment of the birds and an assessment of the animal science parameters and water and ration consumption parameters and, in case clinical signs or evidence of probable cases of AI and NCD are found, the established passive surveillance procedures must be released, such as interdicting the property, collecting samples, and opening a FORM-IN.

On top of putting in place the procedures provided for addressing suspected cases, as established by the PNSA, the Form of this study must be filled out (which will be entered in SIGEP or another system defined by MAPA), recording the following in the “Notes” field: Suspected case of AI and NCD according to FORM-IN number XXXXXXXX. The samples collected must follow the established protocol for passive surveillance and be registered in e-Sisbravet.

5.1. <u>List of the necessary minimum materials for field active surveillance materials:</u>

Collecting blood to obtain serum:

a) disposable sterilized 5-mL syringes;
b) 25x7 or 25x8-mm needles; and
c) micro tubes (Eppendorf-type) with 2-mL capacity.

Collecting swabs:

a) swabs with a plastic flocked-nylon or non-flocked polyurethane or polyester rod;
b) 15-mL tubes (Falcon-type); and
c) transport medium for viral conservation, according to the options specified in Annex 7 to this manual. The medium quantity follows a proportion of one swab/mL of transport medium, thus, for each vial with a pool of five (5) or six (6) swabs, five (5) or six (6) mL of medium.

d) scissors for cutting the swab rods.

Notes:

- Using swabs with wood swabs or with a cotton end is not recommended;
- To freeze the swabs at the -80 ºC temperature, polypropylene (Falcon-type) tubes must be preferred, since the polystyrene ones may break at that temperature;
- Check the options of conservation medium described in Annex 7 to this manual, observing that the formulations comprise a cell culture medium added to an antibiotic solution and, for the MEM culture medium, the formulation also includes bovine serum or fetal bovine serum or BSA (bovine serum albumin); and
- The transport medium for conserving the samples must be kept frozen in a fridge at -20 ^{o}C until the preparations for collection start.

Material Identification:

a) blank collection forms, according to a template defined by the DSA;
b) sample identification tags (observe what is described in this manual);
c) indelible-ink pen for completing the tag information; and
d) Sample storage material: adhesive tape; recyclable ice; isothermal box; hack for organizing samples vertically, and plastic bags for grouping the samples per core.

5.2. Collection procedure for active surveillance samples

In order to minimize potential health risks, it is advisable that the establishment surveillance and sample collection works follow the usual biosecurity procedures. Following good practices in the process of sample collection, conservation, and dispatching constitutes one of the main factors for a successful collection of material for laboratory diagnosis.

5.2.1. Collecting blood sample to obtain blood serum: it will be done so as to avoid contamination, which can make it impossible to conduct and interpret the laboratory tests. For that reason, sterilized and disposable material should be used.

Blood collection from adult birds will be done through puncture in one of the wing veins, the brachial vein (located in the inner face of the wing drumette), as provided below, or even using the jugular vein:

a) place the bird on a lateral support, restraining it from the lower limbs and neck;
b) raise its wing and locate the brachial vein, which is very superficial;
c) introduce the needle with the bevel upwards and pull the plunger gently to avoid hemolysis;
d) with the 5-mL syringe, take out approximately 2.5 mL of blood, which will guarantee at least 1 mL of serum (this is the minimum serum quantity to be sent to the laboratory);
e) pull the syringe plunder completely and place it slightly inclined over a flat surface, protected from light and at room temperature, until complete coagulation and serum release (usually 2 to 3 hours);
f) following serum release, take out the syringe plunder and shed the serum into a properly identified Eppendorf micro tube;
g) the minimum quantity of serum to be sent to the laboratory should be 1.0 mL, subject to a maximum filling level of two-thirds (2/3) of the Eppendorf tube, since upon freezing there will be a volume increase with a risk of content overflow;
h) place the identification tag on the micro tube individually;
i) fasten the micro tube in an appropriate polystyrene holder or support so that it can be kept in an upright position (with the lid upwards); and
j) keep it immediately refrigerated.

Notes:

- In quails, blood collection can be done from either the right jugular vein or from the wing (ulnar), and it can also be collected from the heart or the occipital venous sinus, though with a risk of death of the bird.

- In turkeys and ducks, blood collection can be done using either the ulnar or the medial metatarsal vein, though the occipital venous sinus can also be used.

- The tubes containing the serum samples must be frozen vertically (upright position) and kept at minus twenty degrees Celsius (-20ºC), with any thawing being avoided. Never freeze serum samples in which clotting has formed, since hemolysis will occur and jeopardize the laboratory tests.

