



AVIAN INFLUENZA AND NEWCASTLE DISEASE

REPORT ON THE SURVEILLANCE PLAN
2ND CYCLE – 2023 / 2024

Ministry of Agriculture and Livestock - MAPA

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2nd cycle 2026

Ministry of Agriculture and Livestock
Secretariat of Animal and Plant Health
Department of Animal Health
Poultry Health Coordination Office – COSAV
Esplanada dos Ministérios - Bloco D - Anexo A - Sala 322
Brasilia-DF CEP: 70043 900
Tel.: +55 (61) 3218-2782/2238
E-mail: pnsa@agro.gov.br

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CYCLE – 2023 / 2024

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

Brasília, May 2026.

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LIST OF ABBREVIATIONS

AI: Avian Influenza

ARNS: Avian respiratory and nervous syndromes

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 (HPAI)

IBAMA: Brazilian Institute for the Environment and Renewable Natural Resources

ICMBio: Chico Mendes Institute for Biodiversity Preservation

LFDA: Federal Laboratories of Animal and Plant Health

LP AI: Low Pathogenicity Avian Influenza

MAPA: Ministry of Agriculture and Livestock

ND: Newcastle Disease

OESA: State-Level Animal Health Executing Agency

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

SFA: Offices of the Superintendents of Agriculture and Livestock

SVS: State Animal Health Veterinary Service

WOAH: World Organisation for Animal Health

EXECUTIVE SUMMARY

In 2022, the Department of Animal Health (DSA) of the Ministry of Agriculture and Livestock (MAPA) reviewed national surveillance strategies for Avian Influenza (AI) and Newcastle Disease (ND). As a result of this process, a new National Surveillance Plan was set up, based upon strengthening the early detection system, upon continuous surveillance, and upon preventing these diseases from being introduced into, and spreading within, the territory of Brazil.

Passive surveillance is deemed to be the most effective strategy for the early detection of suspected cases of these diseases. It is based on the mandatory immediate notification of the Official Animal Health Service of any suspected case of AI or ND; the service will carry out the necessary investigation and apply the measures set forth in the Contingency Plan if outbreaks are confirmed. This plan allows for the containment and eradication of disease, and restoration of the country's disease-free status, in accordance with parameters established by the World Organisation for Animal Health (WOAH).

The AI and ND surveillance plan aims to promote passive surveillance in order to boost the capacity for early detection, and, by means of active surveillance activities, to prove the absence of any infection by these viruses in industrial poultry production, in accordance with the international surveillance guidelines for commercial purposes. Additionally, the plan aims to monitor for the occurrence of infections in wild birds by active surveillance in backyard poultry-raising establishments located in areas classified as being at greater epidemiological risk. These actions are fundamental in guiding risk mitigation measures and preventing these diseases from being introduced into domestic poultry.

Since the detection of highly pathogenic avian influenza (HPAI) virus in wild birds on the coast of the State of Espírito Santo (ES) on May 15, 2023, Brazil has had to face a new epidemiological context. Most of the outbreaks that were identified took place in wild birds, and there were sporadic records of outbreaks among domestic poultry and in marine mammals.

Analysis of the epidemiological curve shows that the peak of detection of cases of HPAI was in June 2023, followed by a marked fall in the number of new outbreak reports as of September 2023. Despite this fall, the fact that infection did occur in wild populations poses a continuous epidemiological risk of the introduction and dissemination of the agent among commercial poultry. The risk is particularly significant in that infected wild birds are the main mechanism of the worldwide spread of the virus.

Epidemiological surveillance for Newcastle Disease is continuous, associated with the introduction of biosecurity measures in all links of the poultry production chain, as the principal strategy for preventing, controlling, and mitigating the sanitary risk. Until the moment of evaluation of the second cycle of the Surveillance Plan, Brazil's animal health status was that the country had eradicated the ND virus, in that the most recent records of the disease dated back to 2006, and it had been restricted to backyard poultry in the states of Amazonas (AM), Mato Grosso (MT) and Rio Grande do Sul (RS).

In that context, maintaining and strengthening the AI and ND surveillance system, by integrating passive and active surveillance activities, were essential for mitigating sanitary threats, protecting domestic poultry and ensuring the sanitary status of the

poultry-raising industry. These efforts are strategic in order to ensure the sustainability of Brazil's poultry production industry and the country's position as an important supplier to the international market.

In compliance with the principles of publication and transparency, DSA sets forth in the present document the results obtained in the first cycle of the AI & ND surveillance plan (2023-2024). Activities were coordinated by DSA, which reports to MAPA's Secretariat for Animal and Plant Health, and were executed by the state-level veterinary services, over the period from July 1, 2023 to June 30, 2024.

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 the immediate notification of, and response to, suspected cases (passive surveillance) in populations of domestic and wild birds.

Objective 2 - To demonstrate the 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 WOA and before importing countries.

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

1.1. DEFINITIONS

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

Industrial (commercial) poultry-raising: the entire set of operations carried out by commercial growers who incorporate technological advances in genetics, nutrition, health, and 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.

Backyard poultry production: domestic poultry flocks belonging to non-industrial-scale farmers, the majority of whom are registered in the state-level animal health services, who incorporate only a few technological advancements, and for whom their poultry production is intended for their own consumption (backyard or subsistence-raising).

Small-scale poultry production: domestic poultry flocks belonging to non-industrial-scale farmers who are registered in the state-level animal health services, who incorporate few technological advancements, and for whom poultry production is intended for local trade (small-scale commercial operations), with only limited access to certain production-chain processing and distribution channels.

Wild birds: in the current epidemiological context, in which wild birds play an important role in spreading the HPAI virus to domestic birds, wild birds are of interest to

surveillance. However, priority is given to migratory aquatic birds, defined as birds whose populations, either totally or partially, carry out cyclical and seasonal movements, faithfully returning to their breeding sites, and are associated with aquatic environments. In this group, species belonging to the orders *Anseriformes* (such as ducks, geese and teal) and *Charadriiformes* (such as seagulls, jacanas, sandpipers, and terns) stand out.

