Ministry of Agriculture and Livestock
Secretariat of Animal and Plant Health
Department of Animal Health

Report on the Surveillance plan for Avian Influenza and Newcastle disease

1st cycle

Mission of MAPA:
To promote the sustainable development of Brazil's agribusiness production chains in order to benefit Brazilian society at large

Brasilia
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Ministry of Agriculture and Livestock
Secretariat of Animal and Plant Health
Department of Animal Health
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Report on the Surveillance plan for Avian Influenza and Newcastle disease

Cycle - 2022/2023

DIVISION FOR THE MANAGEMENT OF SURVEILLANCE PLANS — DIGEV

Department of Animal Health - DSA
Brasilia, January 2024

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LIST OF ABBREVIATIONS

AI - Avian Influenza

ARNS - Avian respiratory and nervous syndromes

COA – Official Laboratory Test Certificate

DSA - Department of Animal Health

ELISA - Enzyme-linked immunosorbent assay

e-Sisbravet - Electronic tool of the Brazilian System for Veterinary Surveillance and Emergencies

HI - Hemagglutination Inhibition Test

HPAI - Highly pathogenic avian influenza

IBAMA - Brazilian Institute for the Environment and Renewable Natural Resources

ICMBio - Chico Mendes Institute for Biodiversity Preservation.
IDAF - Espirito Santo State Institute for Livestock and Forest Protection (*Instituto de Defesa Agropecuária e Florestal*)

IPRAM - Institute for Research into, and Rehabilitation of, Marine Animals (*Instituto de Pesquisa e Reabilitação de Animais Marinhos*)

LFDA - Federal Animal and Plant Health Laboratories (*Laboratórios Federais de Defesa Agropecuária*)

LPAI - Low pathogenicity avian influenza

MAPA - Ministry of Agriculture and Livestock

ND - Newcastle disease

OESA - Animal and Plant Health Executing Agency (*Órgão Executor de Sanidade Agropecuária*)

OVS - Official Veterinary Service

PCR - Polymerase chain reaction

PNSA - National Poultry Health Program

RT-qPCR: Reverse Transcription followed by a Real-Time Polymerase Chain Reaction

SDA - Secretariat of Animal and Plant Health (*Secretaria de Defesa Agropecuária*)

SFA - Offices of the Superintendents of Agriculture and Livestock (*Superintendências de Agricultura e Pecuária*)

SVS - State Veterinary Service

WOAH - World Organization for Animal Health

EXECUTIVE SUMMARY

The Ministry of Agriculture and Livestock (MAPA)'s Department of Animal Health (DSA) reviewed the surveillance strategies for Avian influenza (AI) and Newcastle disease (ND) in 2022, and established a new surveillance plan whose main objective is to enhance and promote the surveillance system in order to control, monitor and prevent the occurrence of these diseases within the national territory.

Passive surveillance is the most suitable strategy for the early detection of these diseases, and is to be based on mandatory immediate notification of suspected cases for investigation by the Official Animal Health Service, where the necessary measures will be taken to confirm an outbreak and apply the measures provided for in the Contingency Plan which aims to contain and eradicate the diseases and restore the country's disease-free status.

In addition to promoting passive surveillance in order to extend the early detection capacity, the AI and ND surveillance plan seeks, through active surveillance activities, to demonstrate absence of AI or ND infection in the poultry industry, in accordance with international trade-related surveillance guidelines, to monitor the occurrence of infections in migratory wild birds, through surveillance performed in backyard flocks in areas close to those areas where such species concentrate, and to direct risk mitigation actions and prevent the introduction of these diseases into poultry.

In the epidemiological context of the preparation of the new plan, highly pathogenic avian influenza (HPAI) had not been detected in Brazil. However, on May 15, 2023, the DSA notified the World Organization for Animal Health (WOAH) of the first detection of highly pathogenic influenza Virus A H5N1 in Brazil, detected in three migratory coastal birds, which were two Cabot's terns (*Thalasseus acuflavidus*) and one brown booby (*Sula leucogaster*). Subsequently, on June 27 of the same year, the first outbreak of highly pathogenic avian influenza A in a backyard flock in the municipality of Serra, Espirito Santo, was confirmed.

Infection by HPAI virus in commercial poultry establishments has not been found in Brazil, and for this reason the country remains HPAI-free to the WOAH.

In order to monitor viral changes, the epidemiological impact, and better understand transmissibility and pathogenicity of the HPAI virus, MAPA integrated genomic surveillance into the AI and ND surveillance system. Use of this genomic tool enabled risk assessment for public health. To date there have been no confirmed cases of HPAI in human, in Brazil.

Testing of the complete genomic sequencing of the virus H5N1, clade 2.3.4.4b, obtained from the first highly pathogenic avian influenza A outbreak in *Thalasseus acuflavidus* (Cabot's tern) in Brazil revealed significant similarity to the H5N1 viruses detected in Chile, Peru and Uruguay in 2022 and 2023. Thus the data resulting from genomic surveillance showed that the likely source for the introduction of the virus into Brazil was the migration of infected wild birds along the Pacific route. Among outbreaks in backyard flocks reported up until June 30, 2023, phylogenetic testing of the viruses showed greater similarity of the hemagglutinin gene (99%) to isolated strains of H5N1 detected in Chile and Uruguay, while the viruses isolated from wild birds in Brazil

suggest that the origin of the infection in the backyard flocks was through contact with infected wild birds.

In order to ensure the dissemination and transparency of its activities, DSA presents the results obtained in the first cycle of the AI & ND surveillance plan 2022-2023, in the present document. The activities performed within the scope of the AI & ND surveillance plan have been delineated and coordinated by this Department, which reports to MAPA's Secretariat of Animal and Plant Health, and have been executed by the veterinary services of those States that are involved. The plan was delineated in 2022, and included the preparation of manuals, and the holding of technical meetings for validation and standardization. Field work was carried out between July 2022 and June 2023. The activities that have been performed, and results obtained, are presented below.

Part A. OVERVIEW OF THE SURVEILLANCE PLAN

1. Objectives of surveillance

The major objectives of the AI and ND surveillance plan are:

Objective 1 - To achieve early detection of cases of AI and ND: the goal of early detection of suspected cases of AI and ND is attained by means of immediate notification of, and response to, suspected cases (passive surveillance) in populations of domestic and wild birds.

Objective 2 - To demonstrate absence of infection by AI and ND within the area of coverage of the industrial poultry-raising plan, in accordance with international trade-related surveillance guidelines: the data generated by the active AI and ND surveillance system in commercial poultry are intended to certify the production chain's disease-free status, providing continual support for confirmation of the sanitary status before WOAH and before importing countries.

Objective 3 - To monitor the occurrence of viral strains of AI: in order to underpin the strategies for public health and animal health.

1.1. Description of target population

For the purposes of the present plan, the following definitions apply:

Industrial (commercial) poultry flocks: the entire set of operations carried out by commercial growers who incorporate technological advances in genetics, nutrition, health, biosecurity, and who accompany animal husbandry indices in their production.

The group includes vertically-integrated poultry farmers, members of cooperatives and independent farmers who access the major processing and distribution channels of the production chain.

Small-scale commercial poultry flocks: a poultry for local trade (small-scale commercial operations) with only limited access to certain production-chain processing and distribution channels.

Backyard flocks: a non-poultry backyard flock that belongs to non-industrial-scale farmers who are mainly registered in the state-level animal health services, who incorporate few technological advancements, and for whom production is intended for their own consumption (subsistence).

Wild birds: the wild birds of concern for surveillance are mainly migratory seabirds, in other words those species for which at least a part of whose populations carry out cyclical and seasonal movements that are highly faithful to their breeding sites, and that are associated with aquatic environments, such as Anseriformes (ducks, geese and teal) and Charadriiformes (gulls, jacanas, sandpipers and terns).

1.2. Identification of the components of the surveillance system

Each component of the surveillance system comprises an activity to investigate for the presence of an infectious agent or disease in the target population. The set of the components or activities of surveillance that are able to detect the presence of a particular pathogen or disease comprise a surveillance system.

The surveillance plan for AI and ND is formed of the five components listed below:

1. **Passive surveillance in poultry and non-poultry flocks**
2. **Passive surveillance in wild birds**
3. **Active surveillance in industrial poultry production**
4. **Active surveillance in backyard and small-scale poultry commercial flocks**
5. **Active surveillance in AI-free and ND-free compartments**

1.3. Current epidemiological status

1.3.1 Avian Influenza

After the start of sampling for the cycle 2022-2023 there was a change in Brazil's epidemiological situation for AI. It was due to the first detection of highly pathogenic avian influenza A within the territory of the country, on May 15, 2023. Up until that point, the disease had been deemed to be exotic to the country.

The first detected case occurred in two seabirds of the species *Thalasseus acuflavidus* (Cabot's tern) as well as in one individual *Sula leucogaster* (brown booby) in the state of Espirito Santo. All investigations and sampling were performed as set forth in the AI and ND Surveillance plan, and as per the technical data sheets for AI and ND. The diagnosis was performed in accordance with the provisions of the WOAH Terrestrial Manual and in the official laboratory designated for this purpose. Actions taken to control and address the highly pathogenic avian influenza A outbreak were carried out by the Espirito Santo State Institute for Livestock and Forest Protection (IDAF/ES) in partnership with the Institute for Research into, and Rehabilitation of, Marine Animals (IPRAM), the Ministry of the Environment (ICMBio and IBAMA), and the Program for Monitoring Beaches (PMP), and coordinated by DSA/MAPA. The case was documented in TECHNICAL NOTE NO.11/2023/DSA/SDA/MAPA. The affected municipalities are shown in figure 1, below.

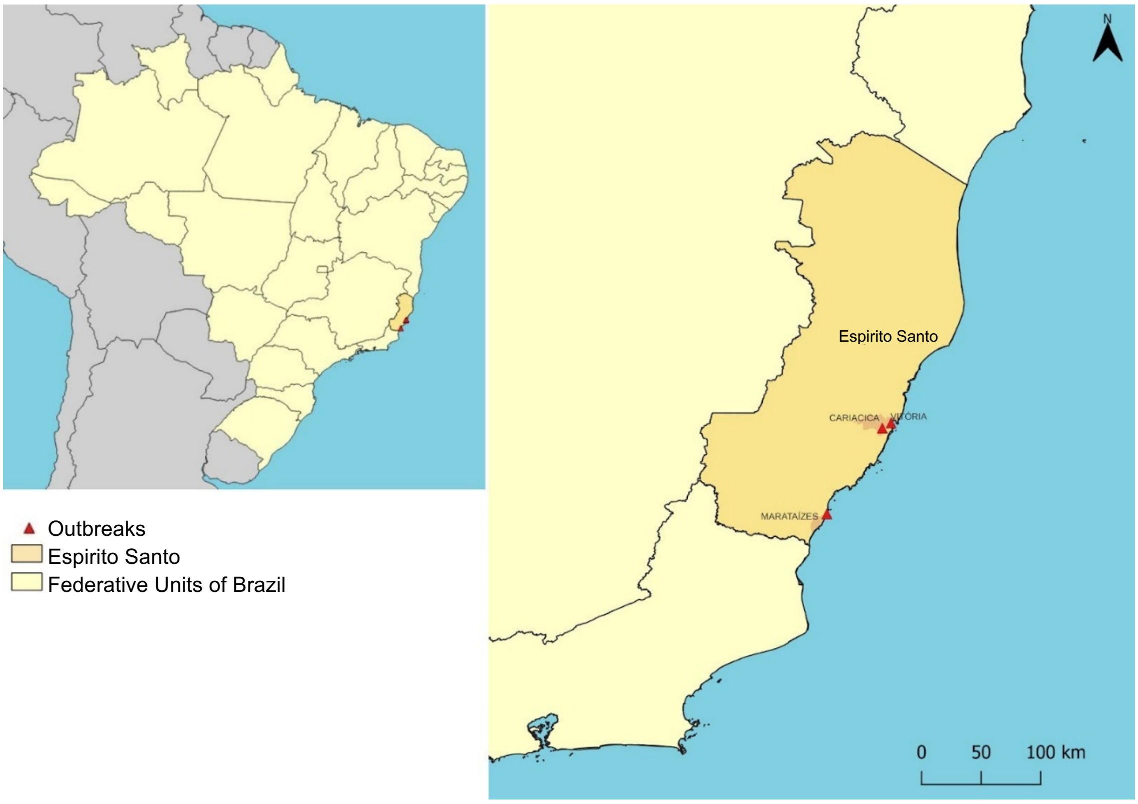

Figure 1. Location of the first outbreaks of highly pathogenic avian influenza (H5N1) in wild birds in Brazil

Just over one month after the first outbreak in wild birds, the first outbreak among backyard flock in Brazil, in the municipality of Serra, Espirito Santo State.

In early June, MAPA published a public panel on AI in order to make public the number of highly pathogenic avian influenza A investigations and outbreaks in Brazil, with all due transparency. Using the public panel and the advanced search filters and the passive surveillance data updated each day, users can read the data on outbreaks by type of bird (wild birds, backyard and poultry), the species of wild and backyard birds affected by AI, and the geographical distribution of the investigations. Access the public panel at: https://mapa-indicadores.agricultura.gov.br/publico/extensions/SRN/SRN.html.

1.3.2 Newcastle Disease

Brazil's epidemiological situation regarding ND is the same as in 2006. So much so that the most recent outbreaks occurred in backyard flock in the State of Mato Grosso, while, in the present case, infections occurred in Columbiformes and eared doves only by pigeon paramyxovirus type 1 (PPMV-1), which poses a low level of threat to the poultry industry because domestic birds are more susceptible to the velogenic strains of avian paramyxovirus type 1 (APMV-1).

Normative Instruction no. 56 published in 2007 states in Article 27 that commercial breeding and egg-laying birds are to be vaccinated systematically against ND, while broilers are authorized to be vaccinated in the event their growers deem this necessary.

1.4. Participation of public and private sector institutions

MAPA's liaison with other federative entities, in particular its joint efforts with health and environmental agencies at federal and state levels, has been of the utmost importance for the effective execution of components 1 and 2 of the AI and ND surveillance plan. For components 3 and 4, the cooperation of the production sector has been an important factor in the financing of material for carrying out epidemiological surveillance and sample-taking activities.

Field activities such as the investigation of suspected cases, establishment inspections, and sample-taking, were performed and funded by the official animal health service in all of Brazil's states.

Surveillance-related sample-taking on farms in the AI- and ND-free compartments was executed by private veterinarians working for the responsible companies. The compartments are audited by Federal Agricultural Inspector/Auditors (AFFAs) working in the state-level Offices of the Superintendents of Agriculture (SFAs) in the states of GO, MG, MS, PR, RS and SP. In the state of Santa Catarina (SC) audits are shared between veterinary officers of the SFA and the Animal Health Executing Agency (OESA - *Órgão Executor de Sanidade Agropecuária*).

As set forth in the plan, the provision of data-gathering computer systems, data analysis, and the drafting of reports, are all the responsibility of DSA/MAPA. The data-gathering and analysis tool used in order to make surveillance system components 1 and 2 operational was e-Sisbravet (Brazilian System for Veterinary Surveillance and Emergencies — *Sistema Brasileiro de Vigilância e Emergências Veterinárias*). The official veterinary service used the data-gathering application Epicollect5 in order to make components 3 and 4 operational. Finally, the data for component 5 were managed using computerized case files in SEI (Electronic Information System — *Sistema Eletrônico de Informações*) and this management was supplemented by the use of Google spreadsheets.

Table 1. Responsibilities of stakeholders in the Avian influenza and Newcastle disease surveillance plan

Stakeholders	**Responsibilities laid down in the Plan**	**Participation in the 1st cycle (effective or otherwise)**
Official animal health service	To standardize and manage sanitary activities, to maintain the database; to analyze and disseminate information; to investigate suspected cases; to inspect birds and harvest samples; to provide financing, capacity-building, education, and communication	Effective
Growers	To give notification of suspected cases, to adopt good practices (of documentation and biosecurity), financing	Effective

Poultry production industry	To give notification to the authorities of suspected cases, to disseminate and provide surveillance information, funding	Effective
Public approved laboratories	To give notification of suspected cases, to disseminate information, to perform screening tests for poultry compartmentalization, and the import/export of avian genetic material (AGM)	Effective
Private laboratories	To give notification of suspected cases, to disseminate information, to ship samples received to the LFDA, to perform screening tests for the active surveillance of wild animals	Effective
Veterinarians	To give notification of suspected cases, to generate relevant information (animal husbandry reports), to take samples for animal health monitoring and certification in compartments, to implement biosecurity, and to disseminate information	Effective
Service providers	To notify authorities of suspected cases; to disseminate information, to provide biosecurity	Effective
Ministry of the Environment and Climate Change (IBAMA and ICMBio) and environmental professionals	To give notification of suspected cases, to follow up investigations in wild animals, to take samples when appropriate, to provide and disseminate information	Effective
Poultry Growers' Association	To disseminate information; to provide funding and institutional support	Effective
Funds for the animal health system	To disseminate information; to provide funding	Effective
Universities	To perform surveillance and screening tests for active surveillance of wild animals, to disseminate information	Effective
Ministry of Science, Technology, and Innovation	To disseminate information, to provide financing, to perform screening tests for the active surveillance of wild animals	Effective
Ministry of Health	To investigate and consult with humans who came into contact with probable cases of HPAI in wild animals and domestic poultry, and disseminate information	Effective

2. Definition of case

The target population for surveillance comprises commercially-farmed poultry, non-poultry, show birds, ornamental birds, companion birds and wild birds. All these birds are the target of surveillance for Avian Respiratory and Nervous Syndrome (ARNS), directed against AI and ND.

The Official Veterinary Service (OVS) is to be notified immediately of all suspected cases of ARNS and probable cases are to undergo laboratory testing.

Definitions of how a case is defined, are given on the Technical Data Sheets for AI and ND and may undergo revision depending on evaluation by DSA.

For the present cycle, the criteria defined below were used:

2.1. Suspected case of Avian Respiratory and Nervous Syndrome

Identification of at least one of the following criteria:

1. mortality greater than or equal to 10% in up to 72 hours, in any commercial poultry-raising establishment or in a single poultry house of a production center in a commercial poultry farm or breeding establishment; or
2. Exceptional mortality (sudden and raised) in populations of backyard flocks, show birds, ornamental birds, companion birds, or wild birds; or
3. abnormal behavior in populations of wild birds, above all migratory aquatic birds;
4. the presence of clinical signs or lesions** (whether neurological, respiratory or digestive) that are compatible with avian respiratory or nervous syndromes (ARNS) in any type of birds; or
5. a sudden drop that is greater than or equal to 10% in the production of eggs, and an increase in deformed eggs, in breeding or in laying birds; or
6. a positive serological test result or the detection of nucleic acid (PCR) for any type of bird.

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

**lesions: to identify the presence of lesions of ARNS, the Official Veterinarian is to necropsy those birds with clinical signs or recently dead.

2.2. Probable case of Avian Respiratory and Nervous Syndrome

Any suspected case that meets at least one of the following criteria:

1. increased rates of mortality without proof of the occurrence of a non-infectious insult***; or
2. the presence of birds showing neurological signs compatible with ARNS; or

3. The association of two or more criteria of suspected cases; or
4. a positive test result for the detection of nucleic acid (PCR) of the agent in approved laboratories; or
5. an epidemiological link to a confirmed case or signs of probable exposure to the agent.

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

2.3. Confirmed case of highly pathogenic avian influenza

A case of highly pathogenic avian influenza is confirmed when there is isolation and identification of the agent, or detection of the specific viral RNA of any type A influenza virus characterized as being highly pathogenic in accordance with Chapter 3.3.4 of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Animal Health Organization (WOAH).

Cases of highly pathogenic avian influenza in wild birds may be confirmed by sequencing a positive sample or by a specific molecular test (RT-qPCR to detect subtype H5 **clade 2.3.4.4**) for simultaneously detecting the virus and determining its pathogenicity.

Once an outbreak of highly pathogenic avian influenza in wild aquatic birds has been confirmed in a municipality, for those species in which laboratory testing has confirmed the disease, the taking of samples for new probable cases that are epidemiologically linked to an existing outbreak may be dispensed with. By a clinical and epidemiological criterion, the new cases may be deemed to be confirmed cases of an existing outbreak up until 30 days subsequent to the latest confirmed case.

2.4. Confirmed case of low pathogenicity avian influenza

The diagnosis of a confirmed case of LPAI requires the isolation and identification of the agent, or detection of the specific viral RNA of any type A influenza virus not characterized as being highly pathogenic.

2.5 Outbreak

The outbreak of highly or low pathogenic avian influenza is established on the basis of the confirmed case, which means an epidemiological unit where at least one case was confirmed, according to the criteria for the definition of established cases.

Note: In an outbreak of highly pathogenic avian influenza all birds presenting compatible clinical signs are deemed to be confirmed cases.

2.6. Ruled-out suspected case

A ruled-out suspected case is a suspected case notified to the Official Veterinary Service that was not classified by the Official Veterinarian as a probable case of ARNS.

2.7. Ruled-out case

A probable case investigated by the Official Veterinary Service, whose laboratory test results do not fit the criteria for definition of a confirmed case of highly or low pathogenic avian influenza.

3. Laboratory diagnosis

3.1. Passive surveillance

In order to characterize a case as a probable case of an officially-controlled disease, there must be accurate observation of clinical signs, and verification of criteria that may determine whether an anomaly is worthy of clinical, epidemiological, and laboratory investigation by the OVS. Therefore, veterinarians working for the official animal health services in the states of Brazil have been responsible for taking samples in probable cases of RNS in both domestic and wild birds.

Passive surveillance has involved taking swabs from birds and sampling their tissues. Samples obtained by means of tracheal and cloacal swabs, as well as organ samples, have been sent for molecular detection of type A influenza virus and ND virus.