- In case there are no means for sample freezing, the samples must be kept under refrigeration and arrive at the laboratory within a maximum of three days.

- The serum samples must be in adequate order, that is, limpid, frozen/cooled, legibly identified, and well stored.

5.2.2. Collecting tracheal swabs:

k) use an adequate swab for the bird's size. Metal swabs are thinner and, for that reason, they are indicated for more delicate birds.
l) open the bird's beak and introduce the swab;
m) rub it on the larynx and trachea walls to collect as many cells as possible, taking care not to hurt the birds;
n) make sure that there are no food remnants on the swap, which may indicate that it was introduced into the esophagus;
o) place the swab in the Falcon-type tube containing a transport medium for viral conservation;
p) break or cut the swab rod along the tube cover and close the tube;
q) shake it with rotating movements to release the cells;
r) add the other swabs (a total of five or six) to the tube to form the pool;
s) place an identification tag onto the tube containing the pool;
t) fasten the tube in an appropriate polystyrene holder or support so that it can be kept in an upright position; and
u) keep it immediately refrigerated.

5.2.3. Collecting cloacal swabs:

a) use an adequate swab for the bird's size. Metal swabs are thinner and, for that reason, they are indicated for more delicate birds.
b) raise the tail feathers and introduce the swab rubbing against the walls;
c) place the swab in the Falcon-type tube containing a transport medium for viral conservation;
d) break or cut the swab rod along the tube cover and close the tube;
e) shake it with rotating movements to release the cells;
f) keep adding the other swabs (a total of five or six) to the tube to form the pool;
g) place an identification tag onto the tube containing the pool;
h) fasten the tube in an appropriate polystyrene holder or support so that it can be kept in an upright position; and
i) keep it immediately refrigerated.

6. Identifying the samples

The samples will be identified with the codes generated by SIGEP or as per instructions defined in advance by MAPA. The sample identification must be entered in the collection form and on the Eppendorg tube.

Following the collection work, all the material used must be removed from the property.

7. Filling out the form

The bird sample collection form must be filled out on an application to be made available by MAPA or on paper with legible handwriting and pen and signed by the OV in charge of collection. That form will be available in SIGEP or such other system as made available by MAPA, and a specific form to be completed will be provided. In case the form is filled out on the application, it must be printed later and sent to LFDA along with the sample.

The original collection form will be filed with the LVU in charge of the selected establishment. The information from the collection form must be entered in SIGEP.

To collect samples at the poultry farm, a form has been defined according to a template that will be available in advance. Correctly using these forms accounts for a fundamental point to the quality and progress of the work, it being worth stressing that all the collection effort may be lost if the information has not been adequately recorded.

8. Storage and dispatching of samples to the laboratory

Prior to submission to the laboratory or the state receiving and verifying center, the OV in charge of collecting and filling out the collection form must perform a last check to verify that all information and due identification relate to the samples collected at that establishment.

Storage and transportation of the samples are very important in maintaining the quality of the collected material and must comply with the biosecurity rules.

The collected material may be sent to a screening center, such a state laboratory, where the samples may be properly stored and jointly forwarded to LFDA. Alternatively, the samples may be immediately sent to the official laboratory as they are collected.

8.1. Storing the samples:

Put the samples in a polystyrene box with recycled ice in the bottom, on the sides, and at the top. Using nine parts of recyclable ice for each sample part stored in the polystyrene boxes is advisable. Support the samples so that they will remain steady inside the polystyrene box (empty spaces may be filled with polystyrene pieces, paper, or similar items) and send them to the official laboratory jointly with the Sample Collection Form.

The LFDA to receive the samples from each federal state will be defined and notified by the DSA, jointly with CGAL/DTEC.

In case the samples cannot be sent immediately, store the vials containing the swabs (tracheal and cloacal) and the serum under refrigeration until the time that they are sent to the official laboratory.

The samples can be kept under refrigeration for a maximum of 96 h (including the period of transportation up to the laboratory) or frozen at -80 °C if they need to be stored for longer periods.

The freezer compartment of household fridges must never be used for storing the materials. Keeping the material under refrigeration or with dry ice is preferable.

Avoid successive cycles of freezing-thawing.

When the distance to be run for transporting the material is long and it can be kept frozen, prefer using dry ice. In this case, care should be taken to use double packaging so that the dry ice will not come in direct contact with the samples. Bear in mind that dry ice transportation has its own specific regulations that need to be complied with.

8.2. Sending the samples

Each completed Sample Collection Form must be in three (3) counterparts:

a) one going along with the sample to the Official Laboratory;
b) one sent to the State Coordinator; and
c) one filed with the local veterinary unit.