1.2. IDENTIFICATION OF THE COMPONENTS OF THE SURVEILLANCE SYSTEM

Each component of the surveillance system comprises an activity that is used in order 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 domestic poultry**
- 2. Passive surveillance in wild birds**
- 3. Active surveillance in industrial poultry production**
- 4. Active surveillance in backyard poultry and small-scale production**
- 5. Active surveillance in AI-free and ND-free compartments**

1.3. EPIDEMIOLOGICAL SITUATION DURING THE 2023/2024 CYCLE

1.3.1. Avian Influenza

According to the WOAHP Terrestrial Animal Code, the term “Highly Pathogenic Avian Influenza (HPAI)” is defined when infection affects poultry (i.e. commercial birds), whereas the term “infection by highly pathogenic influenza A virus” is defined when infection affects birds that are not commercially produced, including wild birds. However, for the sake of simplicity, in the present report, we use the term HPAI alone for an occurrence in any species.

In the period from July 1, 2023 to June 30, 2024, 107 HPAI outbreaks were reported, two of which were in backyard hen-raising, and the rest in wild animals. The months in which the disease occurred most frequently were September and October 2023, in which 28 and 27 outbreaks, respectively, were reported.

After this peak, from two to six outbreaks were reported in the following months, without constant growth.

Thus Brazil’s sanitary status remained the same as in the previous cycle, in other words HPAI-free in commercial poultry without compromising international trade.

In the period, seven States (*Federative Units* or “UF”s), as per Figure 1, witnessed outbreaks. São Paulo state was the most affected by the disease, with 49 recorded

outbreaks, all among wild birds. States that reported outbreaks in domestic poultry were Santa Catarina (SC) and Mato Grosso do Sul (MS), for the municipalities of Maracajá and Bonito, respectively. These two outbreaks took place on small backyard properties and presented a low intra-flock incidence, with few animals showing clinical signs, most likely due to a lower viral load, and a lower concentration/density of birds per square meter, and that had possibly come into contact with wild animals.

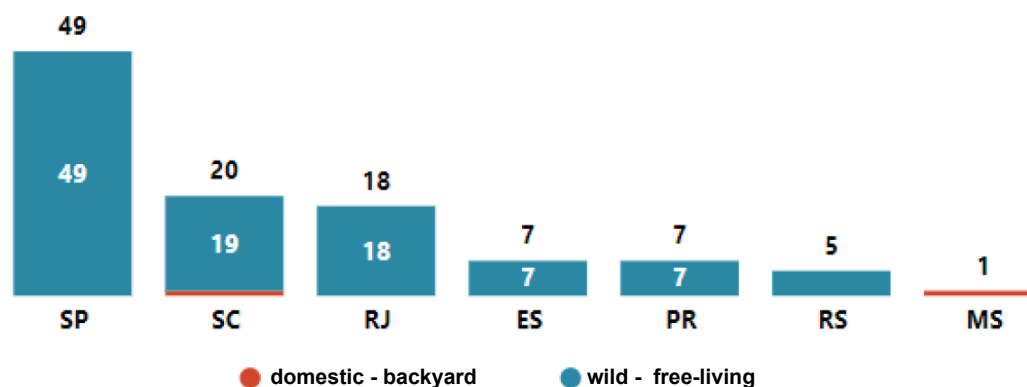


Figure 1. Number of outbreaks by State.

In early June 2023, MAPA published a public panel in order to make public the number of HPAI and ND 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 of HPAI and ND by category—commercial poultry, non-commercial birds and wild animals; the species of wild bird and backyard poultry; 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 (nd) and infection by APMV-1 and variants

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 other production categories such as broilers are authorized to be vaccinated in the event that their growers deem this necessary.

Up until the moment of assessment of the 2nd cycle of the Plan, Brazil had been free of ND, the last outbreak having been reported in 2006 among backyard poultry in Amazonas (AM), Mato Grosso (MT) and Rio Grande do Sul (RS). However, on 17/07/2024, an outbreak of ND among commercial poultry was reported in the municipality of Anta Gorda, RS. Details on that outbreak and the related developments thereof will be debated in the next report on the 3rd assessment cycle.

It should be emphasized that the definition of ND applies only to poultry of commercial flocks. In 2006, backyard poultry were still being taken into account in ND outbreaks, in accordance with WOA criteria. However, in 2021, the nomenclature ND came to be considered only for commercial poultry. Thus for the other categories of bird, backyard

or wild, the disease is defined today as infection by avian paramyxovirus serotype 1 (APMV-1) and its variants (see sections 1.3 and 2.3 Definitions of a case).

In terms of APMV-1 infection, *Pigeon paramyxovirus type 1* (PPMV-1) variant, nine outbreaks in wild birds were reported in the period under analysis. This infection is deemed to be present in Brazil in pigeons and eared doves.

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.

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. Compartments are audited by Federal Agricultural Inspector/Auditors (AFFAs) working in the state-level Offices of the Superintendents of Agriculture (SFAs).

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 spreadsheets.

The responsibilities of each interested party contained in the Plan are shown in Table 1.