All samples deemed positive or inconclusive through molecular detection of type A influenza virus have been submitted to specific assays for the molecular diagnosis of subtypes H5, H5 Clade 2.3.4.4, Neuraminidase N1 by RT-qPCR. Additionally, samples testing positive for influenza A and meeting specific criteria have been tested by means of partial hemagglutinin sequencing with subsequent deduction of amino acids from the cleavage site in order to determine the pathogenicity of the virus. Viral isolation was performed for samples deemed inconclusive after molecular diagnosis techniques to detect subtypes H5, H5 Clade 2.3.4.4, Neuraminidase N1 by RT-qPCR.

Likewise, samples testing positive or inconclusive after molecular detection assay for gene M of ND virus underwent confirmation molecular tests to detect gene F of ND virus.

Samples for passive surveillance were shipped to, and tested at, the official MAPA laboratory, the Federal Laboratory of Agricultural Health (LFDA) in Campinas, in the state of São Paulo. The LFDA/SP Laboratory possesses an NB3 level biological security laboratory and is acknowledged by WOAH as a reference for the diagnosis of AI and ND.

Figure 2 shows the expected flow diagram for laboratory diagnosis of samples taken for passive surveillance for AI and ND.

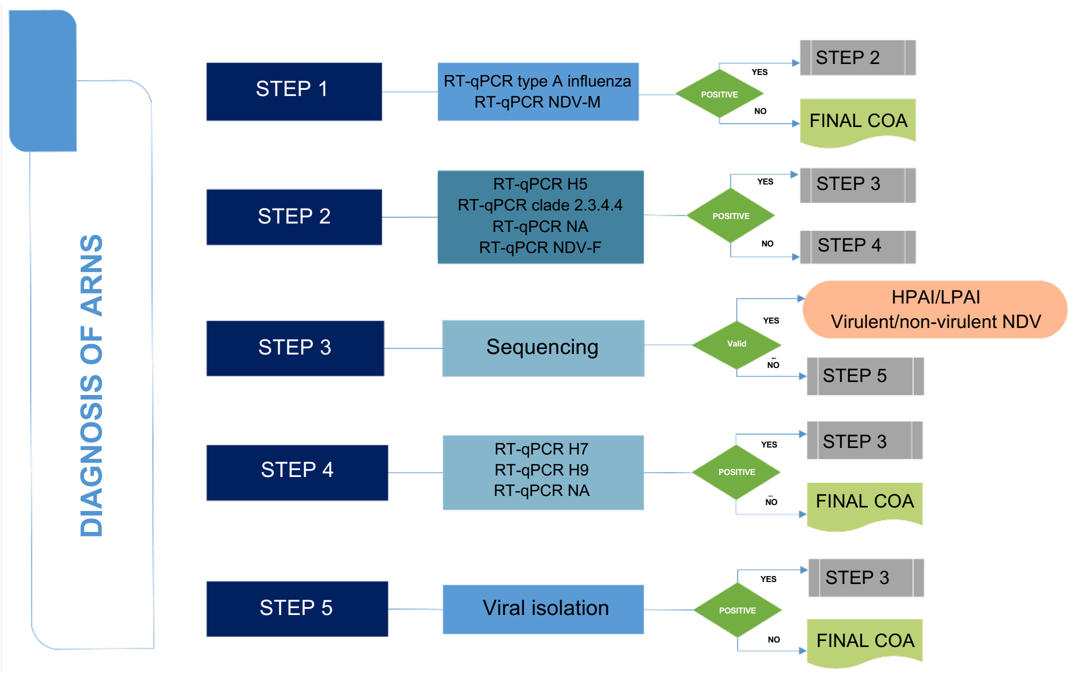

Figure 2. Expected flow diagram for laboratory diagnosis of samples taken for passive surveillance for Avian influenza and Newcastle disease.

3.2. Active surveillance

Samples of blood serum and pools of tracheal and cloacal swabs were taken for active surveillance. Each swab pool started an influenza A detection reaction and an ND detection reaction.

3.2.1 Components 3 & 4

Diagnostic screening to detect AI in the active surveillance components was based on serial Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to type A influenza virus in the harvested blood serum samples. The ELISA test was performed for ND serum screening in component 4. Because of vaccination against ND in the poultry flocks, the serum samples harvested for component 3 do not undergo serological testing for ND.

Serum samples testing positive for AI underwent hemagglutination Inhibition (HI) for the detection of antibodies against the 16 type A influenza virus subtypes (H1 to H16). HI is deemed the gold standard for sub-typifying type A influenza virus antibodies in serum samples.

Samples obtained from the pools of tracheal and cloacal swabs were sent for molecular detection of type A influenza virus and ND virus. A screening RT-qPCR reaction was performed to detect type A influenza virus in order to identify two target-sequences of gene M and one for the nucleoprotein gene (NP). If samples are detected testing positive for type A influenza virus at screening, they undergo specific assays for the molecular diagnosis of subtypes H5, H7 and H9. An RT-qPCR assay was performed for the molecular detection of the ND virus in order to amplify the target-sequence of gene M of APMV-1 virus. Samples testing positive or inconclusive after the screening assay underwent confirmatory molecular tests to detect gene F of the same virus.

All active surveillance testing in components 3 and 4 was performed at the LFDA São Paulo and LFDA Rio Grande do Sul laboratories. The figure below shows the simplified flow and the laboratory tests for active surveillance in components 3 and 4 of the surveillance plan:

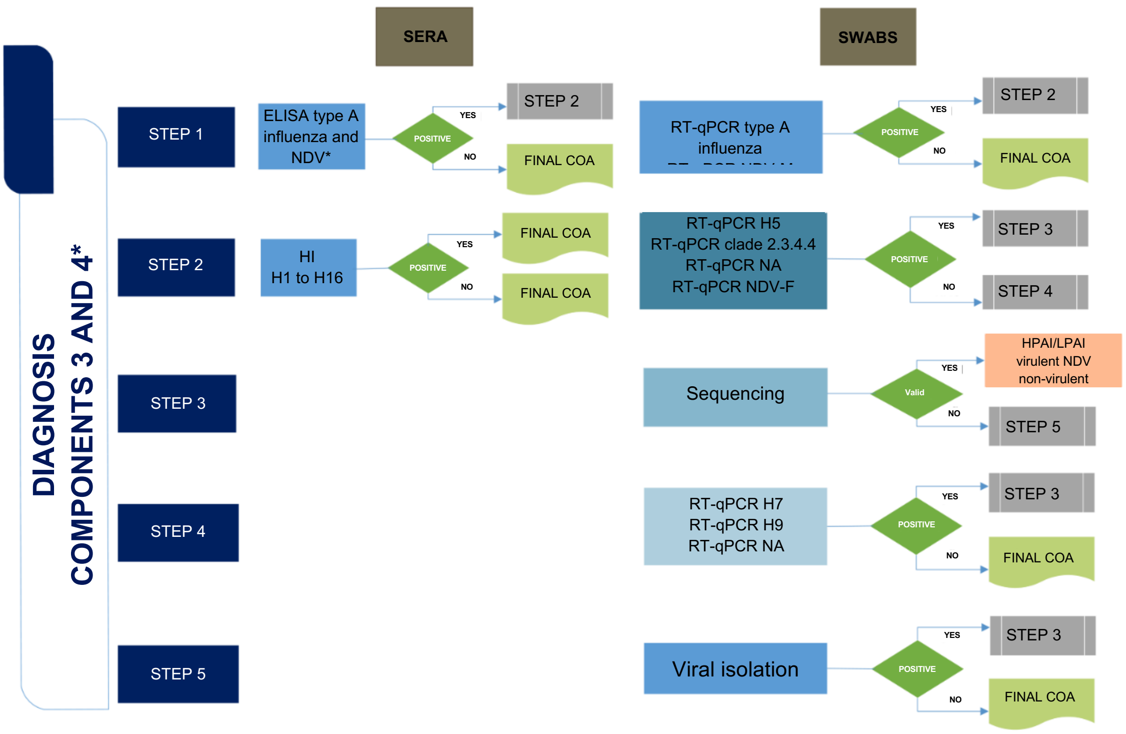

Figure 3. Prescribed flow diagram for laboratory diagnosis of samples taken for active surveillance for Avian influenza and Newcastle disease, for components 3 & 4

3.2.2 Component 5

For component 5, the serum samples underwent ELISA testing to detect type A influenza virus antibodies, or immunodiffusion in Agar Gel (IDAG) for AI.

The ELISA test was performed for ND serum screening. It should be emphasized that the ND diagnostic tests to comply with component 5 are not carried out on poultry stocking production centers where live ND vaccine has been applied. As with components 3 and 4, the tracheal and cloacal swab samples were intended for the molecular detection of type A influenza virus and ND virus (gene M) in component 5.

AI and ND screening tests were carried out at the public MAPA-approved laboratories listed below:

- Centro de Diagnóstico de Sanidade Animal – CEDISA, Santa Catarina;

● Centro de Diagnóstico Marco Enrietti – CDME, Paraná;

● Instituto Biológico – IB, São Paulo;

In the case of molecular tests that were positive, in approved laboratories (Figure 4), the samples were shipped immediately to the São Paulo LFDA for confirmation testing as per NORMATIVE INSTRUCTION NO. 21, DATED OCTOBER 21, 2014.

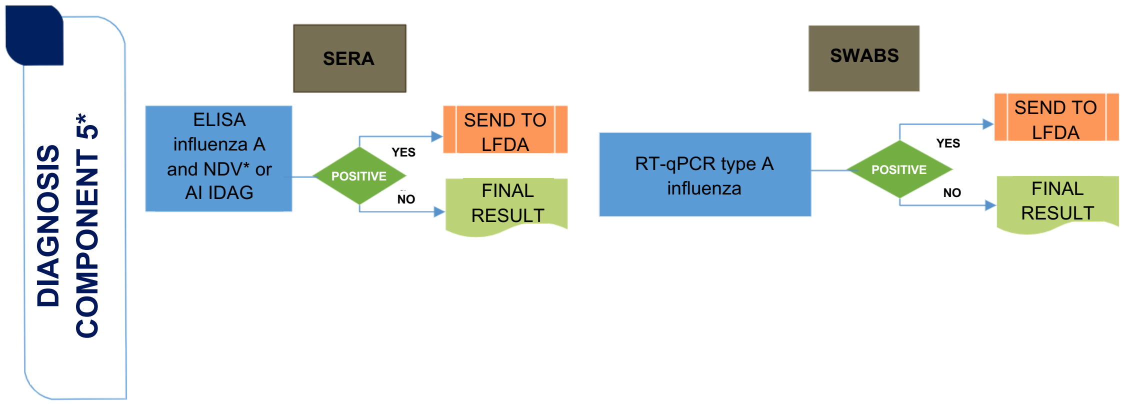

Figure 4. Prescribed flow diagram for laboratory diagnosis of samples taken for active surveillance for Avian influenza and Newcastle disease in component 5

B. DESCRIPTION OF COMPONENTS AND RESULTS OF SURVEILLANCE

COMPONENT 1 - Passive surveillance in poultry and non-poultry flocks

1.1. Objective and source of data

Component 1 of the surveillance system is passive surveillance of domestic birds (poultry and non-poultry) and aims to obtain early detection of AI and ND. Additionally, the data from such passive surveillance helps prove the absence of these diseases in the poultry production system. It is of the utmost importance to the early detection of the diseases that growers and other poultry chain professionals, after awareness-raising and instruction on the signs of AI and ND, should notify the authorities, and this reflects the level of awareness-building and engagement within the production chain. DSA-standardized procedures for investigation and the closing of occurrences of AI and ND include, but are not restricted to the following:

- Visits to, and inspection of poultry and non-poultry flocks, and interviews with farmers;
- Analysis of animal husbandry indices with judicious assessment of mortality records;
- The identification of clinical signs or lesions (whether neurological, respiratory or digestive) that are compatible with avian respiratory or nervous syndromes (ARNS) in any type of birds;
- Epidemiological investigation and sampling of birds;
- e-Sisbravet record of investigation;
- Laboratory testing for AI and ND;
- Emergency actions to control and eliminate outbreaks, in the event of a positive result;
- Phylogenetic tests of the virus genome, in the event of positive results.

The flow of notifications and records of animal health information, the technical procedures for addressing suspected cases, and laboratory testing for AI and ND, are all given in the User's Manual of the Brazilian System for Veterinary Surveillance and Emergencies (*Sistema Brasileiro de Vigilância e Emergência Veterinárias* — e-Sisbravet) as well as in the technical specifications for the diseases.

It should be emphasized that all responses performed within the context of component 1 were recorded in e-Sisbravet, and all notifications were addressed to the state-level veterinary service at the local veterinary unit having jurisdiction over the municipality in which the suspected case was reported, for subsequent call-out.

1.2. Surveillance approach

The passive surveillance approach aims to obtain early detection and speedy elimination of outbreaks of AI and ND based on investigations of notifications provided by farmers, technical specialists of the production chain, veterinarians, or any citizen.

1.3. Risk Indicators

The presence of birds showing clinical signs or lesions (which may be neurological, respiratory or digestive) that are compatible with ARNS, sudden or raised mortality, and an epidemiological link to any confirmed cases.

1.4 Target population

The target population encompasses domestic species susceptible to AI and ND and present within the territory of Brazil. Component 1, which is a passive surveillance strategy, includes all species of poultry, backyard or ornamental birds.

1.5 Sample design

Because it is a component of passive surveillance, there is no sampling design. Investigations are triggered by notifications addressed to the OVS by farmers, technical experts of the production chain, veterinarians or any citizen.

Data on investigations of suspected cases of AI and ND have been extracted from e-Sisbravet, taking into account the date when the initial response was made, within the period from 01/07/2022 to 30/06/2023.

1.6 Sampling strategy

All suspected cases of AI or ND meeting the criteria defined as being a probable case of ARNS underwent sample-taking as per guidance provided in technical data sheets and passive surveillance manuals.

1.7 Type of material harvested

As a rule, for the laboratory investigation of probable cases in poultry and non-poultry flocks, tracheal and cloacal swabs were taken from 30 live birds and organ samples were collected from 5 necropsied birds (showing clinical signs or lesions compatible with AI and ND, or from birds that recently died — without evidence of organ autolysis). Organ samples came from the digestive, respiratory and nervous systems.

1.8 Results

1.8.1 Number and geographical distribution of the clinical and epidemiological investigations of suspected cases of avian respiratory and nervous syndrome

In the period under assessment, the Official Veterinary Service (OVS) carried out 886 clinical and epidemiological investigations of suspected cases of avian respiratory and nervous syndrome (ARNS) among poultry and non-poultry flocks throughout the territory of Brazil, of which 125 investigations were classified as probable cases (for which samples were harvested for AI and ND) and 761 were classified as ruled-out suspected cases.

Of the total number of investigations of ARNS in poultry and non-poultry flocks, 575 were carried out on poultry (industrial flocks), 12 of which were classified as probable cases, while 563 were assessed by the OVS as being ruled-out suspected cases.

The geographical distribution of the clinical and epidemiological investigations involving ruled-out suspected cases and probable cases of ARNS in industrial flocks during the assessment period, are given in figure 5.

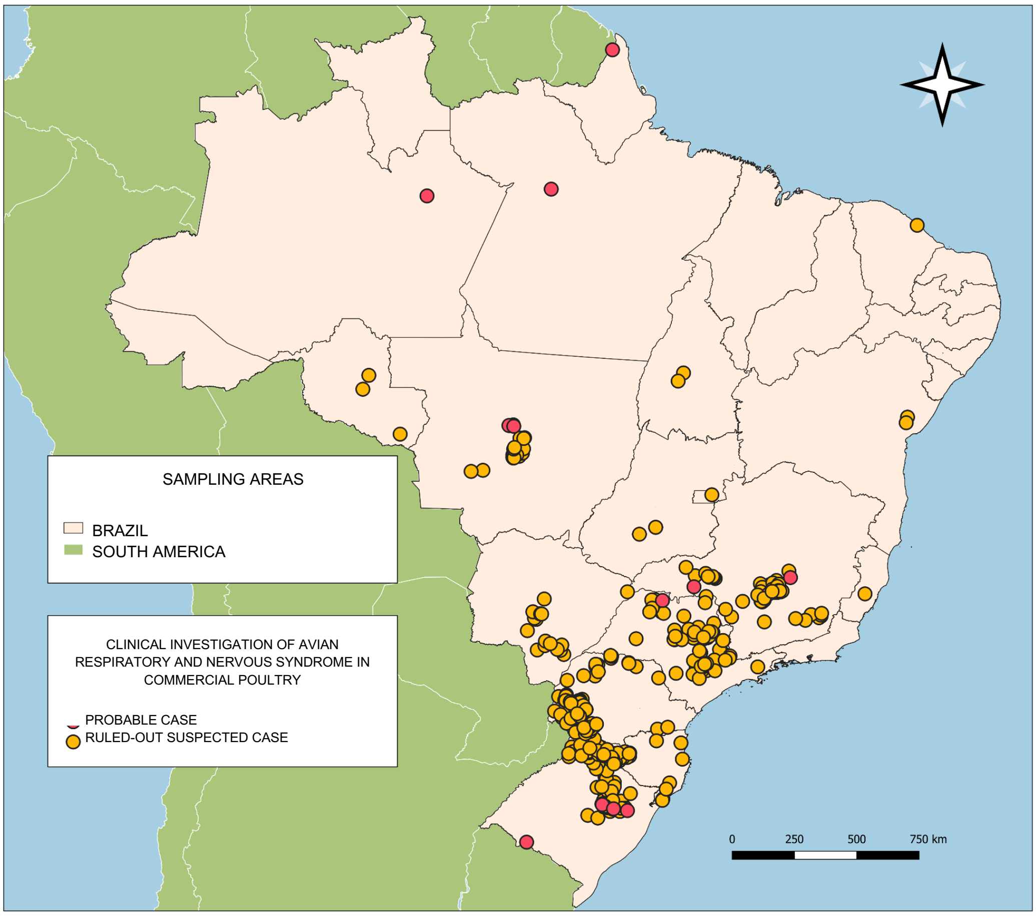

Figure 5. Geographical distribution of probable cases and ruled-out suspected cases of avian respiratory and nervous syndrome among commercial poultry from July 2022 to June 2023

The frequency of probable cases of ARNS in commercial poultry, by State of Brazil, is given in figure 6. The state of Rio Grande do Sul (RS) obtained the largest number of ARNS cases classified as probable, among commercial poultry.

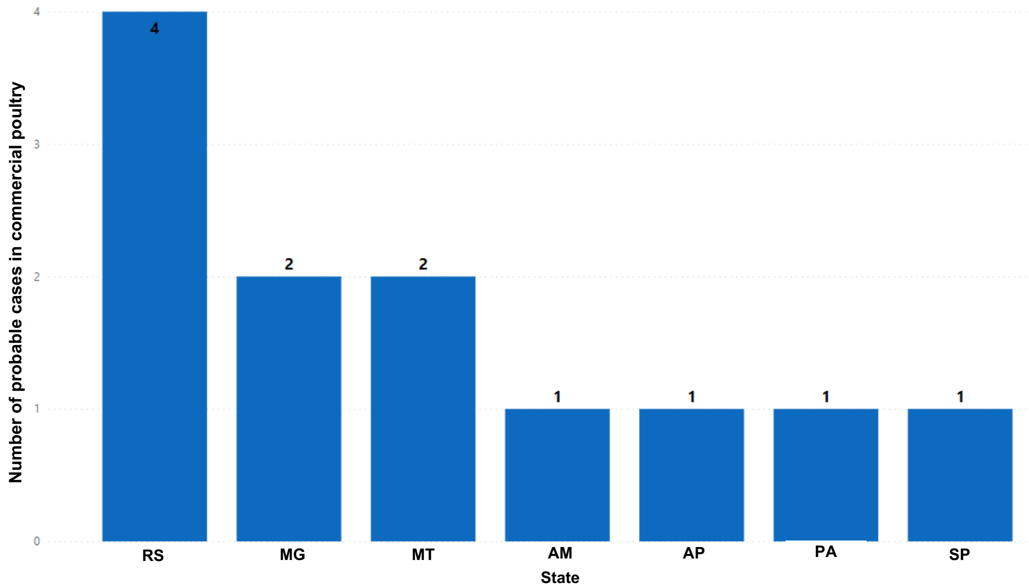

Figure 6. Frequency of the number of probable cases of Avian respiratory and nervous syndrome in commercial poultry in the States of Brazil from July 2022 to June 2023

311 clinical and epidemiological investigations were also carried out in backyard flocks, where 113 call-outs were classified as probable cases, and the other 198 were ruled out, as shown in Figure 7.

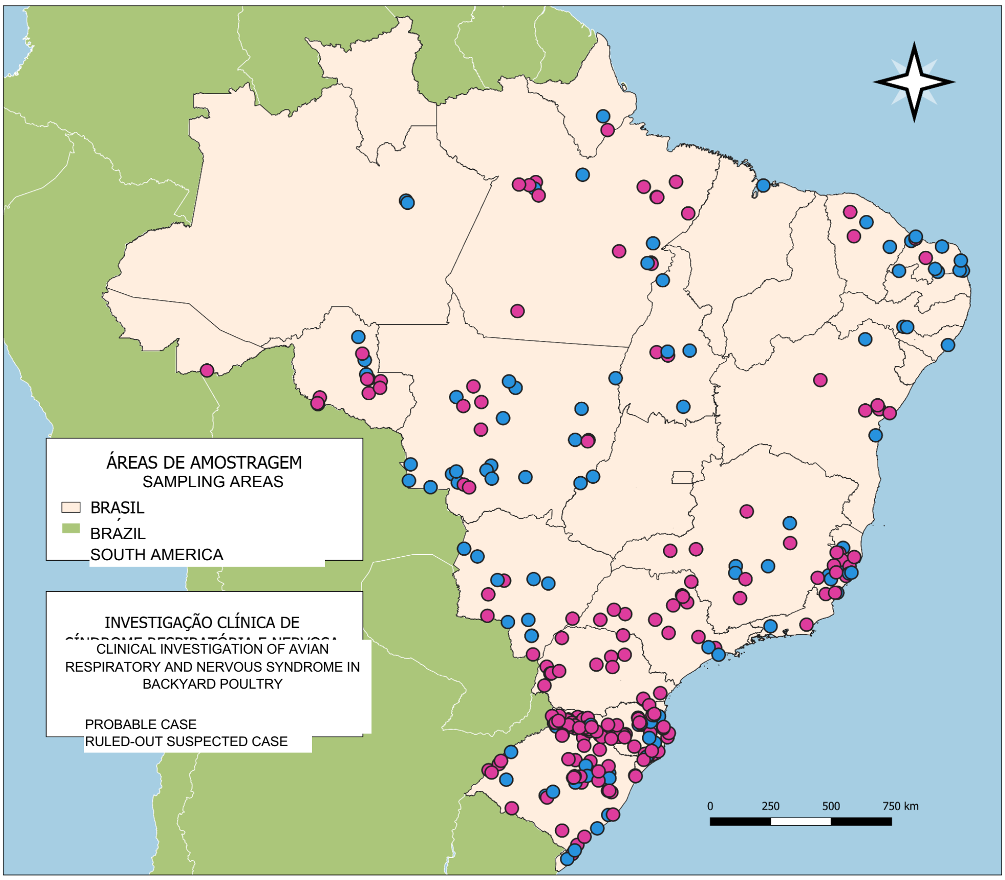

Figure 7. Geographical distribution of probable cases and ruled-out suspected cases of avian respiratory and nervous syndrome among backyard flock from July 2022 to June 2023

It can be seen that there was a greater geographical distribution and larger number of probable cases of ARNS in backyard than in commercial poultry flocks (Figures 5 and 7). Additionally, there were more States with probable cases of ARNS in backyard flocks. In all, 20 States had probable cases of ARNS during the plan's period of application. Figure 8 presents the frequency of the number of probable cases of ARNS per State.