Avoid, except for emergencies, doing material dispatches to the laboratory during weekends and holidays, since the time in transit in these cases can be even longer. Schedule sample dispatches to LFDA so that they will preferably take place between Monday and Wednesday.

9. Use of SIGEP or another system made available by MAPA to manage the study

To monitor and manage the data on establishments and samples from "Industrial Poultry Farming", SIGEP or such other system as defined by MAPA will be used. Entering the data provided in the sample collection forms into the system will be the responsibility of the OESA.

10. Laboratories

The samples will be analyzed by the Federal Farming Protection Laboratory in Campinas – SP.

11. Diagnostic testing

The diagnosis protocols described in the AI and NCD surveillance plan will be used. Thus, upon identifying a positive or inconclusive sampling in ELISA, the Laboratory must notify the DSA to investigate the suspected case.

12. Laboratory result

Every laboratory result must be entered into SIGEP or such other system as defined by MAPA. No test reports will need to be issued and sent. Positive results must, on top of being entered into the system, be sent to the DSA, to the OESA Central Unit, and to the animal health service of the Office of the Federal Superintendent for Agriculture, Livestock, and Supply (SISA/SFA). Only positive cases will be timely communicated.

13. Payment of costs with material purchases and sample submission to LFDA

The costs in connection with purchasing materials and sending the samples to the laboratories, for the AI and NCD Surveillance Plan, must preferably be agreed upon between the OESA and the private sector.

ANNEX 3 – Number of establishments to be sampled in component 3 – ACTIVE SURVEILLANCE FOR INDUSTRIAL POULTRY FARMING by State and type of production

Surveillance areas	Number of establishments to be sampled				
	Broiler farms	Commercial egg production farms	Breeding farms	Others	Total
AREA 1					
RS					
AREA 2					
SC					
AREA 3					
PR					
AREA 4					
ES					
MG					
RJ					
SP					
AREA 5					
BA					
SE					
AL					
PE					
PB					
RN					
CE					
PI					
MA					
AREA 6					
AC					
AM					
AP					
PA					
TO					
AREA 7					
DF					
GO					
MS					
MT					
Total					

ANNEX 4 – REGIONAL CHARACTERIZATION RELATIVELY TO POULTRY PRODUCTION

1.1 South

Brazil's South accounts for 64% of the production of broilers. Paraná is the national leader in production (35.54%), followed by Santa Catarina (14.89%), and Rio Grande do Sul (13.65%). With high biosecurity levels, 62.5% of the fertile-egg parent stock farms are located in the South (13.9% in PR; 4.5% in SC; and 3.2% in RS).

The states in this region account for 99.8% of the national production of ducks (99.75% in SC, 0.03 % in RS, and 0.05% in PR) and 99.9% of the national production of turkeys (56.54% in RS, 39.45% in SC and 3.99% in PR). In the South are also 15% of ostrich breeding establishments.

Commercial egg production in the South accounts for 13% of the national total, and Rio Grande do Sul leads the production of eggs for human consumption.

In Brazil's South are four sites of migratory birds (Estação Ecológica do Taim - RS, Parque Nacional da Lagoa do Peixe - RS, Foz do Rio Araranguá- SC, and Foz do Rio Tijucas - SC), recognized by the Department of Animal Health for active surveillance for avian influenza and Newcastle disease viruses. At these sites, serum tests have already identified subtypes H1, H3, H4, H5, H6, H7, H8, H10, H11, H12, H13, H14, H15, and H16 of the avian influenza virus, without any molecular detection or virus isolation.

1.2 Southeast

Brazil's Southeast (São Paulo, Rio de Janeiro, Espírito Santo, and Minas Gerais) stands out for its commercial egg production and accounts for nearly half of the nation's production of eggs for consumption, with the state of São Paulo as the second largest producer 29.63%; followed by Minas Gerais with 10.54%; and Espírito Santo with 9.17%.

The production of broilers in the Southeast accounts for around 17% of the national total, with 8.32% in São Paulo; 7.44% in Minas Gerais, and 0.68% in Espírito Santo (ABPA, 2022). The region also includes 29.5 % of the nation's fertile-egg parent stock farms, and São Paulo and Minas Gerais concentrate 64% of the ostrich breeding establishments.

In the state of São Paulo are sites of migratory birds (Ilha do Cardoso - SP and Ilha Comprida - SP), recognized by the Department of Animal Health for active surveillance for avian influenza and Newcastle disease viruses. At these sites, serum tests have identified subtypes H3, H6, H8, H12, and H16 of the avian influenza virus, without any molecular detection or virus isolation.