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 collect samples; to provide financing, capacity-building, education, and communication	Effective
Growers	To notify authorities of suspected cases; to adopt good practices (of	Effective

	documentation and biosecurity); to provide funding	
Poultry industry production	To notify authorities of suspected cases; to disseminate and provide surveillance information; to provide funding	Effective
MAPA official laboratories	To perform laboratory tests on passive and active surveillance samples; to generate laboratory data for epidemiological analysis; to disseminate information	Effective
Public approved laboratories	To notify authorities of suspected cases; to disseminate information; to perform screening tests for poultry compartmentalization; and to import/export avian genetic material (AGM)	Effective
Veterinarians	To notify authorities 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 notify authorities 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
Animal health system funds	To disseminate information; to provide funding	Effective
Universities	To perform screening tests for the active surveillance of wild animals; to disseminate information	Effective
Ministry of Health	To investigate and advise individuals who came into contact with probable cases of HPAI in wild animals and domestic poultry; and disseminate information	Effective

2. LABORATORY DIAGNOSIS

2.1. PASSIVE SURVEILLANCE

Passive surveillance for AI and ND is an essential strategy for the early detection of those diseases, and is based upon compulsorily and immediately notifying the official veterinary service of suspect cases.

For passive surveillance, swabs were taken from birds and their tissues sampled. Samples obtained by means of tracheal and cloacal swabs, as well as organs in the neurological, respiratory and digestive systems, were sent for molecular detection of type A influenza virus and ND virus.

All positive or inconclusive samples tested by molecular detection of type A influenza virus were 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 were 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 in inconclusive samples after molecular diagnosis techniques to detect subtypes H5, H5 clade 2.3.4.4, neuraminidase N1 by RT-qPCR.

Samples testing positive or inconclusive after a molecular detection assay for gene M of ND virus underwent confirmation molecular tests to detect gene F of the ND virus. Furthermore, compliance with the criterion of the virulence of the virus is determined by partial sequencing of the F gene to assess for the presence of multiple base amino-acids of the C terminal region of protein F2.

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 NB3Ag level biological security laboratory and is acknowledged by WOA and Agriculture Organization of the United Nations (FAO) as a reference for the diagnosis of Avian Influenza and Newcastle Disease.

Figure 2 shows the expected flow diagram for laboratory diagnosis of samples taken for passive surveillance for Avian Influenza and Newcastle Disease

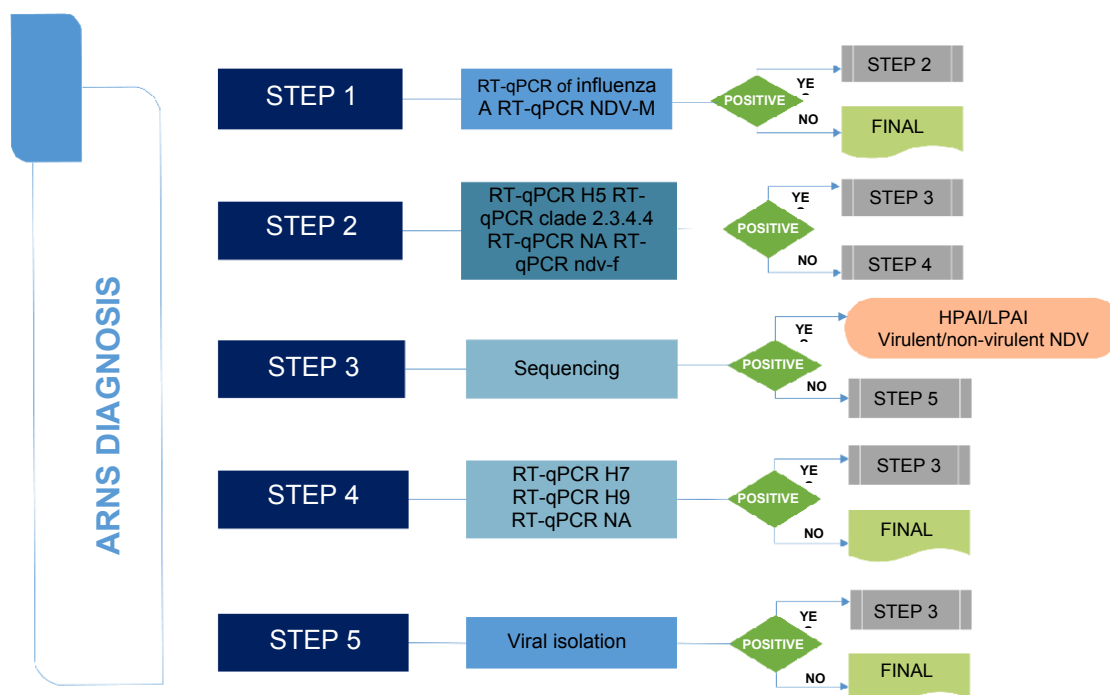


Figure 2. Flow diagram for laboratory diagnosis of samples taken for passive surveillance for Avian Influenza and Newcastle Disease.

2.2. ACTIVE SURVEILLANCE

Continuous, scheduled, active surveillance in annual cycles, systematically applied in all the States of Brazil. The strategy is planned upon the basis of epidemiological and risk analyses; it involves the definition of the sampling criteria and the planning of taking samples from apparently healthy birds.

For active surveillance, samples of blood serum and pools of tracheal and cloacal swabs were taken. Each pool of swabs gave rise to a reaction for influenza A detection reaction and an ND detection reaction.

2.2.1 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 Newcastle Disease in industrial poultry production, 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-typing 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). Whenever samples tested positive for type A influenza virus at screening, they underwent 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 in the screening assay underwent supplementary molecular tests to detect gene F in APMV-1.