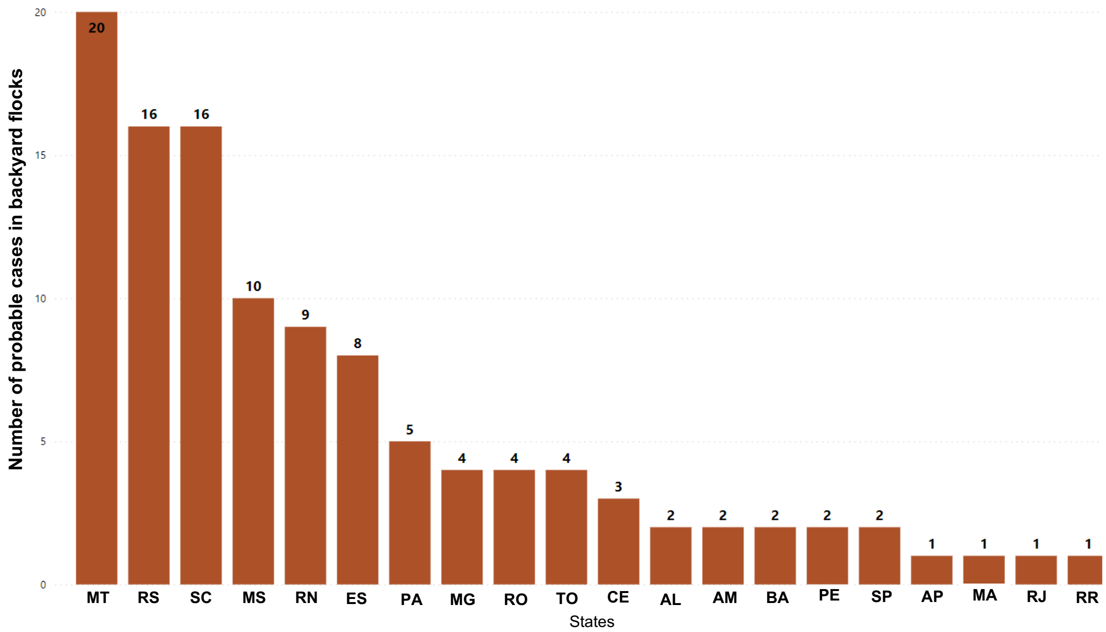

Figure 8. Frequency of the number of probable cases of avian respiratory and nervous syndrome in backyard flocks in the States of Brazil from July 2022 to June 2023

It can be seen in Figure 8 that 20 (17.69 %) investigations classified as probable cases in backyard flocks occurred in the State of Mato Grosso.

Regarding the temporal distribution of the occurrence of probable cases in poultry and non-poultry during the period under assessment, it can be seen that the number of probable cases in commercial poultry remained relatively stable throughout July 2022 to June 2023. However, in regard to probable cases in backyard flock, it is noteworthy that there has been a growing number of occurrences from November 2022, peaking in March 2023. The second peak in the number of occurrences of probable cases coincides with the first outbreak of AI in wild birds in Brazil, showing that the population at large was sensitized, and risk communication was effective (Figure 9)

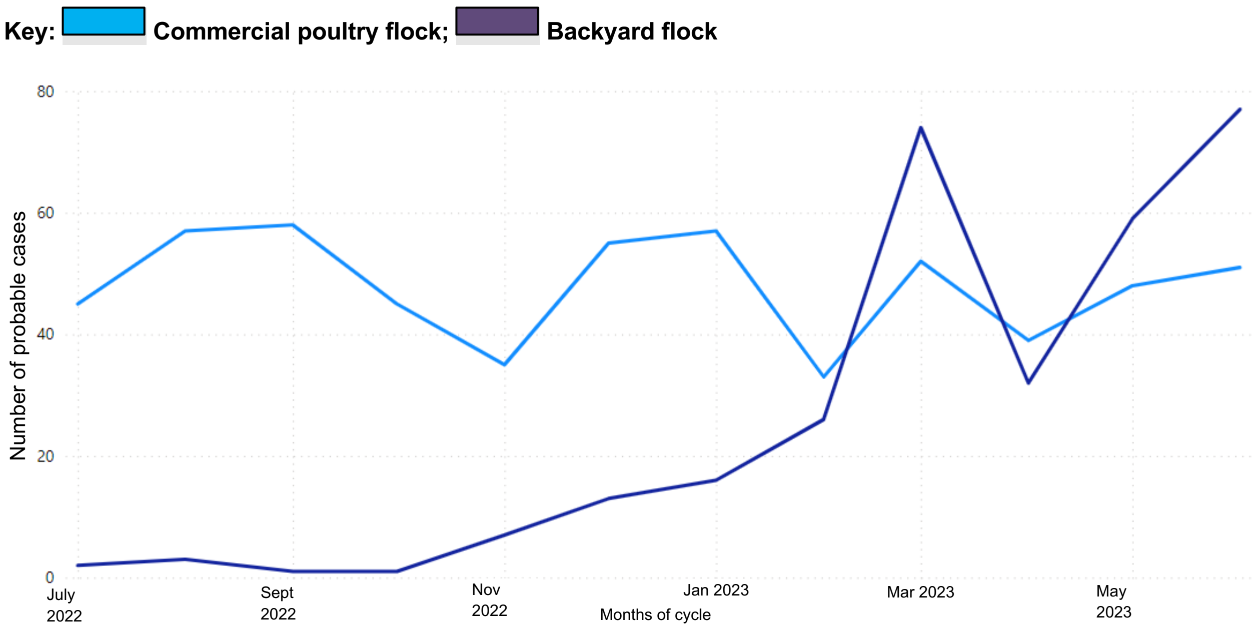

Figure 9. Temporal distribution of probable cases of avian respiratory and nervous syndrome among commercial poultry and backyard flocks from July 2022 to June 2023

1.8.3 Bird species sampled

Data for passive surveillance from July 2022 to June 2023 show that clinical and epidemiological investigations were carried out on the following commercial poultry species: hen/chicken, ostrich, Japanese quail, goose, duck and turkey.

Regarding call-outs concerning backyard species, sample-taking in probable cases was performed for the following species: hen/chicken, quail (American/European/Japanese), pheasant, Guinea Fowl, goose, teal, duck, peacock and turkey. Samples were also harvested in species not commonly classified as backyard, although they can be raised as companion animals, or for other reasons: Roadside Hawk, pigeon, psittacidae and other passeriformes. Other species served, when the request for a response came from backyard raising operations, were: eared dove, caracara, Daurian partridge and emu.

1.8.4 Outbreaks of highly pathogenic avian influenza in backyard flocks

The first outbreak among non-poultry (backyard flocks) in Brazil was reported on June 27, 2023, in the city of Serra in the state of ES, and affected a backyard flock containing ducks, geese, teal, turkeys and hens. For the period under assessment of the surveillance plan, July 2022 to June 2023, only one outbreak of AI took place, in backyard flock. On that farm multiple species of birds were found, including hens, geese, teal, ducks and turkeys, totaling 57 susceptible birds, 8 cases, 3 deaths and 54 birds culled.

The following measures were taken: the farm was banned, all birds were euthanized, and their carcasses destroyed, cleaning, disinfection, and surveillance actions within a radius of 10 Km from the outbreak.

Regarding surveillance actions within a radius of 10 km from the outbreak, 91 farms keeping backyard flocks were inspected, totaling 2,475 birds, while education and risk-communication actions were taken to raise awareness of the disease. No new suspected cases were identified.

1.8.5 Assessment of the results of surveillance

The passive surveillance strategy for domestic birds aimed to obtain an early detection of the introduction of HPAI virus into Brazil through clinical and epidemiological investigation and laboratory testing of wild and domestic birds suspected of being infected with AI and ND.

In the epidemiological investigation performed by the OVS of the outbreak farm on which the first outbreak of highly pathogenicity Influenza A was reported affecting backyard flock, a small lake was found which was frequented by wild birds that came into contact with the premise's backyard flock. Therefore, the results of epidemiological surveillance and genomic investigation of the virus found in the outbreak, showed that the likely cause of the introduction of the virus into domestic birds was direct contact with infected wild birds. Phylogenetic testing of the HA gene also showed that the genes identified in the outbreak in Brazil are similar to the genetic sequences of the isolated AI viruses circulating in neighboring South American countries.

COMPONENT 2 - Passive surveillance in wild birds

2.1 Objective and source of data

Component 2 of the surveillance system is passive surveillance in wild birds, the aim of which is to monitor changes in the disease within the wild environment in order to enable early detection and control a possible introduction of AI and ND into poultry.

Systematic investigation of exceptional mortality events in birds in the wild, above all in areas where migratory aquatic birds concentrate, aquatic stopover points, and other water bodies, aims to understand the sanitary situation of wild populations, and enable the planning of local measures to protect against and mitigate risks to production poultry flocks.

Exceptional mortality events in the wild birds are understood to be situations where dead or sick birds are found in numbers above what is normally observed, for an unknown reason, but excluding anthropic actions such as poisonings, chemical accidents, death caused by weapons, bombs, traps, etc.) and natural phenomena (storms, earthquakes, droughts, floods, hurricanes and toxic algal blooms, etc.).

DSA-standardized procedures for investigating and closing occurrences of AI and ND include but are not restricted to the following:

- Visits and inspections, preferably accompanied by an environmental professional, at locations where wild animals are found or collected, with interviews of the individuals who located it/them;
- The identification of exceptional mortality and any clinical signs or lesions (whether neurological, respiratory or digestive) that are compatible with avian respiratory or nervous syndromes (ARNS) in any type of birds;
- Epidemiological investigation and sampling of birds;
- e-Sisbravet record of investigation;
- Laboratory testing for AI and ND;
- Emergency actions to control and eliminate outbreaks, in the event of a positive resultI;
- Phylogenetic tests of the virus genome, in the event of positive results.

The flow of notifications and records of animal health information, the technical procedures for addressing suspected cases, and laboratory testing for AI and ND, are all given in the User's Manual of the Brazilian System for Veterinary Surveillance and Emergencies (*Sistema Brasileiro de Vigilância e Emergência Veterinárias* — e-Sisbravet) as well as in the technical specifications for the diseases.

It should be emphasized that all responses performed within the context of component 2 were recorded in e-Sisbravet, and all notifications were addressed to the state-level veterinary service at the local veterinary unit having jurisdiction over the municipality in which the suspected case was reported, for subsequent call-out.

2.2 Surveillance approach

The passive surveillance approach aims to obtain early detection and speedy elimination of outbreaks of AI and ND based on investigations of notifications called in by environmental management agencies, technical specialists of the production chain, veterinarians, or any citizen.

Governmental and non-governmental agencies and organizations responsible for the environment and preservation of natural resources are essential players in the detection of suspected cases of AI and ND from exceptional mortality events involving the wild birds of concern and by evaluating unusual behaviors among that population.

2.3 Risk Indicators

The presence of birds showing clinical signs or lesions (which may be neurological, respiratory or digestive) that are compatible with ARNS, unusual behavior, sudden or raised mortality, and an epidemiological link to any confirmed cases.

2.4 Target population

The target population encompasses wild animals or free-living animal species that are susceptible to AI and ND and present within the territory of Brazil. Component 2 is a passive surveillance strategy and therefore includes wild birds in the rehabilitation centers, in zoos, and in scientific collections, etc.

2.5 Sample design

Because it is a component of passive surveillance, there is no sampling design. Investigations are triggered after environmental entities or agencies, farmers, production chain technical experts, veterinarians or citizens at large notifies the OVS, and depending on their links to other epidemiological units, there may be repercussions.

Through liaison with the environmental agencies IBAMA and ICMBio, a network for monitoring mortality events in wild birds, involving the Beach Monitoring Project (*Projeto de Monitoramento de Praias*), the world’s largest beach monitoring project, and all of Brazil’s Federal Conservation Units, as shown respectively in figures 10 and 11.

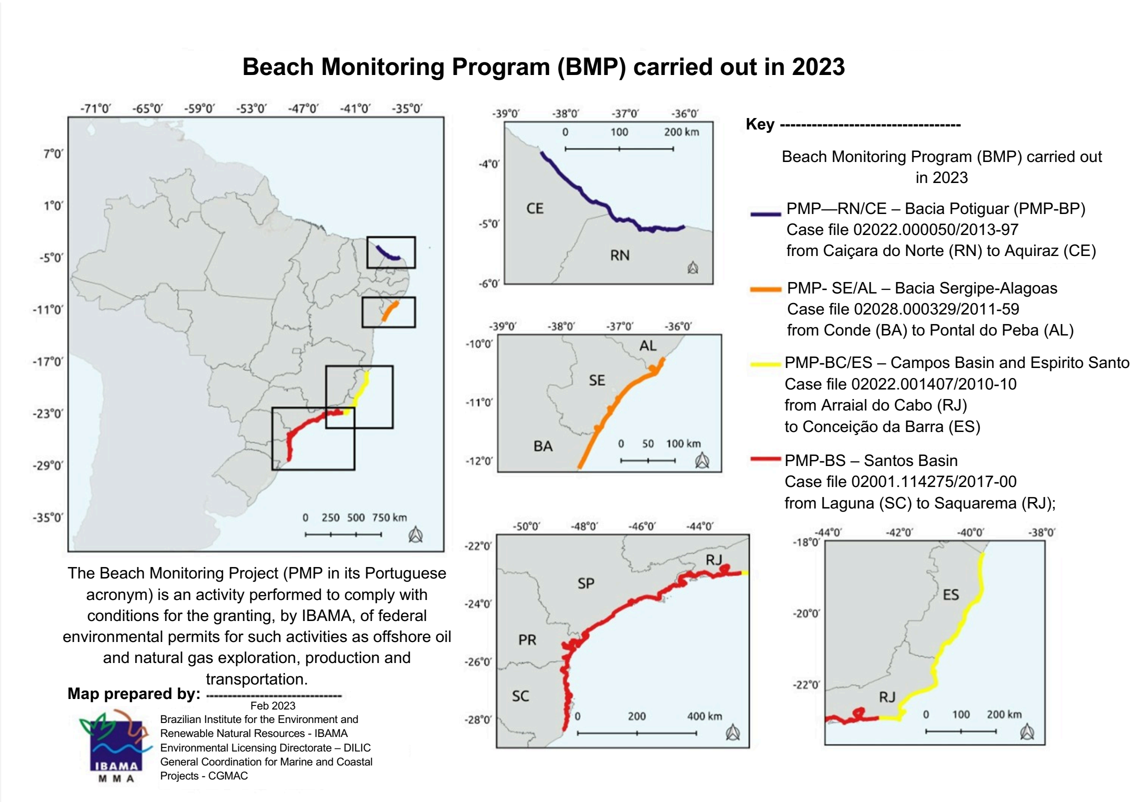

Figure 10. Characterization of the Area of Coverage of the Beach Monitoring Project.

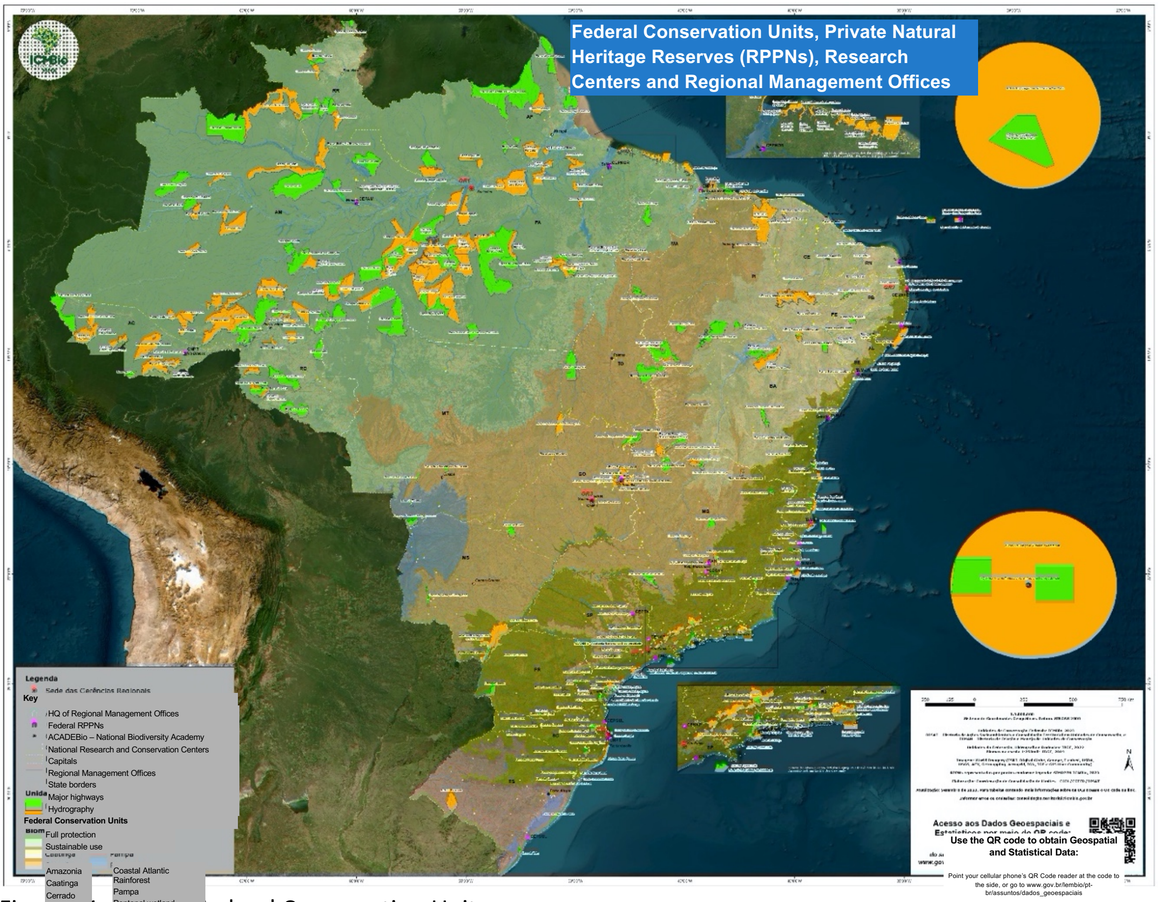

Figure 11. Brazil's Federal Conservation Units

Data on investigations of suspected cases of AI and ND have been extracted from e-Sisbravet, taking into account the date on which the initial response was made, within the period from 01/07/2022 to 30/06/2023.

2.6 Sampling strategy

In this component, all suspected cases of AI or ND meeting the criteria defined as being a probable case of ARNS, underwent sample-taking in accordance with epidemiological criteria laid down by the DSA, and guidance provided in technical data sheets and passive surveillance manuals.

2.7 Type of material harvested

For laboratory investigation of probable cases in wild birds, tracheal and cloacal swabs and organ samples are collected from necropsied individual bird that showed clinical signs or lesions compatible with AI and ND, or from birds that recently died — without evidence of organ autolysis). Organ samples came from the digestive, respiratory and nervous systems.

2.8 Results

2.8.1 Number and geographical distribution of the clinical and epidemiological investigations of suspected cases of avian respiratory and nervous syndrome

In the period under assessment, the Official Veterinary Service (OVS) carried out 221 clinical and epidemiological investigations of suspected cases of avian respiratory and nervous syndrome (ARNS) in wild birds throughout the territory of Brazil, of which 148 investigations were classified as probable cases (for which samples were harvested for AI and ND) and 73 were classified as ruled-out suspected cases. Figure 12 shows the geographical distribution of the clinical and epidemiological investigations involving ruled out suspected cases and probable cases of ARNS in wild birds during the period assessed.