1.3 Central-West

Brazil's Central-West (Goiás, Mato Grosso, and Mato Grosso do Sul) has in its grain abundance a favorable scenario for the growth of poultry farming. Poultry farming in the region's states is growing and currently accounts for 15% of the national production of broilers, with 8.272% in Goiás; 3.76% in Mato Grosso, and 2.75% in Mato Grosso do Sul.

The production of eggs for consumption accounts for 11.5% of the national egg production, with 5.59% in Mato Grosso; 4.63% in Goiás, and 1.71% in Mato Grosso do Sul (ABPA, 2022).

Mato Grosso do Sul further has 2.4% of all fertile-egg parent stock farms. Also in the region are 6% of the ostrich breeding establishments.

In Central-West are three sites of migratory birds (Praias do Rio Paraguai - MT, Praias do Rio Araguaia – MT, and Pantanal - MS), recognized by the Department of Animal Health for active surveillance for avian influenza and Newcastle disease viruses. At these sites, serum tests have identified subtypes H1, H3, H4, H6, and H14 of the avian influenza virus, without any molecular detection or virus isolation.

1.4 Northeast

Brazil's Northeast (Bahia, Sergipe, Alagoas, Pernambuco, Paraíba, Rio Grande do Norte, Ceará, Piauí, and Maranhão) has 2.5% of the national production of broilers, with Pernambuco being a highlight.
Commercial egg production is very expressive in the region, with the states of Pernambuco (8.19%); Ceará (5.26%), and Bahia (1.67%) representing around 15% of the national egg production (ABPA, 2022).
In the Northeast are seven sites of migratory birds (Mangue Seco - BA, Cacha Pregos - BA, Coroa Vermelha - BA, Cetrel - BA, Coroa do Avião - PE, Fernando de Noronha - PE, and Galinhos - RN, Panaquatira - MA, Guará - MA) recognized by the Department of Animal Health for active surveillance for avian influenza and Newcastle disease viruses. At these sites, serum tests have identified subtypes H5, H8, H10 EH16 of the avian influenza virus, without any molecular detection or virus isolation.

1.5 North

Brazil's North (Tocantins, Pará, Amapá, Roraima, Amazonas, Acre, and Rondônia) has a more expressive poultry production in the state of Pará, followed by Rondônia, and Tocantins. The region's poultry farming accounts for 1.5% of the national production of broilers and eggs. Approximately 1.5% of the national production of eggs for consumption is in the North.
In the North are five sites of migratory birds (Ilha de Marajó - PA, Bahia de Marajó - PA, and Salinópolis - PA), recognized by the Department of Animal Health for active surveillance for avian influenza and Newcastle disease viruses. At these sites, serum tests have not identified any H subtypes of the avian influenza virus.

ANNEX 5 – PERSONAL PROTECTIVE EQUIPMENT (PPE)

Guidance on Personal Protective Equipment (PPE) for investigations of suspected SRN cases and exceptional mortality of wild birds

Taking into account the actions of the Official Veterinary Service (OVS) in **response to suspected cases and potential foci of avian influenza** (AI), the DSA provides instructions on the use of personal protective equipment (PPE) that can be used by that service in those actions.

We suggest that the minimum list of PPE to be used during these activities include overall, apron, mask/respirator, boots, glasses, and disposable gloves, according to these characteristics:

I. Overall for full body protection during operations that include risks of contamination by chemical agents, made of high-density, 100% polyethylene fiber, nonwoven fabric, with a front-side opening and zip lock closure, elasticated hand cuffs and hems, no boot, and including a hood, with a simple seam;
II. Apron for chemical and microbiological protection, made of high-density, 100% polyethylene, nonwoven fabric, with a polyethylene cover over the fabric, 71 grams per square meter, front model, with a waist piece measuring approximately 115 centimeters, a neck piece measuring approximately 55 centimeters, and a minimum length of 120 centimeters;
III. PFF2 or superior mask - disposable half-facepiece particulate filter respirator (PFF2 or superior) for dust, fog, fumes, and low concentrations of acid gases. It comprises an inner support shell of non-woven fabric molded into synthetic fibers, a filter medium comprising a layer of electrostatically treated micro fibers and a layer of activated carbon-charged micro fibers to retain acid gases. The outer part of the respirator is made up of a non-woven cover for filter medium protection. The set must contain two elastic bands, a nose adjustment clip, and an exhalation valve;
IV. Hood compatible with a powered air purifying respirator. Made of polypropylene-coated polyester with a double shroud that is as long as back and chest of the user, a system provided with an elastic for a better adjustment to the user's neck. Transparent front side visor made of polyethylene terephthalate glycol in a semi-circular shape. In the inner part of the hood is a system for fastening it to the user's head through a suspension provided with a sweat-absorbing strip and a simple adjustment. Rear opening with a quick-lock system for the tube, made of a plastic material;
V. Boot for chemical protection and water operations, made of PVC. It must be highly resistant to abrasion, tearing, and tensile stress. With lining and anti-slip sole. High calf at least 32 centimeters tall;
VI. Glasses with good skin sealing, flexible PVC structure to easily fit all the outlines of the face, with uniform pressure, adjustable band in order not to become loose during the activity, and indirect ventilation to avoid fogging. It also needs to support prescription glasses. In addition, the lens must be made of a transparent plastic and be anti-fogging and scratch-resistant. The glasses can be either reusable (provided that all adequate decontamination actions are taken) or disposable; and
VII. Glove for protection against biological agents, made of natural latex, non-sterile, disposable, lubricated, with a bioabsorbable powder. Uniform texture, anatomical format, ambidextrous, high tactile sensitivity, good elasticity, resistant to tensile stresses, with wrist cuffs, minimum thickness of 0.22 centimeters. Sizes: small / medium / large / extra-large.

It is worth highlighting that the PPE (whether nationally manufactured or imported) can only be purchased if it has an indication of the Certificate of Approval (CA) issued by the competent national authority for occupational safety and health matters under the Ministry of the Economy.

Each OESA must have a sufficient quantity for routine responses to suspected SRN cases. In the event of AI foci, it is understood that the OESA must permanently have a sufficient quantity of PPE to secure an adequate response for at least 30 days, according to the reality of the production industry in each state. Defining strategies for purchasing, storage logistics, and distribution of said PPE will be responsibility of each OESA.

Active surveillance:

With regard to active surveillance activities, it is expected that inspections and sample collections will be performed on birds without clinical signs of AI. Thus, it is advisable to follow the regular biosecurity rules of the poultry farms, wearing routine overalls or disposable aprons, disposable gloves, shoe covers, disposable masks, and safety glasses, and the indicated PPE does not need to be used when responding to suspected SRN cases.

ANNEX 6 – FORMULATION OF TRANSPORT MEDIA AND PREPARATION OF TUBES FOR COLLECTION

Option 1: Cell culture medium MEM ("Minimal Essential Medium") with 10% bovine serum (or 10% fetal bovine serum) and 0.5X concentration of antibiotic solution.

Formula:
- 850-ml sterile MEM cell culture medium.
- 100-ml sterile fetal bovine serum (or bovine serum).
- 50-ml sterile 10X antibiotic solution (prepared according to the formula below).

Distribute 5 ml per Falcon tube (1 ml per swab) and keep it frozen until use.

Option 2: BHI ("Brain Heart Infusion") medium with a 0.5X antibiotic solution.

Formula:
- Brain infusion: 200g
- Heart infusion: 250g
- Proteose peptone: 10g
- Dextrose: 2 g
- Sodium chloride: 5g
- Disodium phosphate: 2.5g

Hydrate in 1000 ml of deionized water and adjust pH to 7.4±0.2. Autoclave it (121 ºC/15 min). Add 50 ml of the sterile 10X antibiotic solution to 950 ml of the sterile BHI broth. Distribute 5 ml per Falcon tube (1 ml per swab) and keep it frozen until use.

Option 3: Buffered Tryptose Phosphate broth with a 0.5X antibiotic solution.

Formula:
- Tryptose: 20g
- Dextrose: 2g
- Sodium chloride: 5g
- Disodium phosphate: 2.5g

Hydrate in 1000 ml of deionized water and adjust pH to 7.3±0.2. Autoclave it (121 ºC/15 min). Add 50 ml of the sterile 10X antibiotic solution to 950 ml of the sterile Buffered Tryptose Phosphate broth. Distribute 5 ml per Falcon tube (1 ml per swab) and keep it frozen until use.

10X antibiotic solution:

Dulbecco's formula (DPBS):
- Sodium chloride: 8g
- Potassium chloride: 0.2g
- Calcium chloride: 0.1g
- Sodium phosphate dibasic: 1.03g
- Potassium phosphate monobasic: 0.2g
- Magnesium chloride: 0.1g

Hydrate in 1000 ml of deionized water. Autoclave it (121 ºC /15 min) and store it at 4 ºC.