Active surveillance samples for component 3 were tested at the São Paulo state (SP), Pernambuco (PE) and RS LFDAs, chosen because they are closest to the samples' origin States. All samples for component 4 were tested at LFDA-SP. The figure below shows the simplified flow and the laboratory tests for active surveillance in components 3 and 4 of the surveillance plan:

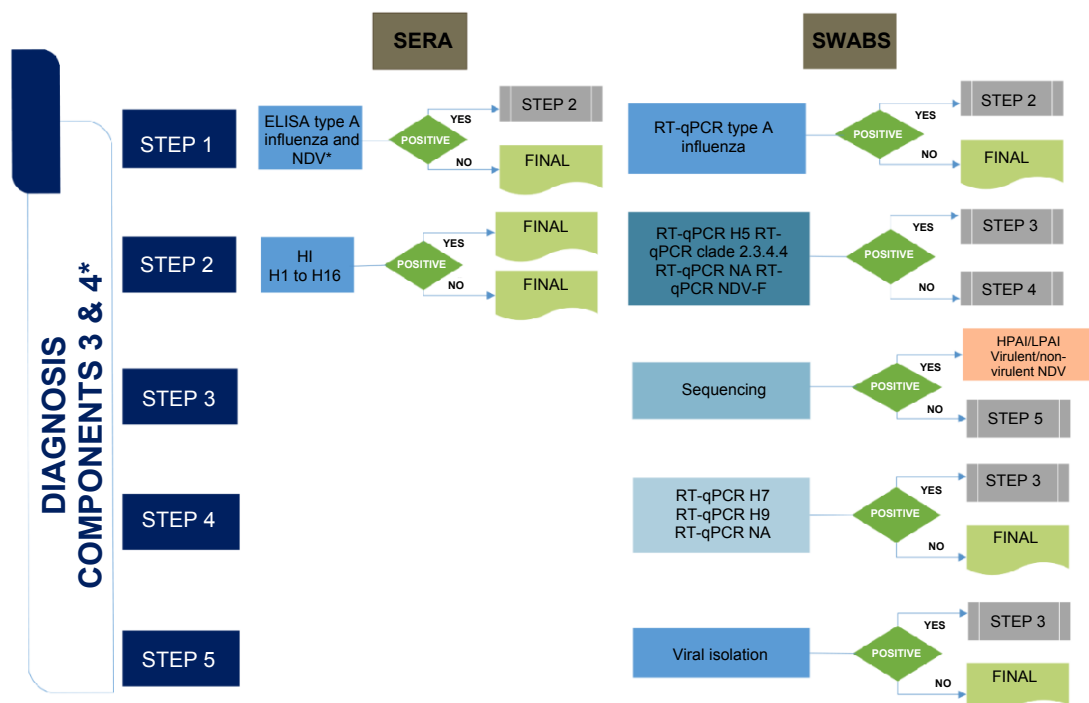


Figure 3. Expected flow diagram for laboratory diagnosis of samples taken for active surveillance for Avian Influenza and Newcastle Disease, for components 3 & 4

*ELISA testing for NDV was performed only in Component 4

2.2.2 Component 5

Sera samples for component 5 underwent ELISA or agar-gel immunodiffusion (AGID) testing for influenza A antibodies.

As with components 3 and 4, the tracheal and cloacal swab samples went for molecular detection of type A influenza virus and ND virus (gene M) by RT-qPCR.

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

- Centro de Diagnóstico Marco Enrietti – CDME, Paraná (PR);
- Instituto Biológico – IB, SP;

For molecular assays testing positive in approved laboratories (Figure 4), the samples were shipped immediately to LFDA-SP for confirmation testing as per NORMATIVE INSTRUCTION NO. 21, DATED OCTOBER 21, 2014.

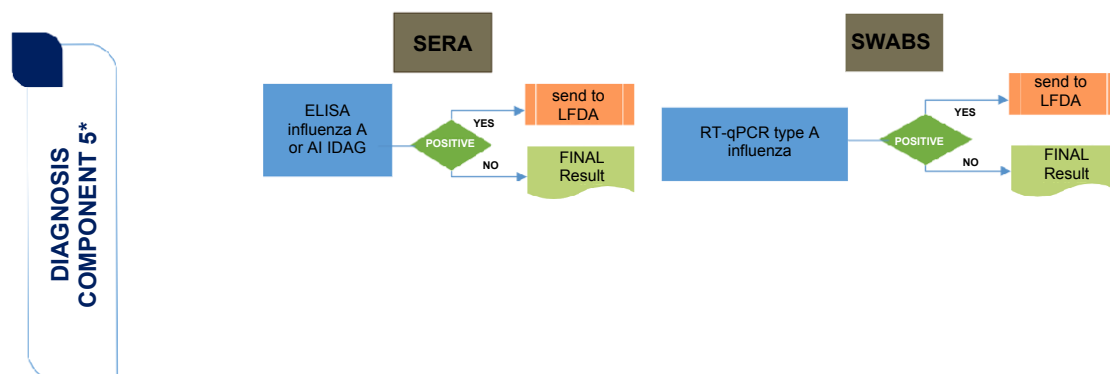


Figure 4. Prescribed flow diagram for laboratory diagnosis of samples taken for passive surveillance for Avian Influenza and Newcastle Disease in component 5

3. GENOMIC SURVEILLANCE

Once the first cases of HPAI were detected in Brazil, a strategy was set up whereby important information could be generated for an enhanced epidemiological assessment and the drafting of disease control strategies in the country. In order to optimize the available resources, it was defined that complete sequencing would be performed on samples that are representative in terms of time, species and geographical location. Additionally, the selected samples, during data analysis, presented qPCR and qC less than or equal to 25. Complete sequencing was performed by the LFDA network, using the installed capacities of LFDAs in the States of SP, Goiás (GO) and Minas Gerais (MG).

From 01/07/2023 to 30/06/2024, thirty (30) samples from 9 States of Brazil underwent complete sequencing. The results that were obtained showed that the A/H5N1 viruses detected in wild birds, backyard poultry and marine mammals belonged to clade 2.3.4.4b, genotype B3.2, which is genetically very similar to the viruses detected in Chile, Uruguay and Argentina. Phylogenetic analysis shows that viruses originating in the USA and coming from Europe and Asia are being transmitted, co-circulating alongside other lineages in the American continent.

Additionally, mutations in gene PB2 (D701N and Q591K) were detected: these are associated with adaptation and transmission in mammals, suggesting a possible zoonotic risk. No mutation in gene HA (Q226L), recently associated with a possible risk of infection in man, was identified.