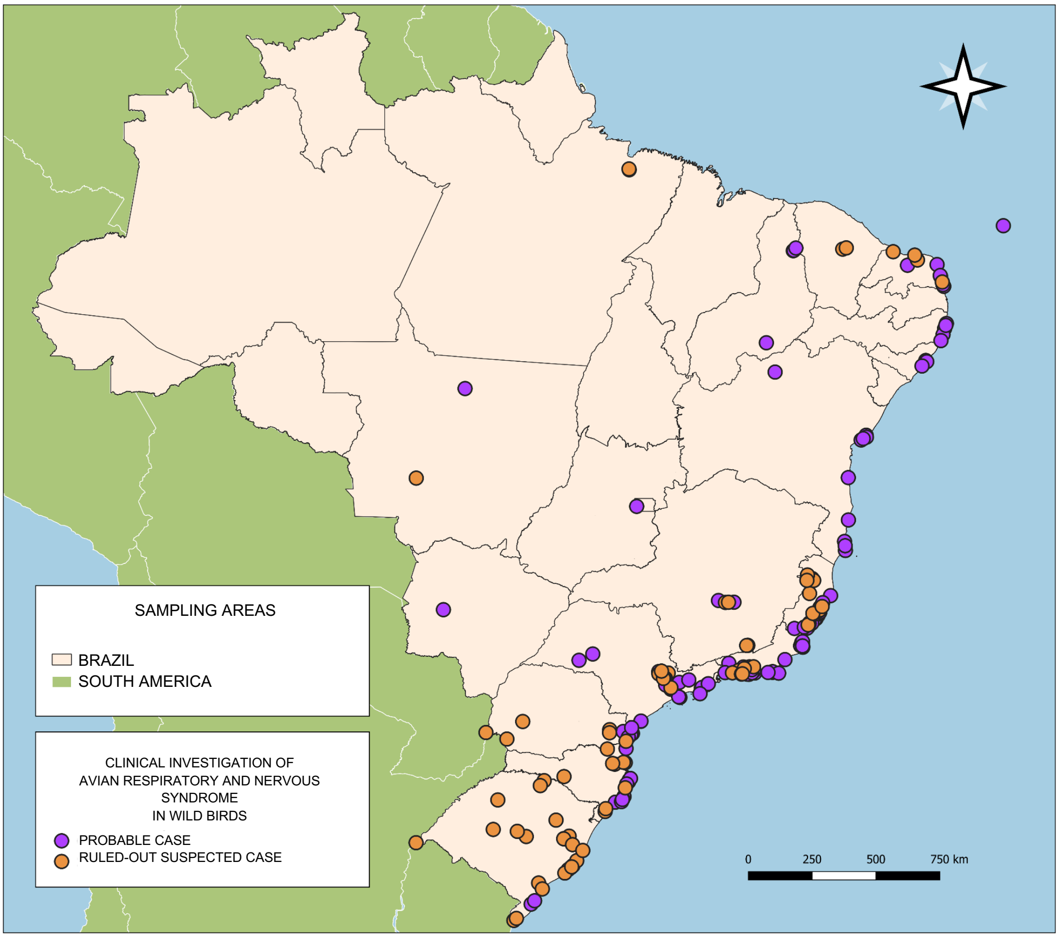

Figure 12. Geographical distribution of probable cases and ruled-out suspected cases of avian respiratory and nervous syndrome in wild birds from July 2022 to June 2023

Figure 12 shows that there was a greater concentration of probable cases among wild birds along the Atlantic seaboard of Brazil, also indicating that most of the investigations were carried out among coastal seabirds in the Atlantic migration route.

In figure 13 it can be seen that the highest frequency of the number of probable cases in wild birds (29 – 19.46%) was in the State of Espirito Santo, the state in which the first outbreak of AI in wild birds took place.

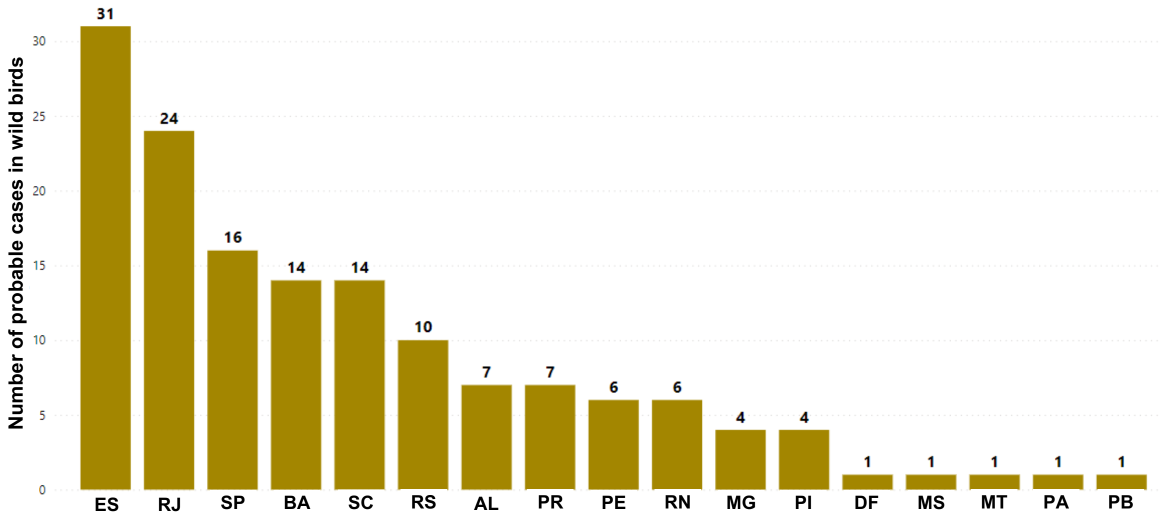

Figure 13. Frequency of the number of probable cases of avian respiratory and nervous syndrome in wild birds in the States of Brazil from July 2022 to June 2023

It is also important to point out a sudden increase in the number of probable cases of ARNS in wild birds beginning in May 2023. In that period the curve was tending towards stability, with a low number of probable cases throughout the assessed period. The noteworthy increase in occurrences among wild birds coincides with the period when the first outbreak of highly pathogenicity Influenza A was diagnosed on May 15, 2023. Up until the end of June 2023, the assessment period of the present report, there was an increasing number of occurrences of probable cases of highly pathogenicity Influenza A in wild birds, as can be seen in figure 14.

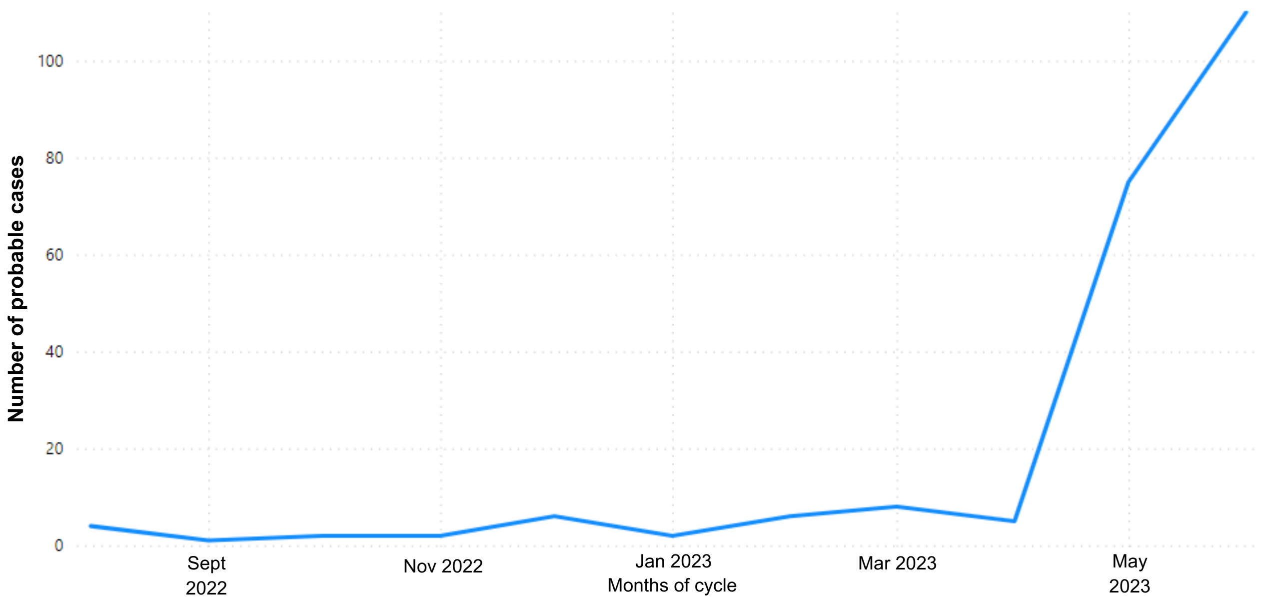

Figure 14. Temporal distribution of probable cases of avian respiratory and nervous syndrome in wild birds from July 2022 to June 2023

2.8.2 Bird species sampled

Data for passive surveillance for the period from July 2022 to June 2023 show that clinical and epidemiological investigations were carried out on approximately 80 species, which are listed in ANNEX 1.

The wild bird species with the largest number of ARNS investigations was *Thalasseus acuflavidus* (Cabot's tern), with 32 call-outs for the OVS in the period under assessment. The aforementioned species is a coastal seabird that nests on islets and islands off the coast of Brazil from Espirito Santo to Santa Catarina, between April and September. Other species of tern can be found among colonies of Cabot's tern, such as South American tern (S*terna hirundinacea*) and Royal tern (*Thalasseus maximus*). In this context we emphasize that the Royal tern second-most investigated wild species for AI, with a total of 25 responses for the species. Breeding behaviors in mixed colonies were deemed a relevant factor for the transmission of highly pathogenicity Influenza A between the two most widely-affected species.

2.8.3 Outbreaks of highly pathogenic avian influenza in wild birds

The first outbreak of highly pathogenicity Influenza A in wild birds in Brazil was reported on May 15, 2023 among three migratory coastal seabirds, two from the species *Thalasseus acuflavidus* (Cabot's tern) and one individual *Sula leucogaster* (brown booby). The OVS response was at a training center in the municipality of Vitoria, in the state of Espirito Santo.

Since then, there has been a growing number of outbreaks among wild birds, mostly in migratory coastal seabirds, but the highly pathogenicity Influenza A virus has also been detected among terrestrial birds. During the assessment period for the surveillance plan, July 2022 to June 2023, there were 56 outbreak of highly pathogenicity Influenza A in wild birds in the States of Espirito Santo, Rio de Janeiro, Bahia, São Paulo, Paraná and Santa Catarina.

Current data for the epidemiological situation of highly pathogenic avian influenza are given at: https://mapaindicadores.agricultura.gov.br/publico/extensions/SRN/SRN.html.

2.8.4 Assessment of surveillance

Passive surveillance in wild birds aims to enable monitoring of changes in the disease in the wild environment, so as to detect and rapidly control a possible introduction into backyard or commercial poultry.

MAPA made a great effort to set up a monitoring network for mortality events among wild birds covering the entire territory of Brazil, demonstrating effectiveness in detecting outbreaks among wild birds.

Epidemiological surveillance data show that the first outbreak of highly pathogenicity Influenza A in wild birds in Brazil occurred in the period when seabirds were migrating to Brazil's coastal region. It should be emphasized that the vast majority of outbreaks took place in Tern populations, because they are species that nest sympatrically, from April to September, and perform regional movements on coastal islands ranging from Espirito Santo to Santa Catarina.

MAPA therefore integrated virus genomic testing to the AI and ND surveillance system as a strategy for elucidating the origin, transmission dynamic, and evolution of the highly pathogenicity Influenza A virus circulating in Brazil. As a result, phylogenetic testing of the H5N1 virus, clade 2.3.4.4b which was obtained in the first outbreak among *Thalasseus acuflavidus* (Cabot's tern) in Brazil showed that the likely source of incursion of the virus was through the migration of infected wild birds along the Pacific route.

It should also be observed that not only seabirds tested positive for highly pathogenicity Influenza A. Land-living wild birds, such as the birds of prey Caracara (*Caracara plancus*) and the tropical screech owl (*Megascops choliba*) were also affected by the highly pathogenicity Influenza A virus. The results of phylogenetic testing showed a great similarity between the viral isolates obtained from seabirds and terrestrial birds, suggesting the same origin for the virus among the infected birds.

COMPONENT 3 - Active surveillance in industrial poultry production

3.1 Objective and source of data

Component 3 of the surveillance system aimed to demonstrate a status of HPAI-virus freedom for industrial poultry production based on epidemiological surveillance and laboratory serological and molecular testing in commercial poultry raising establishments.

All the activities performed to comply with component 3 – "active surveillance in industrial poultry production" were recorded in the Epicollect5 application with the goal of recording, consolidating and sharing data from the study activities.

3.2. Surveillance approach

An active surveillance strategy was used in order to confirm the absence of clinical signs or changes in animal husbandry indicators and animal health indicators compatible with the occurrence of AI or ND within the territory of Brazil, using as a reference the results of visits, clinical inspections, and the assessments of animal husbandry, animal health, and epidemiological indicators, as well as an analysis of the results of serological and molecular tests for industrial poultry-producing establishments.

3.3. Risk Indicator

The types of establishments, and their risk categories, were defined after taking into account the absence of AI and ND in Brazil, the history of occurrence in other countries (European Food Safety Authority, 2017; WAHIS, WOAH), the surveillance plans prepared by other animal health entities, and the environmental and production conditions found in the territory of Brazil. The most important aspects for this categorization were, in order of importance: the susceptibility of the species that are present; the duration of the animals' production cycle; and the impact of handling, health and biosecurity practices.

- VERY LOW RISK → Broiler farms
- LOW RISK → Breeding Hen Farms (parent stock, grandparent stock, great-grandparent stock, or pure lineage animals)
- MODERATE RISK → Laying hen farms
- HIGH RISK → Duck, turkey and quail farms

3.4 Target population

In order to meet the goals of this component, the target population was industrial poultry production, defined as the set of establishments raising hens, turkeys, ducks, teal and quail located within the area of sampling (Figure 15) and with a housing capacity greater than 1000 birds. Component 3 excludes backyard operations, growers of ornamental birds, or of birds for purposes other than food production (meat and eggs).

The databases of the registrations of farms and livestock-raising operations made available by the state-level animal health agencies (OESA) were taken into account for identifying establishments and for estimating the total population in each state of Brazil.

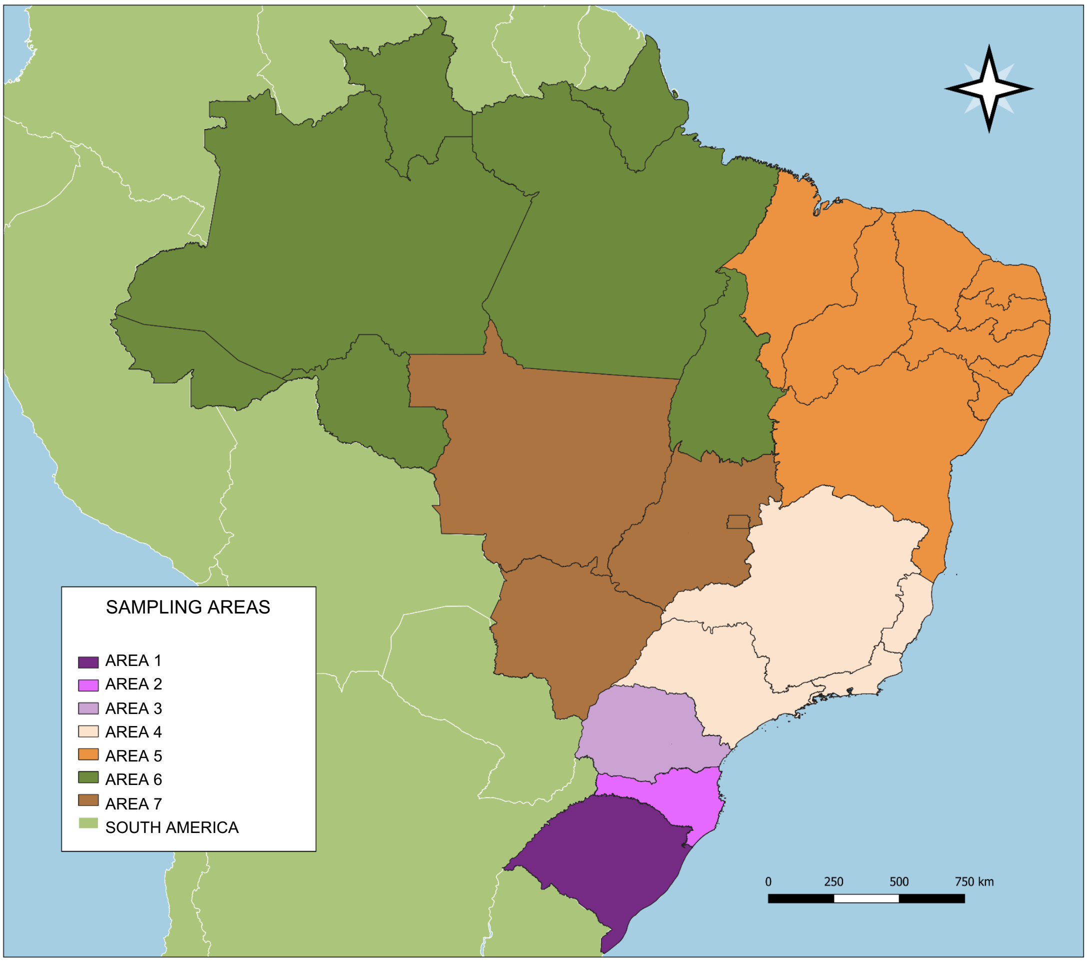

Figure 15. Sampling areas for component 3

3.5 Sample design

The list of sampled industrial poultry establishments was created by DSA on the basis of the sampling calculation described in the AI and ND surveillance plan, summarized as follows: The sample size was calculated by taking seven sampling areas into account Two-step sampling was proposed for each area. In the first step, premises would be randomly selected, and in the second step, for each selected premise all farm, birds would be sampled in order to classify the establishment as to the presence or absence of AI or ND.

In order to define the minimum number of farms to be sampled in the first step, a random draw was held obeying the parameters of 1% prevalence, 95% confidence level and 95% sensitivity for the "AI - ELISA" and "ND - PCR" tests, and 100% specificity.

Regarding the number of animals to be sampled per farm (step two) the sample-size calculation took into account and estimated prevalence on farms of 30% and confidence level of 95%.

3.6 Sampling strategy

A cross-sectional study was planned with a two-step sampling strategy, where the first step was the selection of farms. At this step, after calculating the number of funds to be examined, the premises were selected by taking into account the several activities that are carried out on poultry farms, and adopting a risk-based strategy taking into account the model described in the surveillance plan.

In the second step, eleven (11) animals were sampled for each epidemiological production unit (nucleus) in the poultry farm. In the case of epidemiological production unit (nucleus) possessing several sheds, the prevalence among sheds was deemed to be 30%, and therefore in those epidemiological production unit (nucleus) the sampled animals would have to be distributed among the several sheds. In the case of poultry farm possessing several epidemiological production unit (nucleus), the OVS had to randomly select the maximum of five (5) epidemiological production unit (nucleus) to sample collection.

The distinction between establishments taking part in the random draw for each component abate the standardization given in MAPA Normative Instruction no. 56, published December 4, 2007, which, for the purposes of registration, defined raising activities numbering up to 1000 birds as small-scale poultry-raising.

Taking into account the variety of locations where Nearctic migratory birds are found in Brazil, and particularly the presence of groups of animals from the species Charadriiformes and Anseriformes, a supplementary sampling was carried out exclusively in municipalities located nearby these places in order to increase the sensitivity of the component. The increment was equivalent to 10% of the initial sample.

Thus, the final sample size was 2,385 establishments.

3.7 Type of material harvested

Eleven (11) birds from each epidemiological production unit (nucleus) on a farm were selected at random for sample taking. When there were several sheds forming one epidemiological production unit (nucleus), sampling was distributed between the sheds as far as possible.

In order to obtain serum, blood samples were harvested on an individual basis by venipuncture from 11 live animals. Tracheal and cloacal swabs were taken from each selected bird. The provisions of the plan were that swab samples would be packed in groups as pools. For the chicken species, the swab pool could be formed as follows: one pool of 11 tracheal swabs and another pool of 11 cloacal swabs, or the 11 cloacal swabs could be split into two pools, one with 5 cloacal swabs and the other with 6 cloacal swabs. The same distribution was accepted for the tracheal swabs.

It should be emphasized that when swabs of different species were sampled, such as turkeys, ducks, quail and teal, the 11 samples of cloacal and tracheal swabs from each epidemiological production unit (nucleus) were necessarily split into four pools.

3.8 Individuals responsible for sample-taking

Veterinarians of the official animal health services in each State were responsible for all inspections and activities, supported by technical auxiliaries and employees of the poultry-raising establishments.

3.9 Activities performed

3.9.1 Identification of establishments

Each inspected establishment received a unique identification (designated as the MAPA Code), generated by the DSA in accordance with the number of farms defined for each State, or “Federative Unit”.

3.9.2 Records of the data

The responsible veterinarian taking the samples completed the electronic forms using the Epicollect5 application software, and the information was shared with LFDA-SP, tested the samples; with DSA and with the OESAs, using the electronic spreadsheets.

Annex 2 shows the minimal script for information audited during inspections of the breeding establishments and commercial poultry farms.

3.9.3 Schedule for sample-taking

Epidemiological surveillance and laboratory testing activities ran from July 19, 2022 to June 5, 2023, with a greater concentration of sample-taking in the initial period (Figure 16).