Concerning antivirals, no resistance to neuraminidase inhibitors (NAIs) N292S, H275Y and T438I, was identified, although continued surveillance remains indispensable for detecting any possible emerging resistance.

The study mapped the spread of the virus in the Southern Hemisphere, and identified possible entrance pathways and emphasized the importance of surveillance for preventing outbreaks and protecting human as well as animal populations

More information is given at:

[First report and genetic characterization of the Highly Pathogenic Avian Influenza A\(H5N1\) virus](#)

[in Cabot's tern \(*Thalasseus acuflavidus*\), Brazil](#)

[Phylogenetics of avian influenza A\(H5N1\) viruses from outbreaks in Brazil](#)

PART B

DESCRIPTION OF THE COMPONENTS AND RESULTS OF SURVEILLANCE

1. COMPONENT 1 - PASSIVE SURVEILLANCE IN DOMESTIC POULTRY

1.1. Objective, approach and source of data

Component 1 of the surveillance system is passive surveillance of domestic poultry and aims to achieve early detection of AI and ND, the target diseases of ARNS Surveillance. Passive surveillance for ARNS depends on laboratory diagnosis to confirm cases because the clinical signs of the target diseases are similar. Therefore, probable cases of ARNS are to be sent for laboratory diagnosis of AI and ND (see section 1.2. Definitions of a case).

It is of the utmost importance to the early detection of the diseases that growers and other poultry chain professionals notify the authorities, and this reflects the level of awareness-building and engagement within the production chain. DSA-standardized procedures for investigation and the confirmation or ruling-out of occurrences of AI and ND include, but are not limited to, the following:

- On-site visits to, and inspection of, domestic poultry-raising establishments, and interviews with growers;
- Verification of records of follow-up of poultry flocks with a thorough analysis of mortality and of production parameters;
- The identification of clinical signs or lesions (whether neurological, respiratory or digestive) that are compatible with ARNS;
- Epidemiological investigation;
- Taking samples from the birds;
- e-Sisbravet record of investigation;
- Laboratory testing for AI and ND;
- Emergency actions for control and eradication;
- Phylogenetic genomic analysis of the virus.

The flows for notifications, animal health information, and procedures for recording and sending for investigation are available in the user's manual in [e-Sisbravet](#) as well as in the Technical Data Sheets for the diseases.

All notified suspected cases and investigations (call-outs) performed by the official veterinary service are to be recorded in e-Sisbravet, which is the main source of data for passive surveillance.

1.2. Definition of a case

Definitions of a case have been established in the AI and ND Technical Data Sheets. The definitions of cases as adopted during the period of execution of the surveillance plan (2023–2024) were subsequently reviewed in the corresponding Technical Data

Sheets. For the present report the criteria in force at that time are therefore to be taken into consideration. Among such criteria a suspected case of ARNS was defined as $\geq 10\%$ mortality within 72 hours in commercial poultry; this criterion was amended to $\geq 5\%$ in the definition currently in force.

1.3 Target population

The target population encompasses domestic poultry 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 domestic industrially-produced poultry, backyard poultry or ornamental birds.

1.4 Results

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 July 1, 2023 to June 30, 2024.

1.4.1 Number and geographical distribution of the clinical and epidemiological investigations of suspected cases of avian respiratory and nervous syndrome (henceforth “ARNS”) in domestic poultry

In the period under assessment the OVS performed 1,119 ARNS investigations among domestic poultry, 174 (15%) of which were classified as probable cases, and samples were taken for laboratory testing for AI and ND (Table 2).

Of the total number of investigations of ARNS in domestic poultry, 692 were carried out on commercial birds, 12 (2%) of which were classified as probable cases, while 680 (98%) were assessed as ruled-out suspected cases. There were 427 investigations in backyard flocks, of which 162 (38%) were probable cases and 265 (62%) were ruled-out suspected cases (Table 2). A greater sensitivity of the commercial production industry can be seen in passive surveillance. This happens because flocks of birds are often monitored by the industry's private technicians and veterinarians, leading to a larger total number of investigations, albeit with fewer probable cases.

Table 2. Number of ARNS investigations and type of domestic poultry production – Cycle 2023/2024

Poultry production	Probable case	Ruled-out suspected case	Total investigations
Commercial	12	680	692
Backyard	162	265	427
Total	174	945	1119

The geographical distribution of the clinical and epidemiological investigations classified as ruled-out suspected cases and probable cases of ARNS in commercial poultry are given in figure 5.

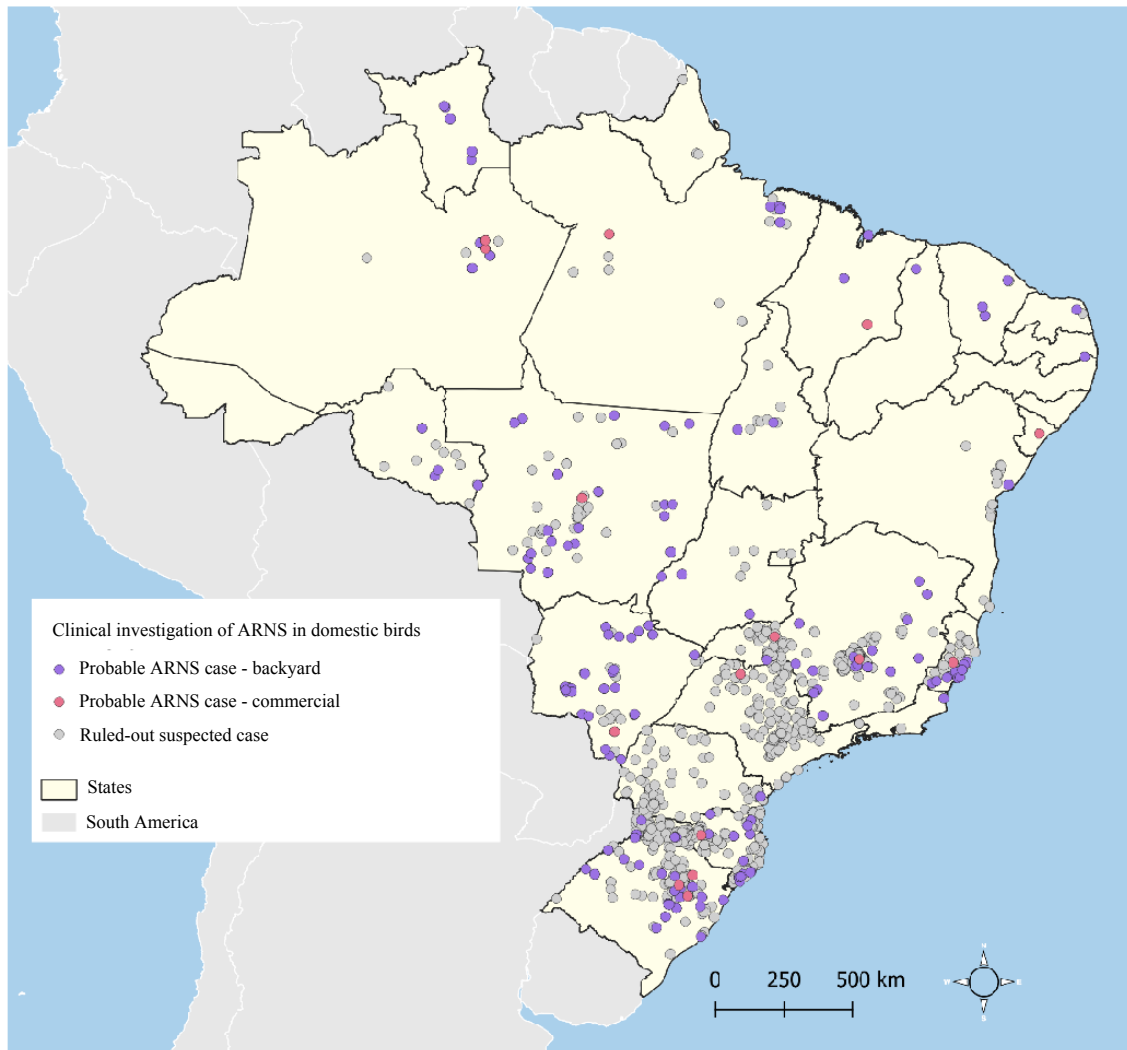


Figure 5. Geographical distribution of ARNS investigations among domestic birds.

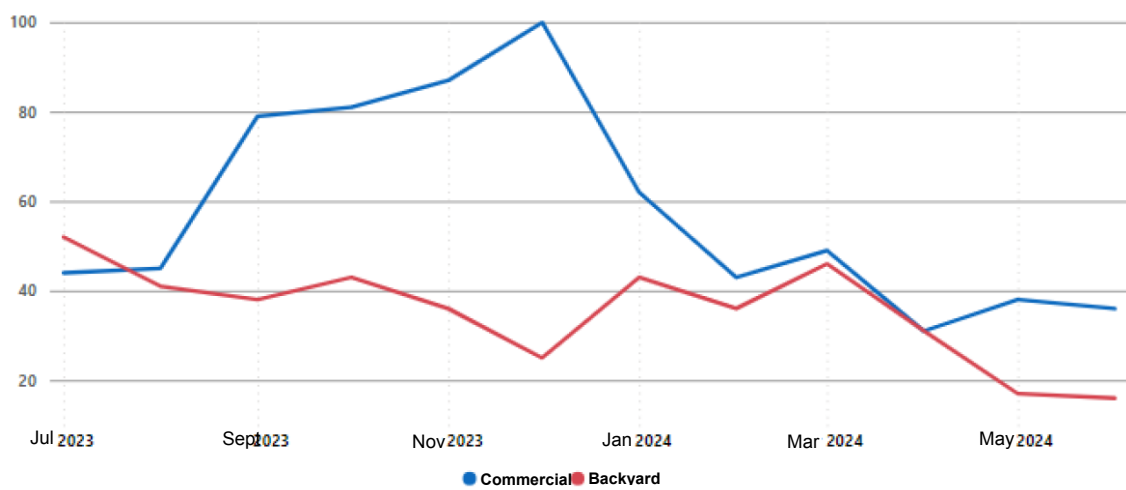


Figure 6. Temporal development of total investigations in domestic birds.

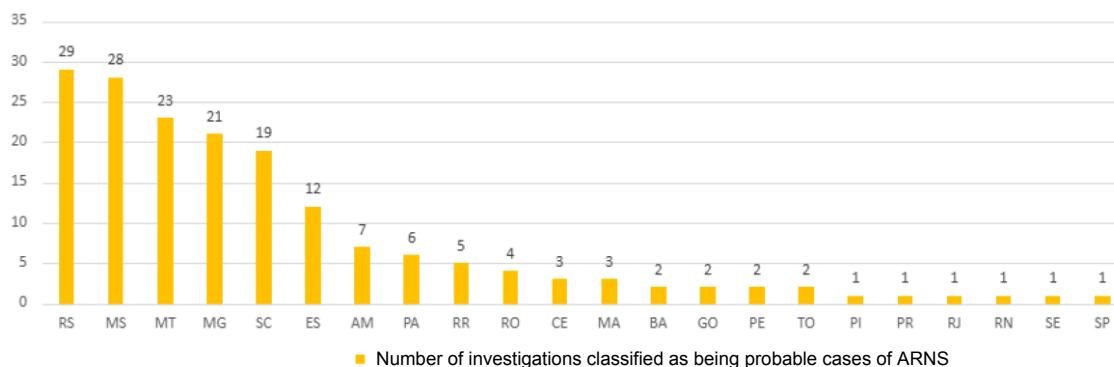


Figure 7. Number of investigations involving probable cases of ARNS in domestic poultry by State.