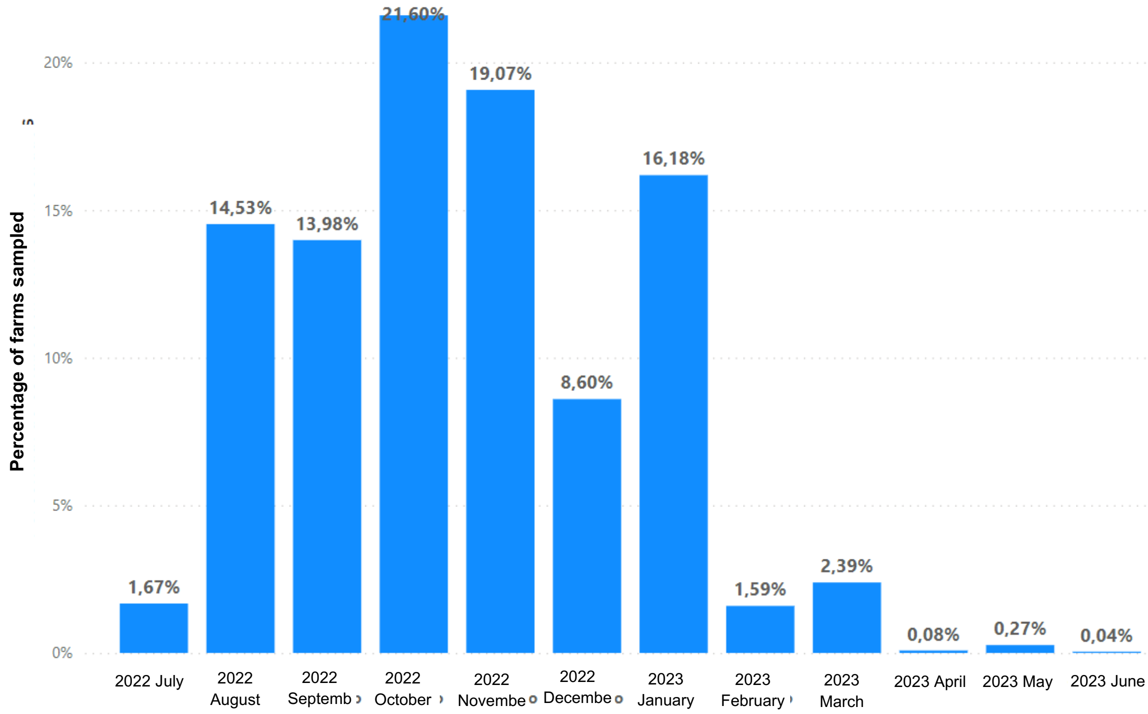

Figure 16. Temporal distribution of sample-taking for component 3 from July 2022 to June 2023

3.10 Results

3.9.1 Number and geographical distribution of sampled farms

Component 3 of the surveillance system is aimed at detecting the presence of ND and AI, in the event they are present in industrial poultry production in Brazil. Therefore, to meet the goals of the present study, 2,385 poultry farms were visited, including breeding and broiler raising establishments, for the performance of epidemiological surveillance and sample-taking for laboratory testing of the AI and ND viruses.

Taking into account the sampling area criteria defined in the surveillance plan, the states of Rio Grande do Sul (Area 1), Santa Catarina (Area 2), and Paraná (Area 3), had the largest number of establishments sampled, totaling 347 establishments each (Figure 17).

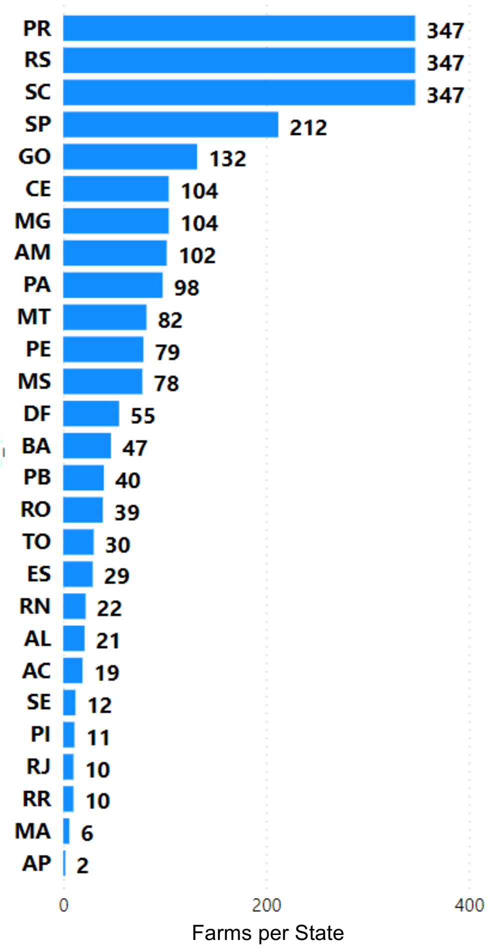

Figure 17. Distribution of the number of sampled poultry establishments, by State, for component 3

Figure 18 presents the geographical distribution of the poultry establishments sampled in the distinct sampling areas of component 3, in accordance with the sampling plan.

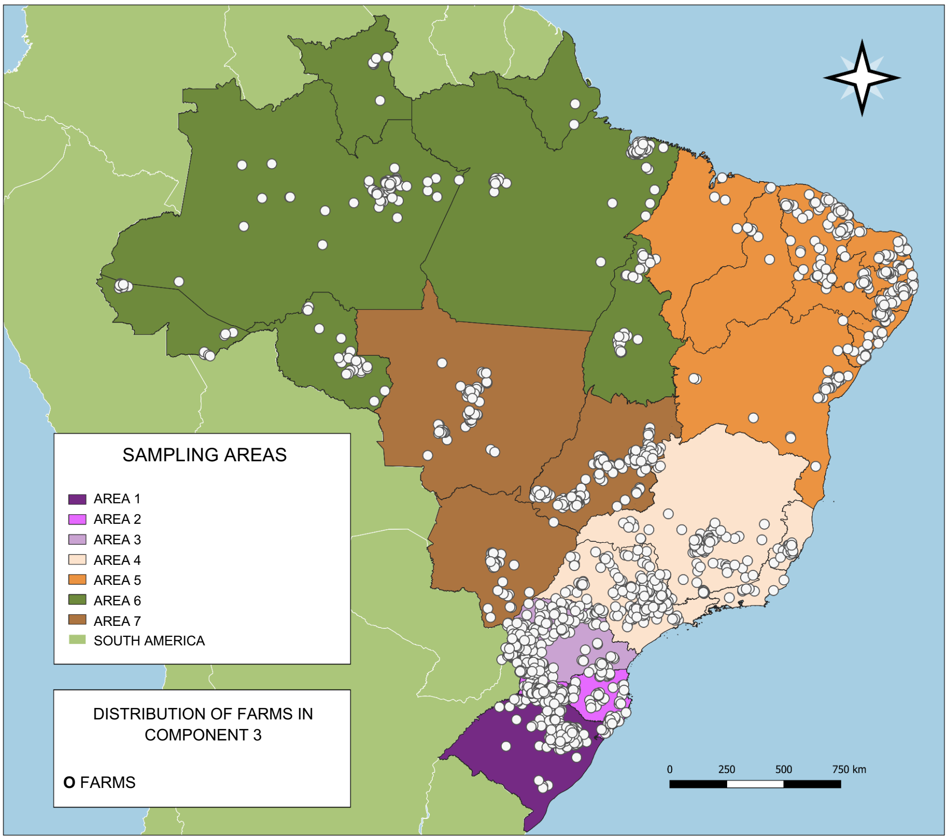

Figure 18. Geographical distribution of poultry establishments sampled in the distinct sampling areas of component 3

3.9.2 Number of samples tested

Of the total number of samples taken for component 3, 44,202 laboratory tests were performed. In order to meet the scheduled provisions of the plan, the set of 11 animals sampled in each epidemiological production unit (nucleus) of a poultry farm was to generate at least 13 laboratory tests, encompassing one assay for each blood serum sample, and one assay for each pool of swabs. The variability in the number of laboratory tests per farm that can be seen in the present study was the result of the fact that there were a varying numbers of epidemiological production unit (nucleus) on the sampled farms, different sampled species (number of pool), as well as samples being rejected or due to laboratory losses.

The distribution of samples analyzed per state in component 3 is shown in figure 19. The state of Rio Grande do Sul (Area 1) obtained the largest number of laboratory tests performed, totaling 6,750 (15.27%), as shown in figure 19.

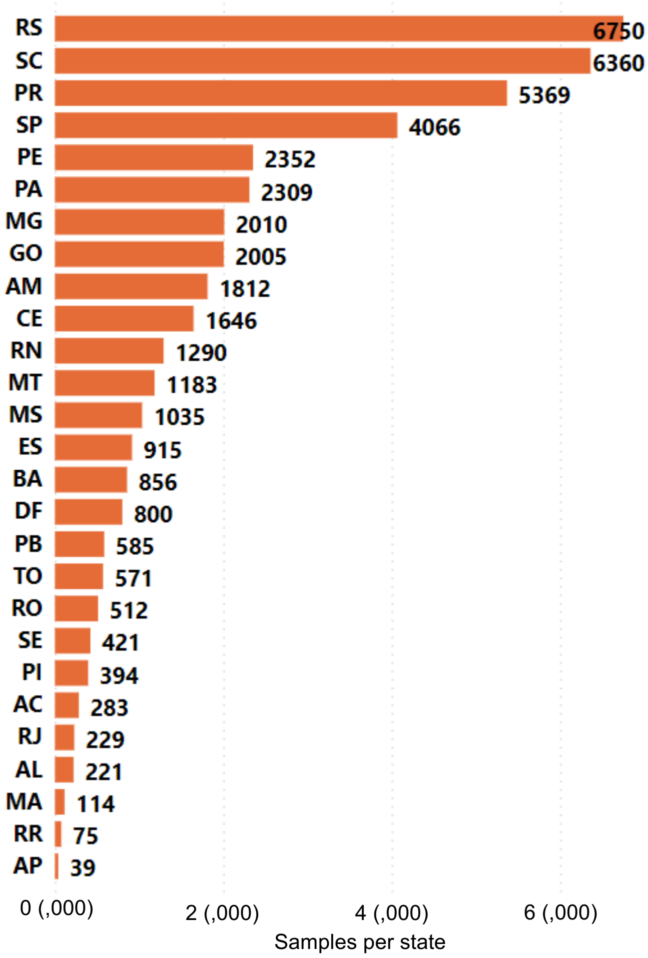

Figure 19. Frequency of the number of samples tested, by State, for component 3

Area 5 obtained the largest share of laboratory tests performed (Figure 20), totaling 7,879 (17.82%). It is important to emphasize that area 5 possesses the largest number of states (PE, CE, RN, BA, PB, SE, PI, AL, MA).

Key: 1 (Rio Grande do Sul); 2 (Santa Catarina); 3 (Paraná); 4 (Southeast region); 5 (Northeast region); 6 (North region) and 7 (Center-West region)

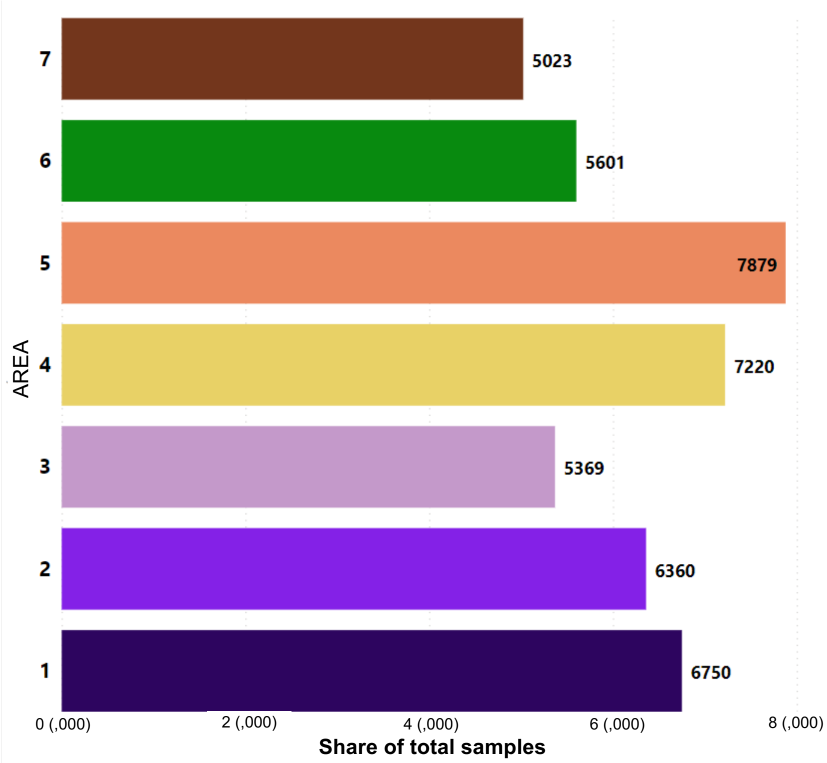

Figure 20. Distribution of the number of laboratory tests per sampling area in component 3

3.9.3 Category and species of birds sampled

During the period in which epidemiological surveillance and sample-taking for component 3 was happening, samples were taken of 4 distinct types of poultry-breeding and poultry-meat raising establishments, to wit: broilers, commercial egg-laying, parent and grandparent birds, as shown in figures 21 and 22.

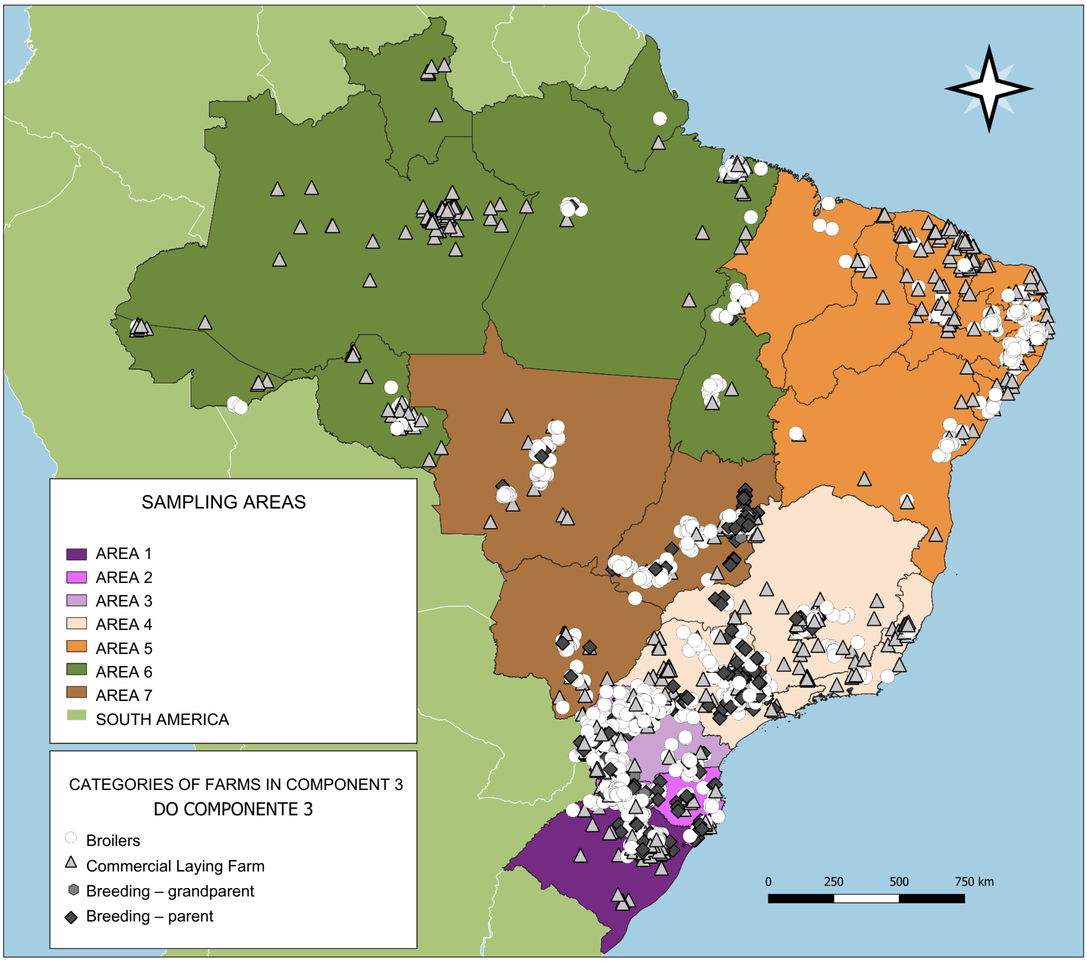

Figure 21. Geographical distribution of the several types of poultry establishment that were sampled in component 3

The vast majority of samples taken were harvested in commercial broiler chicken-producing establishments, totaling 55% of the establishments sampled for that category. Figure 22 shows the frequency of the number of sampled establishments in component 3 by several category of poultry production.

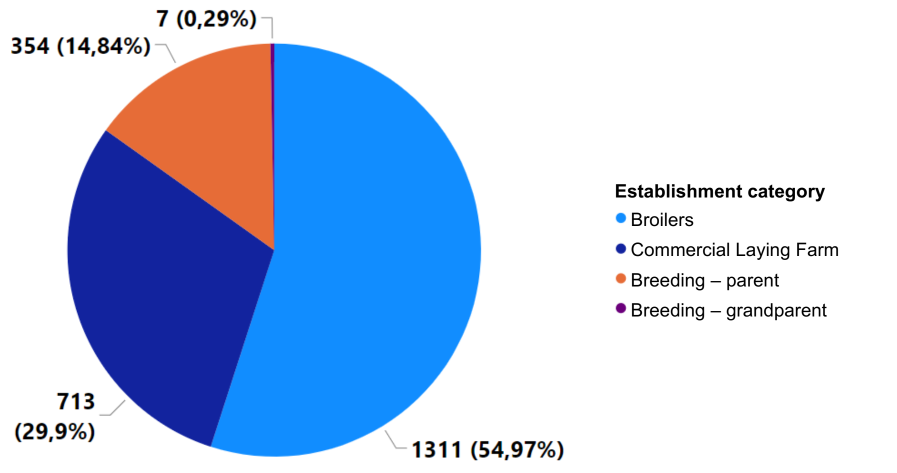

Figure 22. Frequency of the number of sampled establishments in component 3 by several category of poultry production

Regarding species of poultry sampled, chickens were the majority with 2,100 (88.05%) production farms being sampled. The second-most-commonly sampled species of farm was turkey farms, with 219 (9.18%), as shown in Figure 23. Additionally, other species like quail, ducks and teal were sampled in component 3. Figure 23 shows the distribution of species in relation to the number of poultry establishments that were sampled.

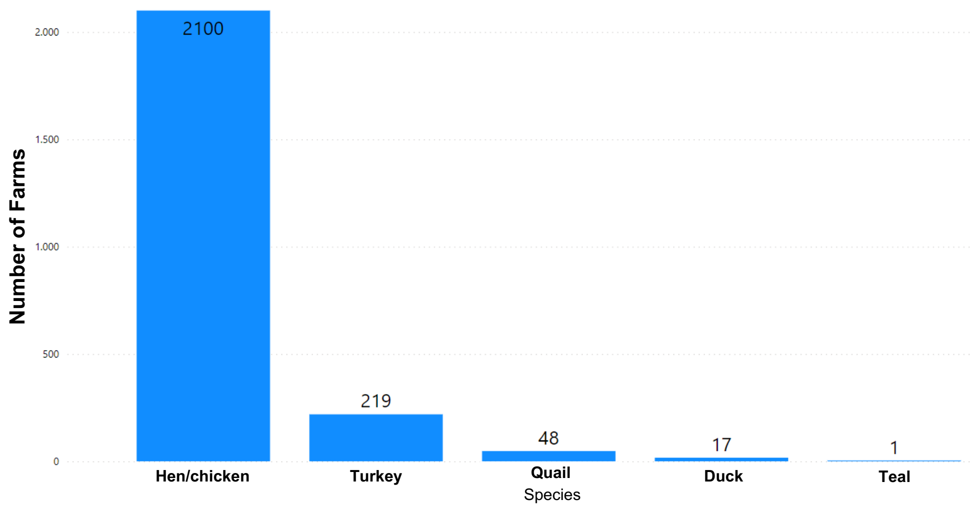

Figure 23. Frequency of the number of establishments holding distinct species of poultry and sampled in component 3

Chickens were sampled in all 27 Brazilian states (Figure 24). Sampling of commercial quail was more frequent in the states of Rio Grande do Sul, Minas Gerais and Santa Catarina (Figure 25). The vast majority of farms keeping ducks were in the state of Santa Catarina with 14 commercial duck farms being sampled (Figure 26). Turkeys were sampled only in the states of the South of

Brazil (RS, SC and PR). Regarding teal, sampling took place on only 1 farm in the state of São Paulo.