In accordance with Figure 7, the States with the most investigations involving probable cases of ARNS, with sample-taking for laboratory testing, were RS, MS, MT and MG. The total number of investigations fell as of December 2023 (Figure 6).

1.4.3 Bird species investigated

Passive surveillance data for the period from July 1, 2023 to June 30, 2024 showed that investigations were carried out for the following domestic poultry species: hens, quail, guinea fowl, geese, teal, ducks and turkeys.

1.4.4 Outbreaks of Highly Pathogenic Avian Influenza and Newcastle Disease in domestic poultry

In the period under assessment, two outbreaks of HPAI were reported in domestic backyard flocks on small farms. The first was responded to in July 2023 in Maracajá on a farm containing 220 fowl of multiple species, 152 of which presented clinical signs.

The second outbreak occurred in Bonito, Mato Grosso do Sul, in September 2023. In this outbreak, 10 hens out of 92 hens being raised on the farm displayed clinical signs. In the surveillance actions carried out within a 10-km radius of the outbreak, 91 farms that were raising birds were identified. All of them possessed the characteristics of backyard operations and underwent multiple inspections. Two farms were identified as displaying mortality of birds, from which samples were taken for laboratory diagnosis: the tests came back negative for AI. For the period under assessment, this was the first outbreak occurring at a greater distance from the coastline in Brazil, in the rural interior of the country.

There were no outbreaks among commercial flocks.

Brazil was ND-free up until June 2023. Thus, for the period under assessment, no confirmed cases of ND, or of APMV-1 infection, occurred for backyard poultry.

1.5 CONCLUSIONS OF PASSIVE SURVEILLANCE IN DOMESTIC POULTRY

The passive surveillance strategy for domestic birds was able to obtain an early detection of occurrences of HPAI virus into Brazil, from its introduction in May 2023, through the notification of suspected cases of ARNS, up to clinical and epidemiological investigation and the laboratory testing of wild and domestic birds suspected of being infected with AI and ND.

The epidemiological investigations carried out by the OVS on farms with HPAI outbreaks showed that there was a possibility that backyard poultry might come into contact with wild birds owing to the low biosecurity standards of such livestock-raising operations. Furthermore no other outbreaks were identified in domestic poultry, even in the areas surrounding the confirmed outbreaks. This therefore suggests that the likely cause of the introduction of the virus among domestic poultry in the period under assessment was direct contact with infected wild birds.

2. COMPONENT 2 - PASSIVE SURVEILLANCE IN WILD BIRDS

2.1 OBJECTIVE, APPROACH 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 domestic poultry. As in domestic birds, passive surveillance of wild birds depends upon laboratory diagnosis in order to confirm cases, owing to the similarity and non-specificity of the clinical signs of AI vis-à-vis infection by APMV-1.

Passive surveillance aims to achieve the early detection and eradication of outbreaks of AI based on investigations of notifications called in by environmental management agencies, technical specialists of the production chain, veterinarians, or any citizen.

Agencies and organizations (both governmental and non-governmental), working in the environmental field and the preservation of natural resources, are essential players in the detection of suspected cases of ARNS, in a context of exceptional mortality events involving the wild birds of concern, and in the evaluation of unusual behaviors among that population.

The aim of the systematic investigation of birds presenting clinical symptoms compatible with ARNS, and any exceptional mortality events in birds in the wild, above all in areas where migratory aquatic birds concentrate, aquatic stopover points, and other water bodies, is 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. Surveillance in wild birds not only provides essential information on the dynamics of viral circulation in the natural environment, but also strengthens and raises awareness in the OVS for timely and effective surveillance activities among domestic poultry.

Exceptional mortality events in 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 poisoning, chemical accidents, death caused by weapons, bombs, traps, etc.) and natural phenomena (storms, earthquakes, droughts, floods, hurricanes and toxic algal blooms, *inter alia*).

Notification by environmental professionals working in federal, state-level, municipal and private-enterprise institutions is of the utmost importance for the early detection of the occurrence of diseases in wild animals.

DSA-standardized procedures for investigating and closing occurrences of ARNS include, but are not limited to, the following:

- On-site 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;
- A finding of exceptional mortality and 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 diagnosis for AI and APMV-1 virus infection;
- Emergency actions to control and eliminate outbreaks, in the event of a positive result for HPAI;
- Phylogenetic tests of the virus's genome, in the event of positive results for HPAI.

The flows for notifications, animal health information, and procedures for recording and sending for investigation are all available in the user's manual in e-Sisbravet as well as in the Technical Data Sheets for the diseases.

All notified suspected cases and investigations (call-outs) performed by the official veterinary service are to be recorded in e-Sisbravet, which is the main source of data for passive surveillance.

2.2 DEFINITIONS OF A CASE

Definitions of a case have been established in the AI and ND Technical Data Sheets.

2.3 TARGET POPULATION

The target population encompasses wild animal species (in captivity or free-living) that are susceptible to infection by AI and APMV-1 virus, and that are 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.

Through liaison with the environmental agencies IBAMA and ICMBio, a network was set up 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.