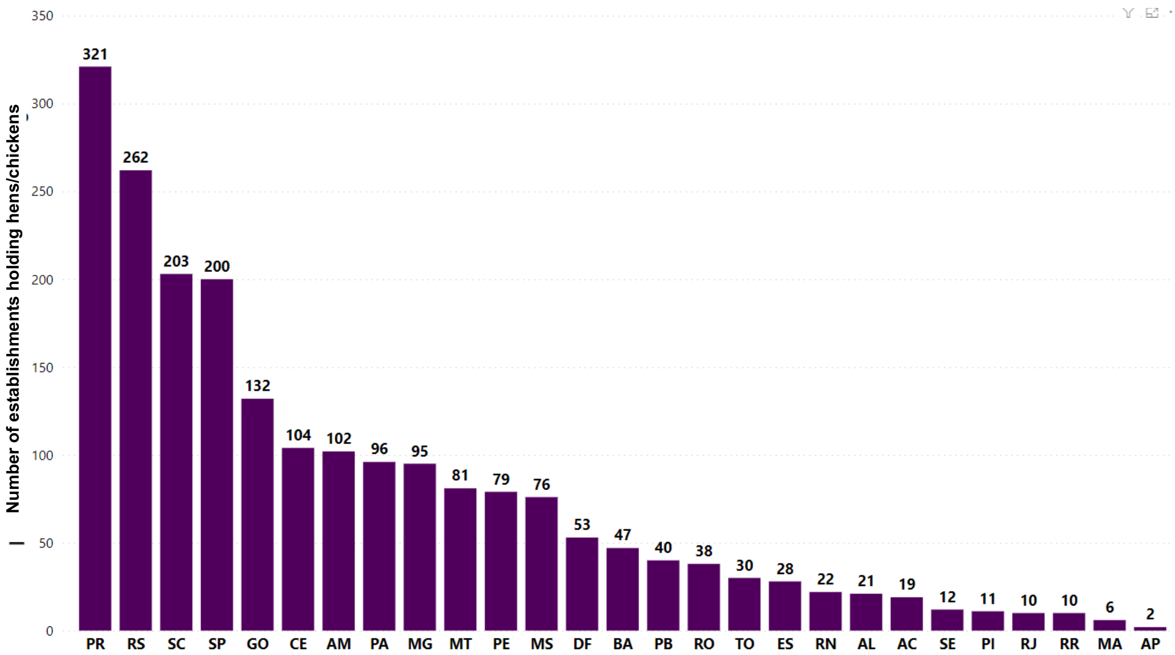

Figure 24. Distribution of poultry establishments holding chickens, by State, and sampled for component 3

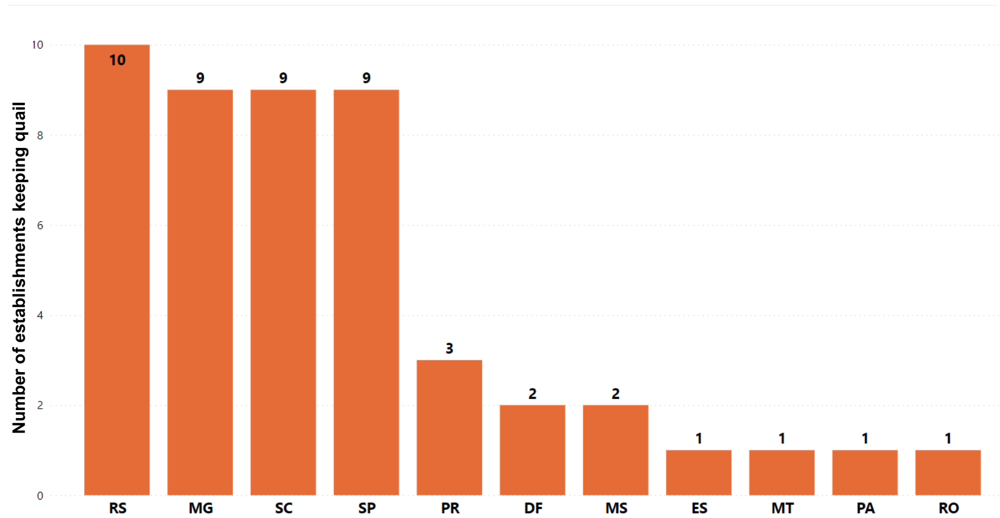

Figure 25. Distribution of establishments holding quail, by State, and sampled for component 3

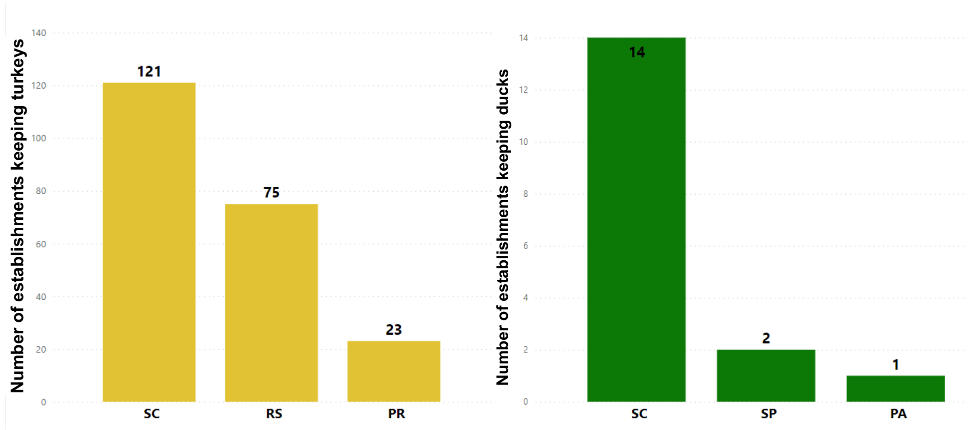

Figure 26. Distribution of establishments holding turkeys and ducks, by State, and sampled for component 3

3.9.4 Analysis of serological tests

In order to obtain the serological profile, 25,237 ELISA tests were performed in order to detect influenza A antibodies, of which 90 samples (0.35%) were positive. Figure 27 shows the total number of ELISA blood serum tests performed in each state of Brazil.

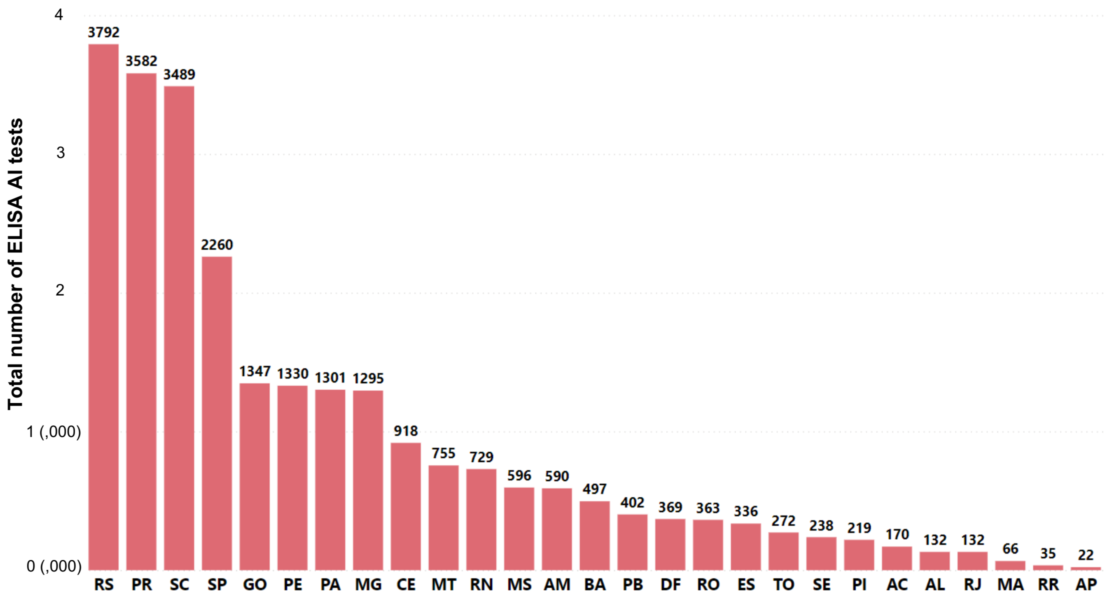

Figure 27. Total number of serological ELISA tests performed for avian influenza, by State, in component 3

Of the total number of 90 positive samples, 67 underwent HI in order to research for antibodies to subtypes H1 to H16 of type A influenza virus (Figure 29). The remaining 23 samples did not present a serum volume sufficiently large for the assay, and for this reason were not tested.

Figure 28 shows the frequency of samples testing positive in serological ELISA tests for AI, by State.

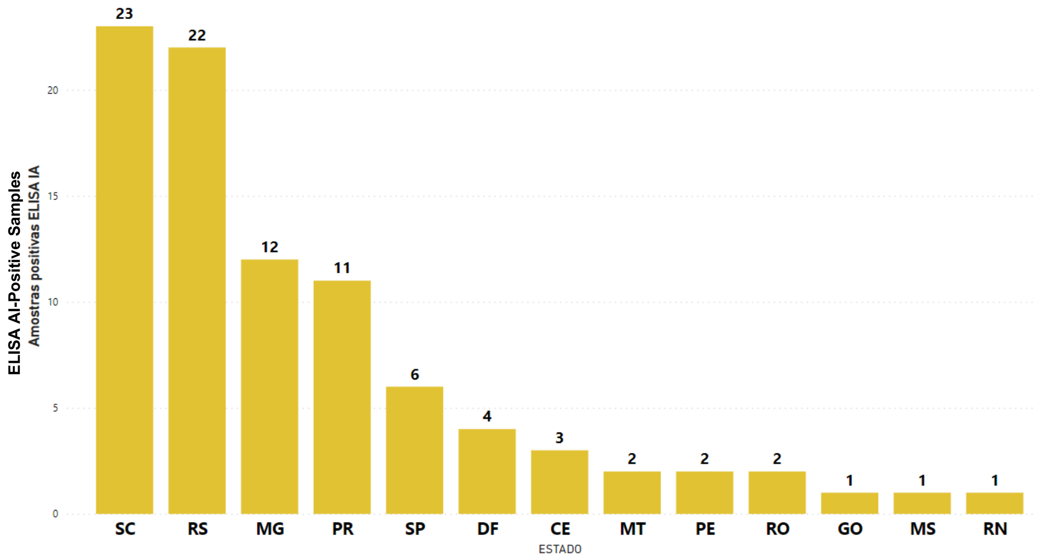

Figure 28. Frequency of samples testing positive in serological ELISA tests for avian influenza, by State, in component 3

Typification of seropositive samples in the ELISA screening test showed only 3 samples containing antibodies to different subtypes of type A influenza virus hemagglutinin (Figure 29). The results showed that two samples from hens raised on two commercial egg-laying establishments in the state of Rio Grande do Sul presented antibodies to subtypes H1 and H16 of influenza virus A. In the state of Santa Catarina, antibodies were detected to subtype H13 in a sample taken from a turkey farm.

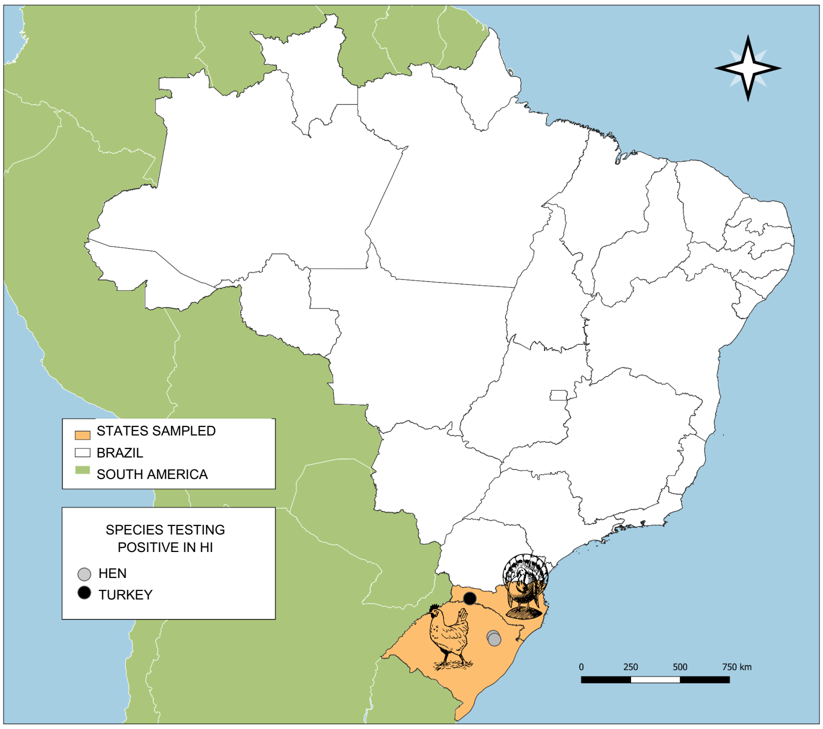

Figure 29. Geographical distribution and species showing the presence of hemagglutinin subtypes of avian influenza virus in component 3

3.9.5 Analysis of molecular tests

8,363 molecular tests were also carried out on pools of tracheal and cloacal swabs in order to detect type A influenza virus through RT-qPCR reactions. Figure 30 shows the number of RT-qPCR tests performed to detect matrix (M) and nucleoprotein (NP) genes of type A influenza virus in the different states.

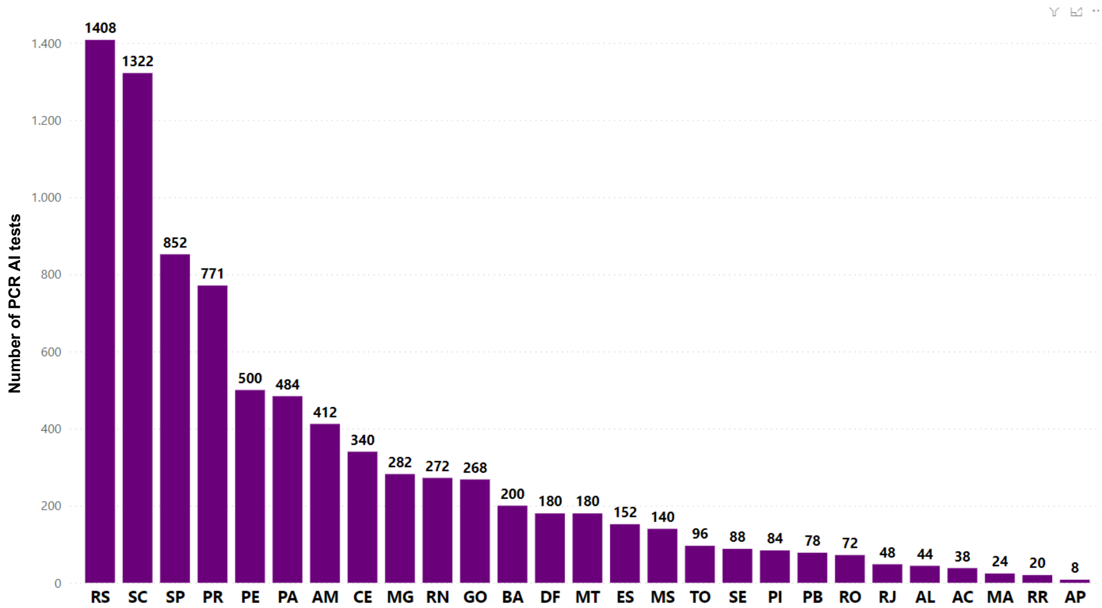

Figure 30. Total number of molecular screening tests performed for avian influenza, by State, in component 3

No sample obtained from commercial poultry tested positive to the molecular test for detecting type A influenza virus. Therefore, no specific assays were performed for subtypes H5, H7 and H9.

In order to detect ND virus, 8,387 molecular tests of pools of tracheal and cloacal swabs targeting matrix gene (M) were performed. The difference between the number of reactions in RT-PCR to AI and ND was found as a result of rejected samples and losses in the laboratory process.

204 positive samples for gene M were found, from 123 distinct industrial poultry establishments. It should be clarified that the detection of gene M identifies pathogenic and nonpathogenic strains originating from vaccines or from the field virus. It should be emphasized that ND vaccination is mandatory for egg-laying flocks and breeding flocks in accordance with Normative Instruction no. 56, published December 4, 2007, and therefore the use of live vaccines may lead to the identification of samples testing positive for gene M.

Table 2 shows the numbers and distribution of samples testing positive for ND virus gene M by state.

Table 2. Frequency of identification of matrix gene for Newcastle disease in samples in distinct States

State	Samples identifying Newcastle disease virus matrix gene
PR	117
SC	25
PA	10
RS	9
BA	7
ES	7
GO	6
MS	5
PE	5
SP	5
CE	3
PB	2
AL	1
AP	1
RJ	1

The greatest prevalence of positive samples for gene M of ND virus was in Paraná, with 117 detections. After this, the states of Santa Catarina and Pará presented the next-highest frequency of positive samples for gene M.

Samples testing positive for ND virus matrix gene M only in hen/chicken and turkey flocks. In the case of in the case of hens/chickens samples testing positive were found on 110 farms, 79 being broiler farms, 18 commercial egg-laying farms and 13 breeding farms (parent). Regarding turkey farms, there were 12 meat-producing farms and 1 parent farm (Figure 31). Figure 31 shows the number of farms containing animals identified with ND virus gene M (dark blue) compared with the number of sampled farms (light blue), as presented above in figure 23.

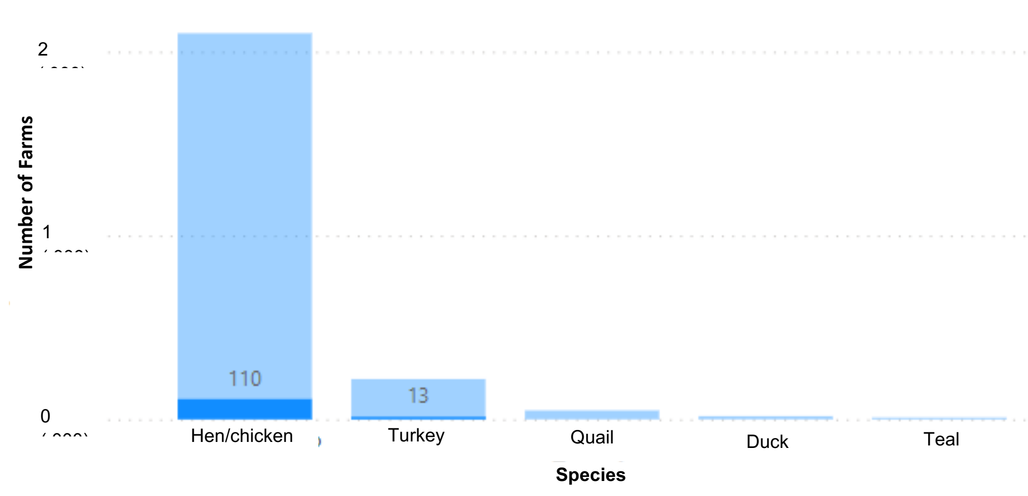

Figure 31. Frequency of identification of matrix gene for Newcastle disease in distinct species of bird

Positive samples for gene M of the ND virus underwent a further RT-qPCR reaction to amplify the gene F region in order to detect only those samples containing mesogenic or velogenic strains of APMV-1 virus. Of the total of 204 samples tested to detect gene F of the ND virus, all tested negative. It should be emphasized that an ND outbreak is configured only on identifying the pathogenic ND virus.

3.10 Interpretation of the surveillance

Active surveillance was carried out in order to monitor and identify circulating AI and ND in industrial poultry populations.

In serological monitoring for AI, antibodies to three different viral subtypes were found in 3 samples, two involving chickens and one of a turkey. HI testing identified antibodies to subtypes H1 and H16, H1 and H13. The serological data indicates that the population of sampled birds had been exposed, in the past, to known low pathogenicity type A influenza viruses. It should be emphasized that samples testing seropositive belonged to an egg-laying and turkey farm, which, according to the AI and ND surveillance plan, are deemed moderate-to-high risk categories for the introduction of HPAI virus into production systems. The most important aspects for this risk categorization were, in order of importance: the susceptibility of the species that are present; the duration of the animals' production cycle; and the impact of handling, health and biosecurity practices.

Regarding the interpretation of molecular testing, the absence of samples testing positive for type A influenza virus, combined with data from epidemiological surveillance, showed that there is no AI infection in commercial populations of poultry.

As regards molecular testing for ND virus in industrial poultry flocks, gene F (the gene indicating pathogenicity of the ND virus) was found to be absent in samples testing positive for gene M, which shows that the samples originate from vaccine standards or nonpathogenic ND virus. Therefore, it can be inferred that there is no ND infection among industrial poultry populations.

COMPONENT 4 - Active surveillance on backyard farms

4.1 Objective and source of data

Component 4 aims mainly to seek to detect AI and ND in backyard populations in at-risk areas; in other words, areas where there is a greater likelihood of exposure to migratory birds. Additionally, poultry flocks (smaller than 1000 birds) for local trade in at-risk areas was also object of surveillance for this component.

Epidemiological surveillance as well as serological and molecular investigation of this population enable early detection of these diseases, because most cases in other countries of the introduction of the virus and AI outbreaks had the same origin, enabling measures to be taken to reinforce biosecurity and protect industrial poultry production.

Epidemiological surveillance by means of the inspection of backyard facilities provides an opportunity to detect susceptible animals showing clinical signs or changes in animal husbandry rates that can lead one to suspect the occurrence of the target diseases of the present study. Additionally, inspection of facilities enables their registries to be brought up to date, allowing the OVS to engage with growers, and enabling animal health education actions to be taken in order to extend the sensitivity of passive surveillance.

All the activities performed in order to fulfill “component 4 – active surveillance in backyard flocks” have been recorded in the Epicollect5 application software.

4.2. Surveillance approach

Active surveillance strategies were used in order to confirm the absence of clinical signs or changes in animal husbandry indicators and animal health indicators compatible with the occurrence of AI or ND within the territory of Brazil, using as a reference the results of visits, clinical inspections, and the assessments of animal husbandry, animal health, and epidemiological indicators, as well as an analysis of the results of serological and molecular tests in backyard and small-scale commercial poultry establishments.

4.3. Type of risk indicator

The study design built in the concept of risk-based surveillance, where sampling targets farms and birds at greater risk of exposure to AI in the several sampling areas of Brazil.

In order to define active surveillance activities from the standpoint of risk-based surveillance, criteria provided for in the AI and ND surveillance plan were used. An extensive database on locations where migratory birds had been reported present either by direct citing or by field research was made available by CEMAVE/ICMBio for this purpose. Owing to the large number of species and sites where sightings have taken place in Brazil, four criteria have been adopted for selecting locations where surveillance is to take place:

1. the presence of migratory birds from families of greater epidemiological importance for transmitting AI, particularly the Anseriformes (*Anatidae*) and Charadriiformes (*Charadriidae*);

2. species in which the pattern of migration involves routes originating in the northern hemisphere (Nearctic species), because they pose the greater threat of introducing AI into Brazil;

In the case of the southern region of Brazil, routes specifically related to the South American continent were taken into account, and municipalities where migratory birds from this region were present were included;

3.the concentration of the raising of poultry flocks in the region; and

4. the presence of industrial poultry production (establishments housing more than one thousand birds) in the municipalities in which such sites are located.

4.4 Target population

To meet the objectives of this component, the target population was backyard flocks located in at-risk areas, because of their greater likelihood of exposure to migratory birds, and where such populations were located near to industrial poultry establishments.

Regarding the selection criterion for sampled birds, the diversity of the birds found in the backyard flocks was considered, taking into account the most common species of gallinaceous and the species that pose the greatest risk of AI infection.

In addition to the most common species of gallinaceous, described herein as “hen/chicken”, three further options of other gallinaceous birds were added: guinea fowl, turkeys and quail. Regarding the species at greatest risk for AI, three domestic Anseriform species were included, all of them of the family Anatidae: ducks, geese and teal. Sample-takers were asked to sample the greatest diversity of species for farm, albeit limited to these seven species.

The target population was defined by taking into account the species that are susceptible to AI and ND and present within the territory of Brazil.

4.5 Sample design

The study design incorporates the concept of risk-based surveillance in the several sampling areas within Brazil.

The sample size estimate took into consideration the backyard population or small-scale production.

The parameters that were used for the calculation were: expected prevalence, sensitivity and specificity of the diagnostic tests; number of sampled areas; assessment of the risk associated with the epidemiological units.

The sample size was calculated using a two-step strategy. In order to perform these calculations, we assumed a 1% prevalence between epidemiological units, and a 95% confidence level. Additionally, it was assumed that if AI were present at a farm, 30% of the birds would be infected.

The parameters associated with the performance of the diagnostic tests, taken into account for the calculation, were 95% sensitivity for "ELISA to detect AI" and "PCR to detect ND". Specificity was deemed to be 100% for the diagnostic protocol, associated with clinical and epidemiological investigation procedures and with the supplementary tests allowed for in the plan.

Regarding sampling areas, unlike component 3 with 7 areas are delimited by the State borders, for this component 4 regions related to the 3 main migratory routes (Central Brazil, Atlantic/Northeast and Amazon) that cross Brazil as a whole were taken into account; therefore, the political limits (borders) of the 'federative units' (States) were not taken into account.

Finally, for each region that was associated with each of the migratory routes (regions of influence) a sample size of at least 322 epidemiological units, in which 11 birds would be sampled, was defined, and the selection of Anseriformes was prioritized.

4.6 Sampling strategy

The sampling strategy of this component aimed to achieve detection of AI and ND in backyard populations or small-scale production flocks located in act-risk areas. These birds are more likely to be exposed to migratory birds, and therefore the execution of this component not only identifies the presence and circulation of the virus in populations at greater risk, but also may provide warnings of occurrences in locations that can impact the sales production systems, enabling measures to reinforce biosecurity and protect industrial poultry production to be taken.

4.7 Type of material harvested

Whatever the size of the epidemiological unit, 11 (eleven) birds are chosen at random from the establishment for sample-taking. In order to obtain serum, blood samples were harvested on an individual basis by venipuncture from 11 live animals. Tracheal and cloacal swabs were taken from each selected bird. It should be emphasized that in the case of farms keeping different species of birds the sample-takers were asked to sample the most diverse possible range of species for farm, albeit limited to the seven species cited in item 4.4, and that each pool of swabs be separated and identified by species.

4.8 Individuals responsible for sample-taking

Veterinarians of the official animal health services in each State were responsible for all inspections and activities, supported by technical auxiliaries and employees of the establishments.

4.9 Activities performed

4.9.1 Identification of establishments

Each inspected establishment received a unique identification (designated as the MAPA Code), generated by the DSA in accordance with the number of farms defined for each State, or "Federative Unit". The selection of municipalities for sampling was carried out by DSA, but the selection of farm was carried out by the veterinarian in charge of the local veterinary unit (LVU) in accordance with the criteria described in the AI and ND Surveillance Plan - ANNEX 1 - SAMPLING PLAN FOR THE DETECTION OF AI AND ND IN BACKYARD ESTABLISHMENTS AND IN AREAS AT GREATER RISK OF THE INTRODUCTION OF AI – COMPONENT 4. Apart from these characteristics, the search for farms presenting the following features was prioritized:

- points attracting wild birds (lakes, reservoirs, etc.);
- the presence of Anseriform birds;
- evidence of close contact between migratory birds and domestic commercial poultry;
- birds raised free-ranging (not confined in enclosures);
- the use of surface water as drinking water for the poultry; and
- the presence of more than one species of bird living on the same holding.

4.9.2 Records of the data

The responsible veterinarian taking the samples completed the electronic forms using the Epicollect5 application software, and the information was shared with the LFDA laboratories that tested the samples; with DSA and with the OESAs, using the electronic spreadsheets.

4.9.3 Schedule for sample-taking

The component 4 samples were harvested from January to June 2023. It can be seen in figure 34 that in March there was a large number of farms sampled along the Central Brazil and Atlantic/Northeast migration routes.

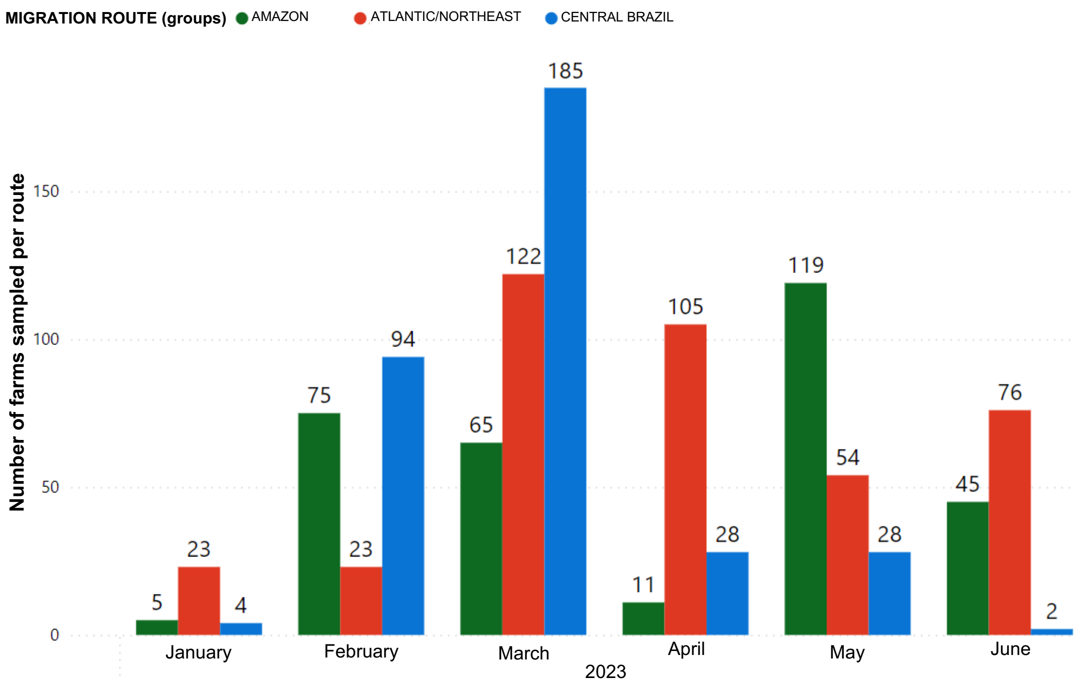

Figure 32. Temporal distribution of sample-taking for component 4 by migratory route

4.10 Results

4.10.1 Number and geographical distribution of sampled farms

To assess for the circulation of ND and AI viruses in backyard farms or small-scale poultry farms, samples were taken from 1,064 farms under the three migration routes in question for serological and molecular testing.

Figures 33 and 34 show the distribution of the farms that were sampled, located all along the wild bird migration routes. On the Atlantic/Northeast Route, 403 farms were sampled, accounting for 37.87% of the total number of sampled establishments. There were 341 farms on the Central Brazil route, which has the second-largest number of sampled establishments on it.

The state containing the largest number of sampled establishments with inspected backyard poultry-raising operations, was Pará, with 142 (13.34 %) of all establishments, as shown in Figure 33. The feature differentiating this state from the others, is that owing to its geographical position within Brazil and its size, it was involved in sampling for all three migratory routes.

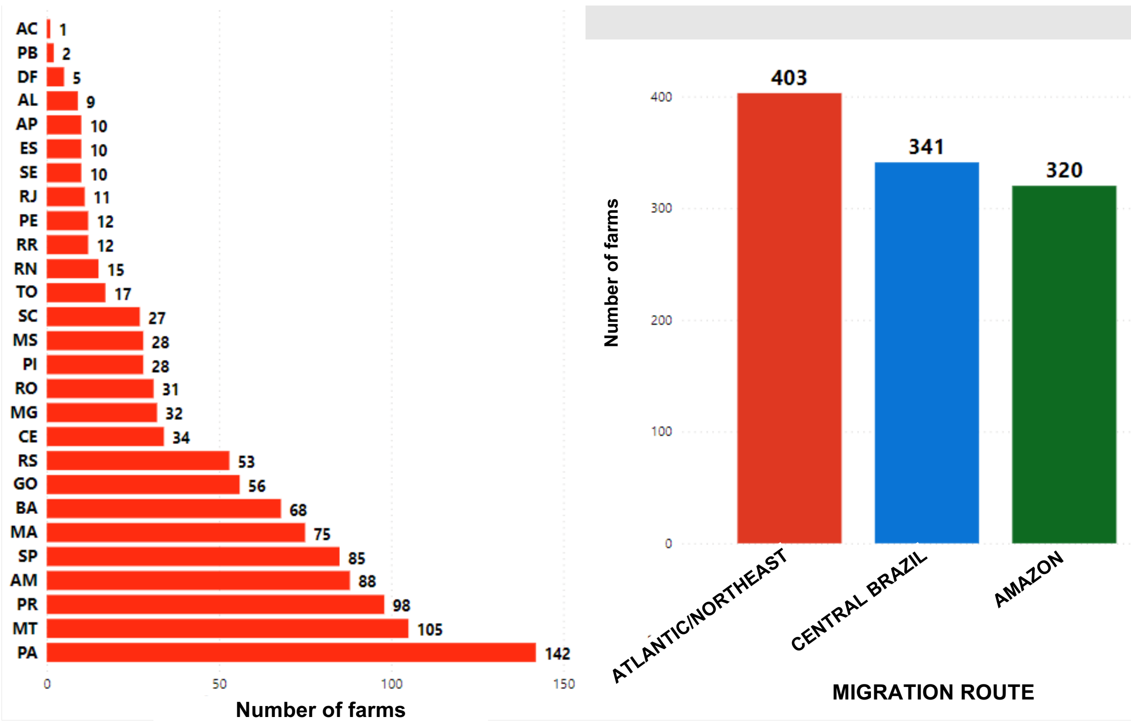

Figure 33. Frequency of the number of farms sampled per State and migration route in component 4

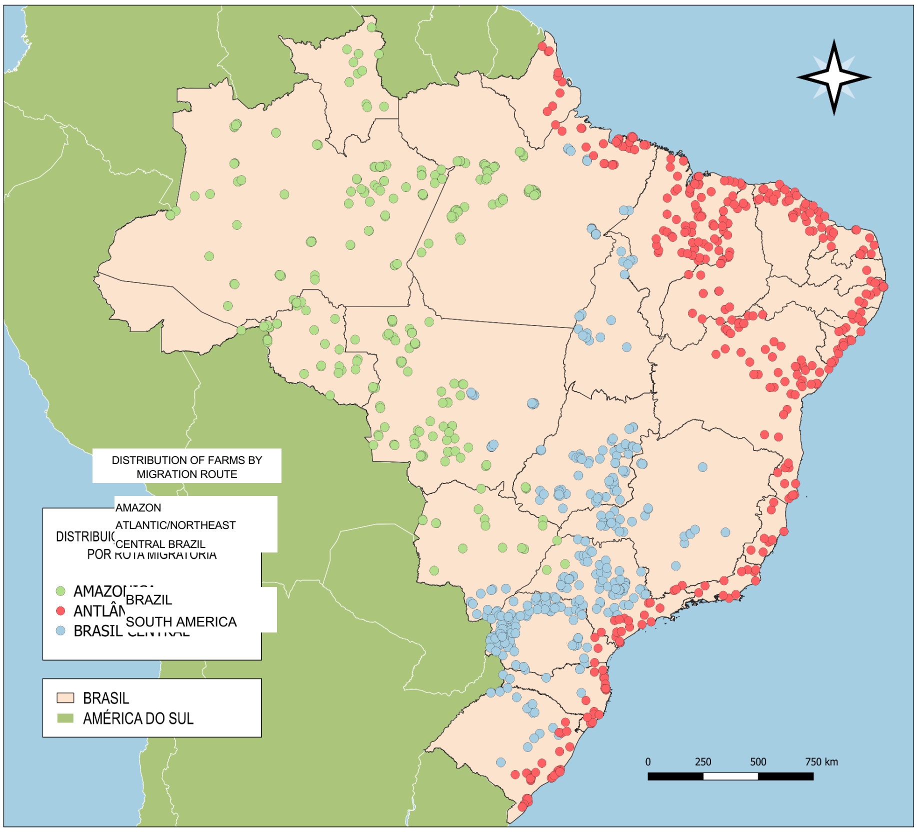

Figure 34. Geographical distribution of farms sampled per route, in component 4

4.10.2 Number of samples tested

Epidemiological and laboratory surveillance for component 4 resulted in 28,408 laboratory tests. To comply with the provisions of the plan, the set of 11 animals sampled on each farm was to generate at least 25 laboratory tests, including blood sera and pools of swabs, given that there was serology for ND in this component. The variability in the number of laboratory tests per farm that can be seen in the present study was the result of the number of birds of different species that were sampled, as well as samples being rejected and laboratory losses.

The state of Pará recorded the largest number of laboratory tests, with 3,730 (13.13 %) of the tests, as shown in figure 35.

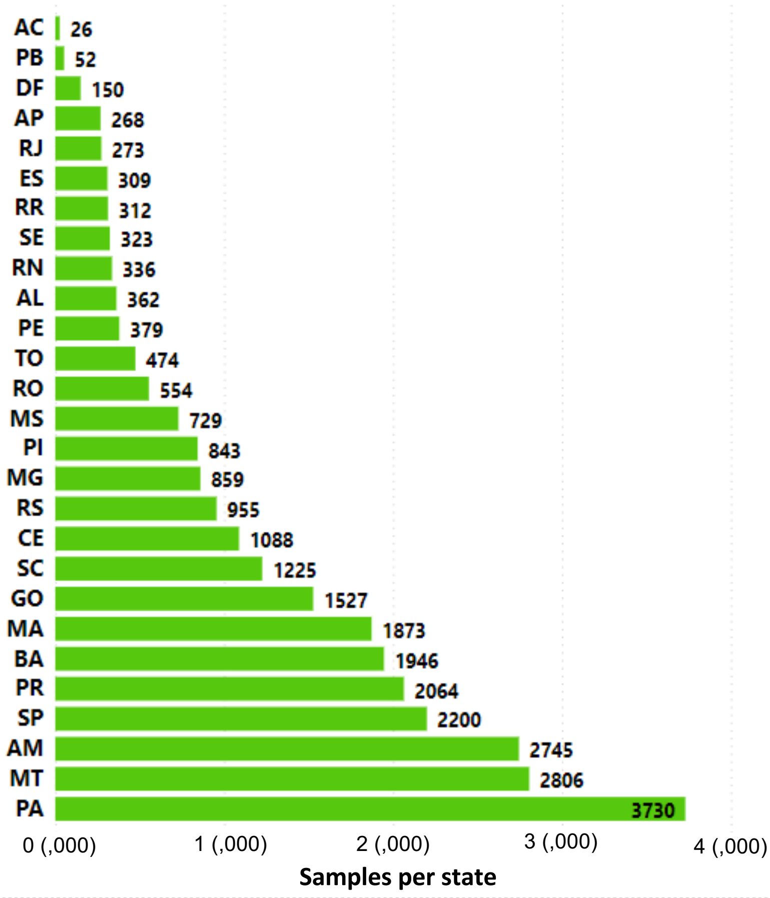

Figure 35. Frequency of the number of laboratory tests, by State, for component 4

4.10.3 Bird species sampled

The majority of birds that were sampled in component 4 kept populations of hens/chickens, specifically 998 (93.8%) farms, of which 66 farms (6.2%) did not have hens/chickens among the flock. Of the total number of 1,064 inspected farms, 812 (76.32%) kept mixed populations including other gallinaceous species.

Concerning the risk of infection by and spread of AI, 542 farms had populations of Anseriformes. Figure 36 shows the distribution of farms on which Anseriformes were present, by their location in relation to the migration routes.

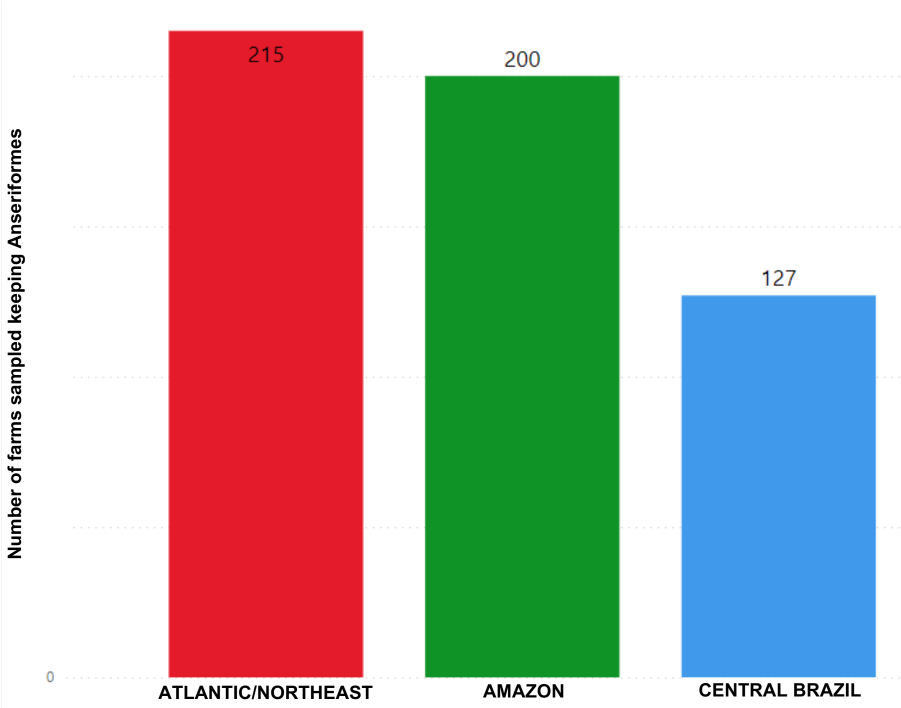

Figure 36. Distribution of the number of sampled farms holding Anseriformes by migratory route

It should be noted that the majority of farms with a presence of Anseriformes were observed under the Atlantic/Northeast and Amazon routes.

4.10.4 Analysis of serological tests

Serological investigation for ND viruses involved 9,886 ELISA tests. According to results obtained, antibodies to ND virus were identified in 224 farms, totaling 682 samples.

The presence of antibodies to ND virus in samples from the flocks, along with negative results to confirmatory molecular testing, may show a vaccine immune response given that the sampled birds may have been purchased after being vaccinated against ND. The result may also demonstrate the circulation of vaccine strains or nonpathogenic viruses in the population. Figure 37 shows the ratio of farms that had samples that tested positive in the serological ELISA test for ND virus as against farms that were sampled in the several States

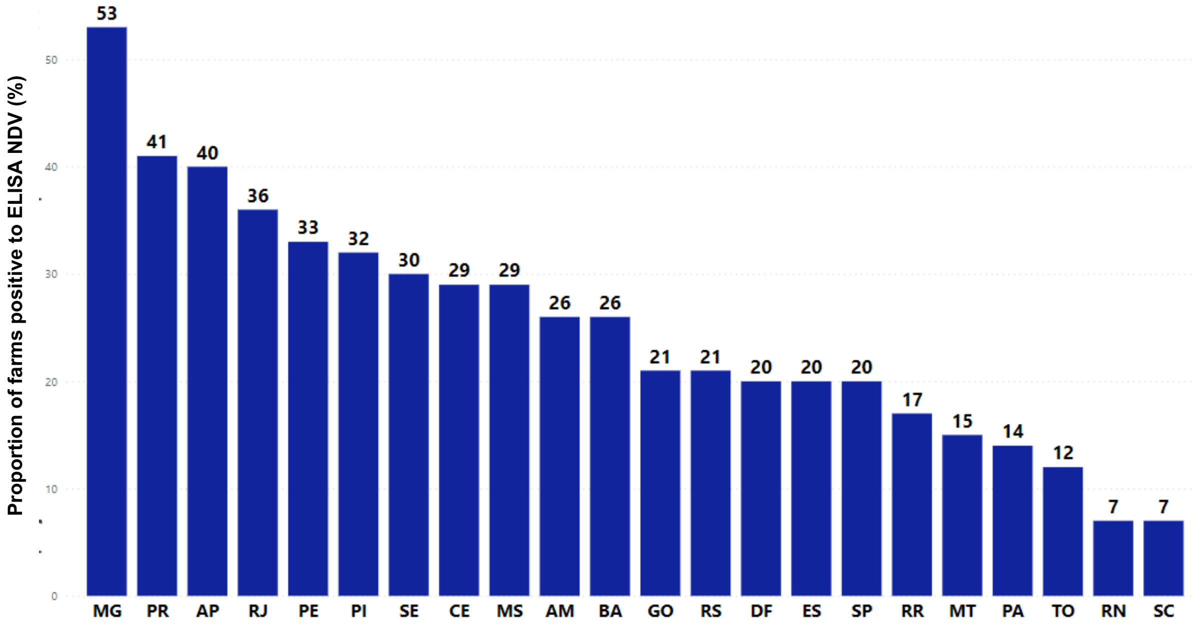

Figure 37. Ratio of farms that had samples that tested positive in the serological ELISA test for Newcastle disease virus as against farms total number that were sampled in the several States for component 4.

The state with the largest ratio of seropositive samples for ND virus to the number of sampled farms was Minas Gerais with 53%.

Notice that in many of these farms there were mixed populations, such that out of 224 farms in Brazil with positive ELISA test results, 214 were raising chickens and on 49 of them, other galliformes (turkeys, quail and guinea fowl) were also being raised alongside the chickens. Only one farm with a seropositive sample result was exclusively raising other galliformes, in other words without chickens and Anseriformes.

Anseriformes (ducks, teal and geese) were being raised on 110 of the 224 farms, 9 of which were raising Anseriformes exclusively. It is noteworthy that 43 of the farms returning seropositive sample results for ND virus held chickens, other galliformes and Anseriformes being raised all together.

In order to test for antibodies to the AI virus, 10,557 ELISA tests were performed. The number of samples testing seropositive for influenza A upon ELISA testing was 0.27% with 28 positive samples. The state with the largest number of positive samples found in the ELISA screening test for AI was Rio Grande do Sul with 12 samples (Figure 38).

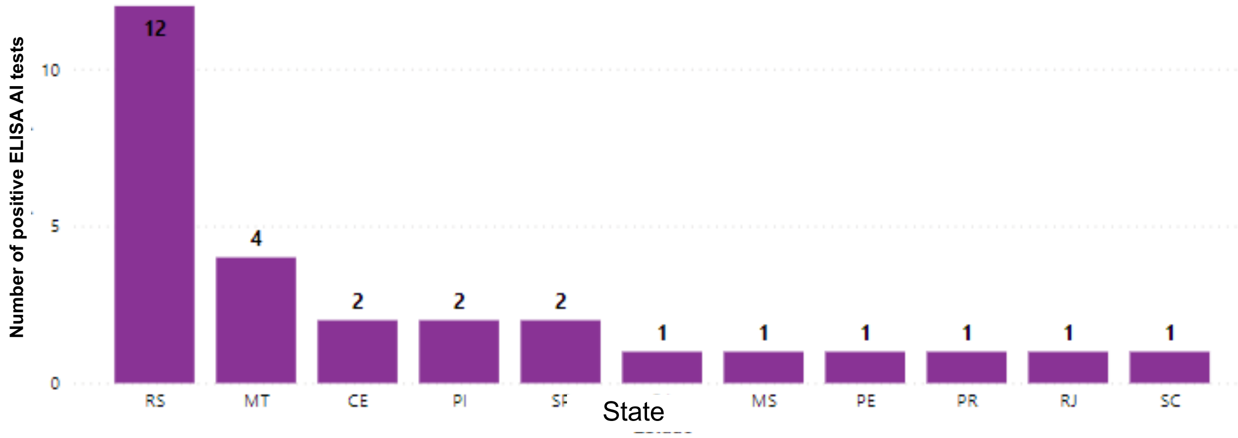

Figure 38. Frequency of samples testing positive in serological ELISA tests for avian influenza, by State, in component 4

All samples testing positive or inconclusive for AI in ELISA underwent HI testing for antibodies against the several subtypes of AI virus (H1 – H16). In the HI test, hemagglutinin was typified in four samples, as shown in table 3.

Table 3. Samples testing positive for the Hemagglutination Inhibition Test (HI) in component 4

State	Species	HI typification
Bahia	Hen/chicken	H7 & H10
Mato Grosso	Hen/chicken	H1
Pernambuco	Hen/chicken	H4
Rio Grande do Sul	Domestic goose	H9

The typification of seropositive samples in ELISA screening tests resulted in the detection of antibodies to subtypes H7 and H10, H1 and H14 in hens/chickens. The test also showed the presence of antibodies to subtype H9 in a sample taken from goose. Geographical distribution and species showing the presence of hemagglutinin subtypes of avian influenza virus is shown in figure 39

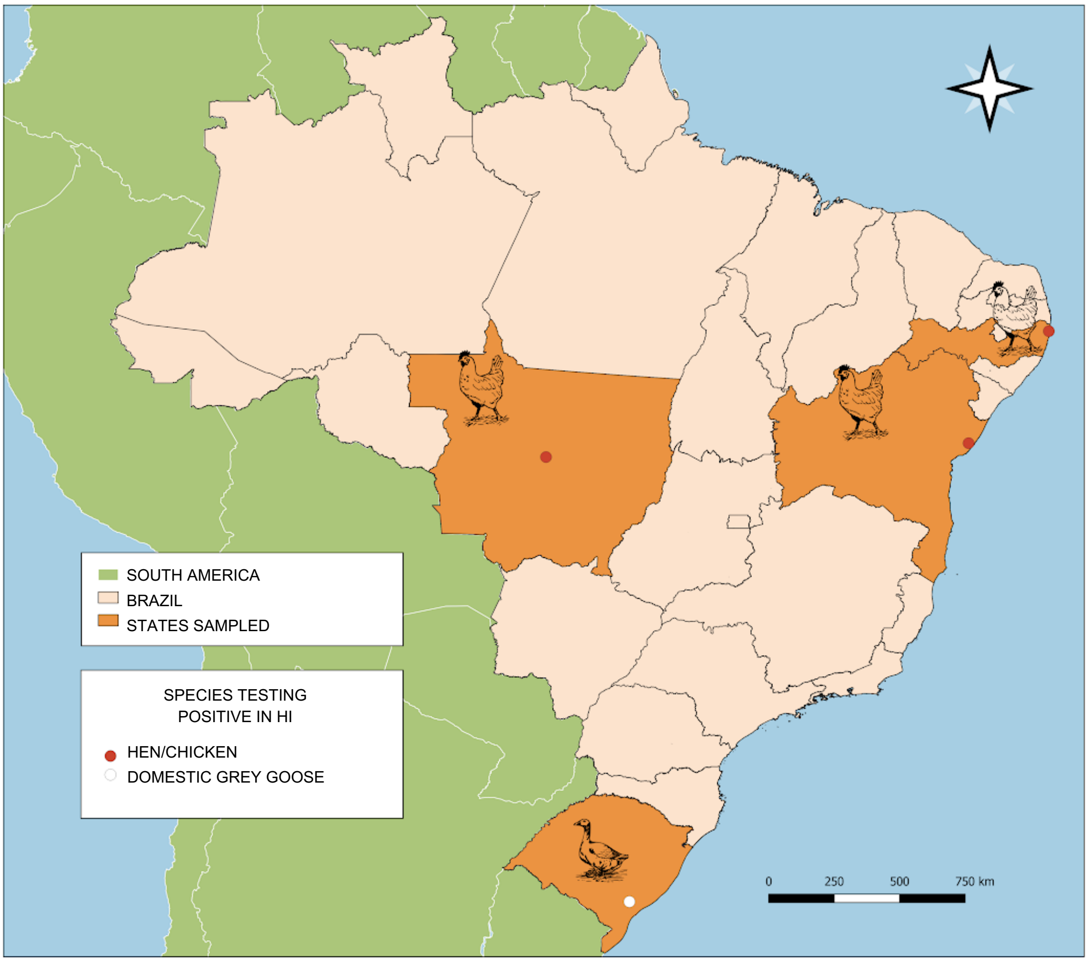

Figure 39. Geographical distribution and species showing the presence of hemagglutinin subtypes of avian influenza virus in component 4

4.10.4 Analysis of molecular tests

Figure 40 shows the frequency of the number of molecular tests carried out on pools of tracheal and cloacal swabs in order to detect type A influenza virus through RT-qPCR reactions. In all, 3,947 tests to detect type A influenza virus by RT-qPCR were carried out.

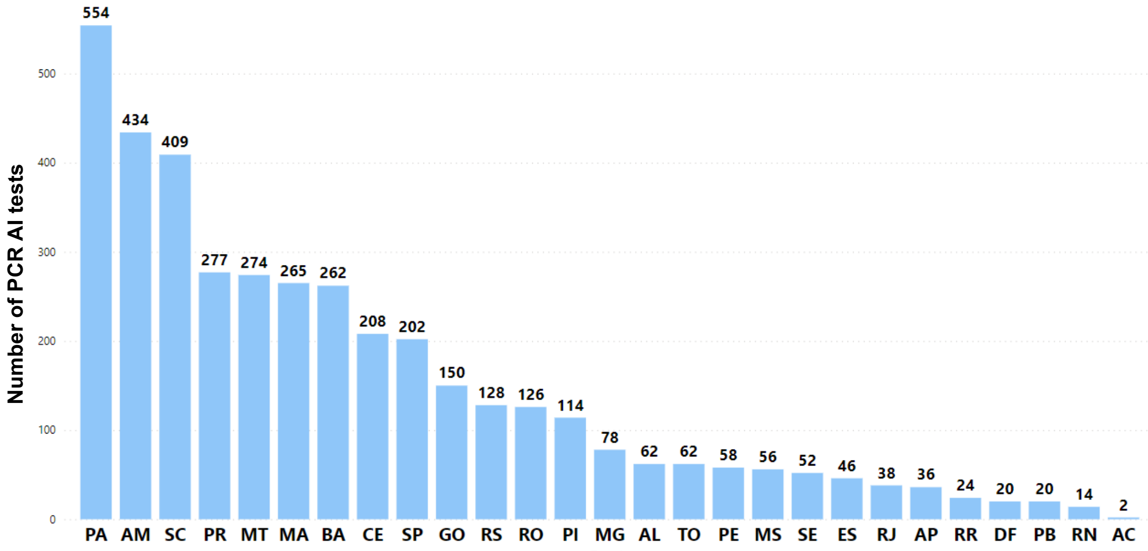

Figure 40. Frequency of the number of molecular screening tests performed for avian influenza, by State, in component 4

According to the tests, there was no detection of positive samples for the M or NP genes of type A influenza virus.

Likewise, in order to detect ND virus, 4,018 molecular tests of pooled tracheal and cloacal swabs were performed. The difference between the number of reactions in RT-PCR to AI and ND was found as a result of rejected samples and losses in the laboratory process.

Of the total tested, 3 samples tested positive by RT-qPCR reaction to the matrix gene for ND virus, two of which were taken from farms in the state of Amazonas, and one from Paraná. In Amazonas the affected species were hens and ducks, while in Paraná the affected specie was a duck.

The 3 positive samples underwent confirmation tests to identify gene F, related to the velogenic ND virus. We emphasize that all samples tested negative for gene F.

4.11 Interpretation of the surveillance

Epidemiological and laboratory surveillance for component 4 sought to detect AI and ND virus among the higher risk domestic birds flocks located in act-risk areas.

The prevalence of antibodies found to ND virus in the flocks was 6.89 % (682) of the tested samples. The presence of these antibodies may indicate that a part of the domestic birds populations that were sampled may have been purchased already having been vaccinated for ND, or exposed to the nonpathogenic ND virus.

It was only possible in the molecular testing to identify the presence of gene M (screening test) in just 3 samples, one from a chicken and two from ducks. It should be emphasized that all 3 samples tested negative for gene F (confirmatory virulence test) of the ND virus, giving proof that virulent strains of the ND virus are not circulating among the target population for this component.

Only 0.27% of the samples tested for type A influenza virus were seropositive on ELISA testing, which is a low frequency. In the analysis to typify antibodies to type A influenza virus, subtypes H7 and H10, H1 and H14 were identified in hens/chickens and subtype H9 in a sample taken from a goose. Additionally, molecular tests carried out on the same samples did not identify any presence of the type A influenza virus, showing absence of AI viral circulation among these flocks.

COMPONENT 5 - Active surveillance in AI- and ND-free compartments

In 2014, MAPA published NORMATIVE INSTRUCTION no. 21, DATED OCTOBER 21, 2014, which governs the technical standards for Sanitary Certification on the Compartmentalization of the Poultry Production Chain in breeding and broiler farms and hatcheries for chickens or turkeys, regarding infection by the AI and ND viruses. The goal of the standard is to recognize and attest to a poultry subpopulation with a distinct sanitary status of being AI- and ND-free, by adopting additional biosecurity procedures, epidemiological surveillance, oversight and audits. Application of the standard is voluntary and only applies to the companies that are interested in being recognized and have received the sanitary certificate.

The farms making up the compartments represent a production system with a higher level of biosecurity, which may be associated with a lower risk of the occurrence of AI and ND as a result of the strictness of the prevention measures that are adopted. These farms were also introduced as a component of the AI and ND surveillance plan.

AI- and ND-free compartments are formed of associated production and functional units, whereby two models of compartments are provided for:

1) Breeding compartment: made up of breeding farms and hatcheries as well as associated functional units
2) Meat production compartments: made up, at least, of parent breeding farms, their hatcheries, broiler farms and associated functional units.

There are currently 7 compartments certified by MAPA, all breeding-related. For the period under assessment, there was an additional compartment for the production of meat, surveillance data for which have been included in the present report. Figure 41 shows the geographical distribution of AI- and ND-free compartments in Brazil.

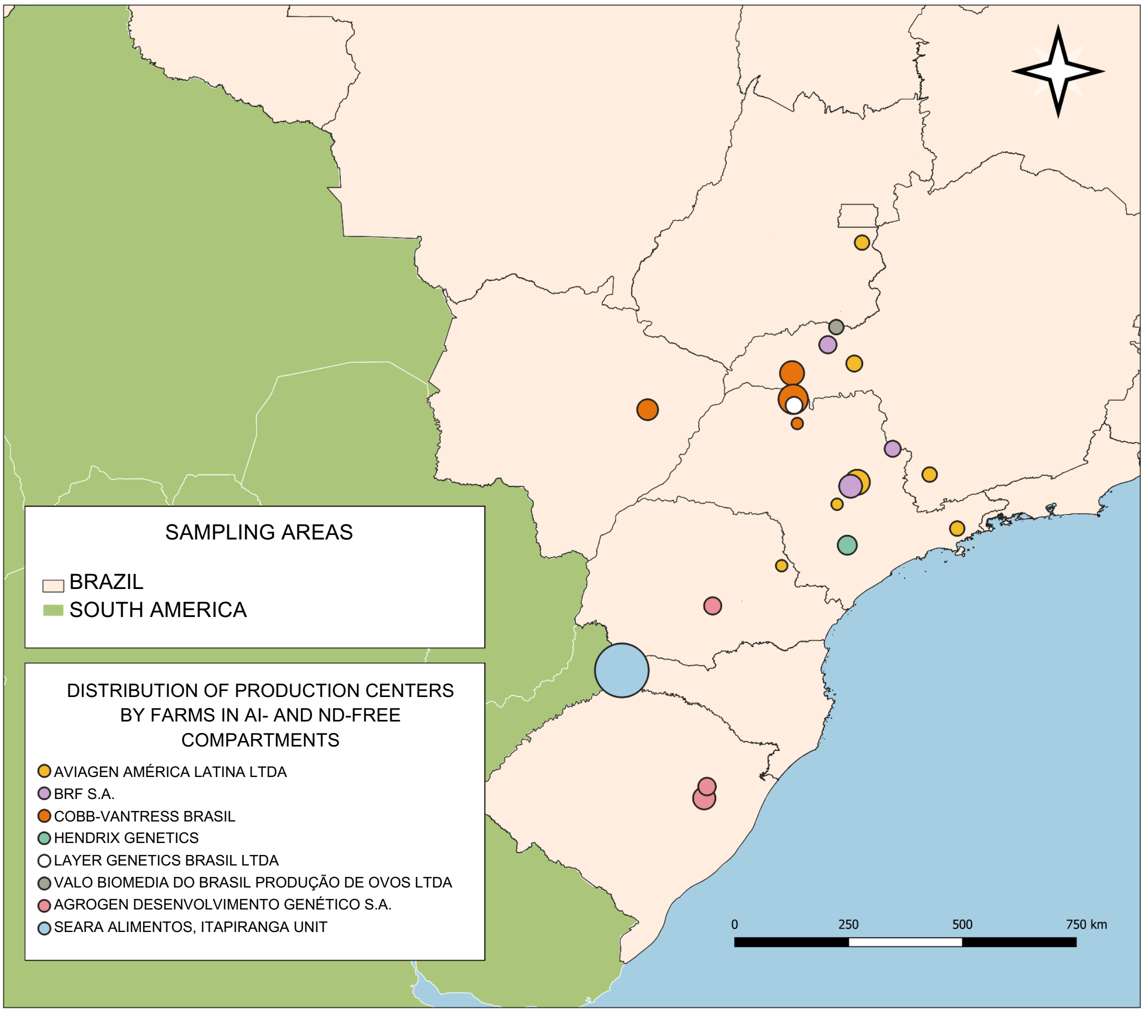

Figure 41. Geographical distribution of epidemiological units (nucleus) of farms with Sanitary Certification of Compartments for the Poultry Production Chain for Avian influenza and Newcastle disease in the first cycle of the surveillance plan.
On the map, circles of greater diameter represent a larger number of epidemiological units (nucleus) sampled per compartment.

5.1. Sampling strategy

All the farms with sanitary certification as compartments were included in sampling to detect the presence of AI and ND viruses.

Epidemiological and laboratory surveillance for AI-free and ND-free compartments is carried out in accordance with Chapter VI of Normative Instruction no. 21 dated October 21, 2014, which recommends serological (ELISA) and molecular testing for AI and ND.

Birds from all the epidemiological units (nucleus) operating on the farm present at the moment of sample-taking, having been housed for at least 21 days, are sampled, whereby blood serum, tracheal swabs and cloacal swabs are harvested from 10 birds per epidemiological units (nucleus), twice annually. It should be recorded that broilers or birds raised for stocking purposes that may have been given a live ND vaccine, are not tested for ND. The laboratory tests to screen for AI and ND are performed in public, MAPA-approved laboratories:

- Centro de Diagnóstico de Sanidade Animal – CEDISA, Santa Catarina;
- Centro de Diagnóstico Marco Enrietti – CDME, Paraná;
- Instituto Biológico – IB, São Paulo.

If positive or inconclusive sample results are identified in the screening testing process, OVS is notified of this fact, and the samples are shipped to LFDA-SP in order for confirmatory testing to detect AI and ND.

5.2 Laboratory diagnosis

On breeding and broiler farms, the surveillance program is performed periodically through clinical examination of the birds and samples are taken for laboratory testing to detect AI and ND. Table 4 presents the number of epidemiological units (nucleus) of the sampled compartments during the AI and ND Surveillance Plan assessment period

Table 4. Number of epidemiological units (nucleus) sampled within the surveillance plan assessment period

Compartment/ Purpose	Semester	
	2022/2	2023/1
AGROGEN DESENVOLVIMENTO GENÉTICO S.A./Breeding	16	12
AVIAGEN AMÉRICA LATINA/Breeding	24	4
BRF S.A./Breeding	11	13
COBB-VANTRESS BRASIL/Breeding	37	26
HENDRIX GENETICS/Breeding	6	4
LAYER GENETICS BRASIL LTDA./Breeding	3	2
SEARA ALIMENTOS LTDA/Broilers	56	34
SEARA ALIMENTOS LTDA/Parent breeding	8	2
VALO BIOMEDIA DO BRASIL LTDA/Breeding	3	2

Differences in the number of epidemiological units (nucleus) that were sampled in these two periods are due to factors that influence the harvesting of the samples, such as the birds being too young to be sampled, or there being a standstill in progress on the farm, among others.

It should be emphasized that no samples testing positive to confirmatory tests for AI and ND were detected among the sampled poultry.

5.3 Interpretation of the surveillance

The purpose of Sanitary Certification for Compartmentalization of the Production Chain involving breeding farms, broiler farms and hatcheries for hens or turkeys, is to recognize and attest to a subpopulation of poultry having the status of being AI- or ND-free through adopting additional biosecurity procedures and epidemiological surveillance which are duly supervised and audited.

In the AI and ND surveillance cycle that was analyzed, samples testing positive for influenza A or gene F of the ND virus were not detected. Thus the 8 MAPA-certified compartments at the time presented both AI- and ND-free status.

6. Results of surveillance

During the AI and ND surveillance cycle from 01/07/2022 to 30/06/2023, the OVS carried out 1,107 clinical and epidemiological investigations on suspected cases of ARNS throughout Brazil, identifying 273 probable cases of AI and ND among domestic and wild birds. In addition, 44,202 laboratory tests to diagnose AI and ND were performed for component 3 (industrial poultry) and 28,408 for component 4 (backyard and small-scale commercial flocks). Regarding component 5, 263 epidemiological units (nucleus) associated with the compartments were sampled.

We emphasize that no positive samples to AI or ND were detected among commercial poultry in the first cycle of the AI and ND surveillance plan, and therefore Brazil maintains its HPAI-free and ND-free sanitary status for WOAH and for its trading partners.

ANNEXES

Table 1. Species of birds sampled for passive surveillance – Components 1 and 2

Wild/ Free-living	Non-poultry	Poultry
White-capped albatross	Eared dove	Ostrich
Atlantic yellow-nosed Albatross	Caracara	Japanese quail
Squirrel cuckoo	Northern bobwhite/ Virginia quail	Hen
Grey-breasted martin	Common or European quail	Domestic grey goose
Sick's swift	Japanese quail	Duck
Guira cuckoo	Emu	Turkey
Speckled chachalaca	Pheasant	
Red-footed booby	Hen	
Masked booby	Guinea fowl	
Brown booby	Domestic grey goose	
Ostrich	Roadside Hawk	
Eared dove	Teal	
Semipalmated plover	Passeriformes	
Grey plover	Duck	
Great kiskadee	Muscovy duck	
Neotropic cormorant	Congo peafowl	
Manx shearwater	Common Peacock	
Wood stork	Daurian partridge	
Ferruginous pygmy owl	Turkey	
Cory's shearwater	Pigeon	
White-faced ibis	Psittacidae	
Caracara		
Black-necked swan		
Japanese quail		
Burrowing Owl		
Barn owl		
Striped owl		
Tropical screech owl		
Buff-necked ibis		
Magnificent Frigatebird		
American purple gallinule		
Gray-hooded gull		
Brown-hooded gull		
Kelp gull		
Common moorhen		
Guinea fowl		
Domestic grey goose		

Snowy egret
Cocoi heron
Roadside Hawk
Great black hawk
Atlantic petrel
Rufous hornero
Sanderling
Short-billed dowitcher
White-rumped sandpiper
Maguari stork
Chilean skua
Brazilian teal
Fulvous whistling duck
Blue-winged teal
Lake duck
Great grebe
Blue-fronted Amazon
Grey petrel
Great shearwater
White-chinned petrel
Passeriformes
Duck
Common Peacock
Turkey
Magellanic Penguin
Picazuro pigeon
Pigeon
Psittacidae
Southern lapwing
Ruddy ground dove
Rufescent tiger heron
Black-crowned night heron
Southern screamer
American golden plover
Arctic tern
Common tern
Cabot’s tern
South American tern
Royal tern
Roseate tern
Red-breasted toucan
American black vulture
Common potoo, or urutau

List of mandatory questions on the Epicollect5 Form

Geographical identification data	Birds production data	Geolocation	Information on samples taken
State	Selected holding or alternate	Latitude	Number of production center actually sampled
Name of Municipality	Species being raised	Longitude	Sample serial number
IBGE Municipality Code	Category of the poultry farm		Type of sample (serum, tracheal swab, cloacal swab)
MAPA code (sent by DSA)	Number of production centers		Remarks
Farm code	Total farm stocking capacity		
	Sampling date		

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Epicollect5. Epicollect5 - Free and easy-to-use mobile data-gathering platform.