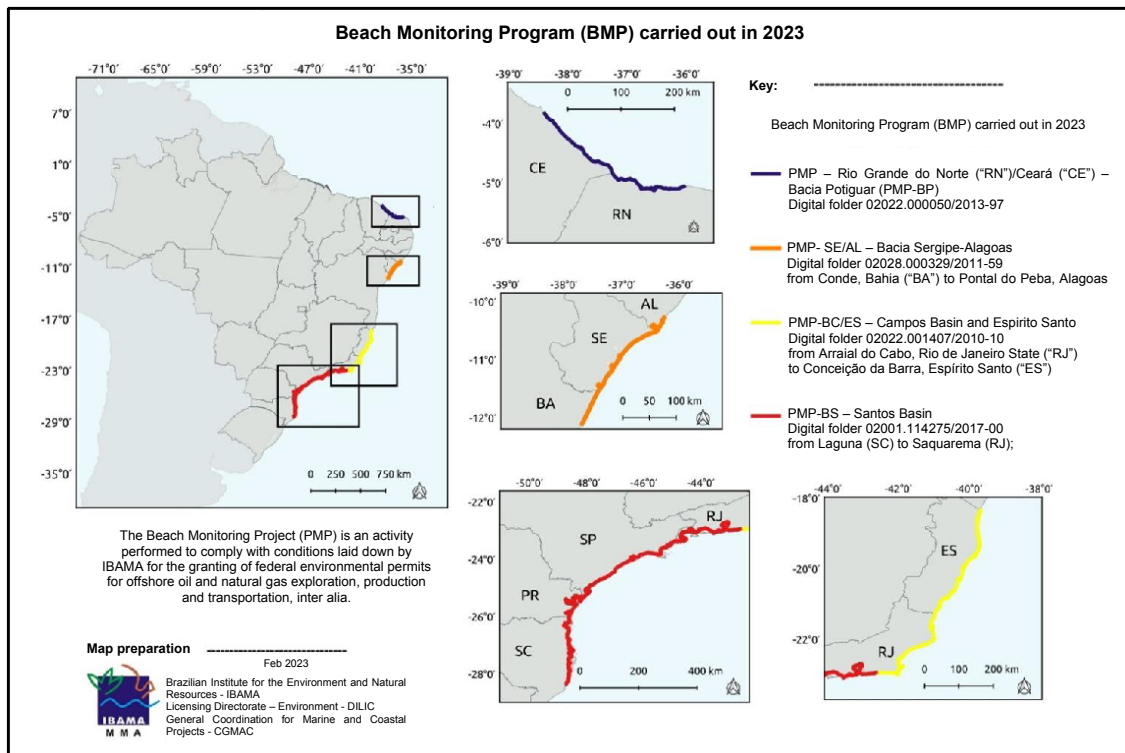


Figure 10. Characterization of the Area of Coverage of the Beach Monitoring Project.

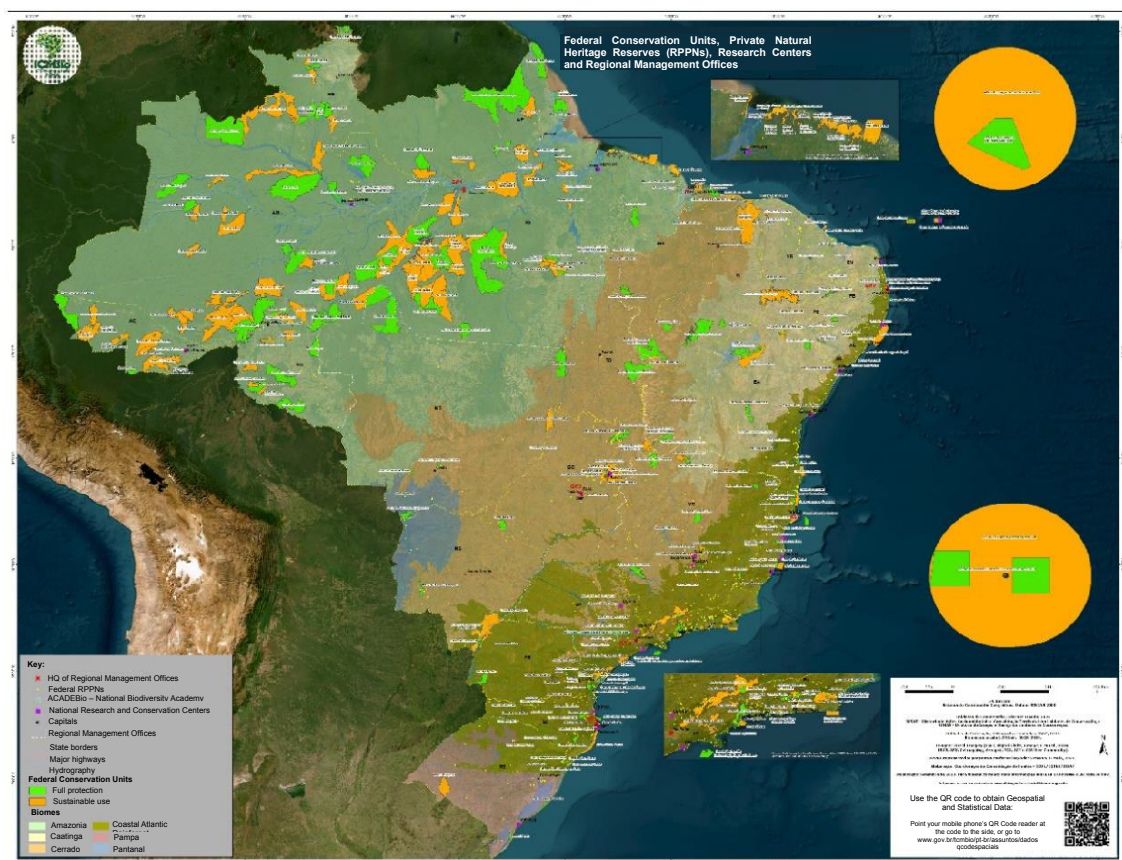


Figure 11. Brazil's Federal Conservation Units

2.4 RESULTS

2.4.1 Number and geographical distribution of the clinical and epidemiological investigations of suspected cases of avian respiratory and nervous syndrome (ARNS) in wild animals

In the period under assessment, the OVS carried out 529 clinical and epidemiological investigations of suspected cases of ARNS in wild animals throughout the territory of Brazil, of which 399 investigations were classified as probable cases (for which samples were harvested for AI and APMV-1 virus) and 130 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 animals during the period assessed.



Figure 12. Geographical distribution of probable cases and ruled-out suspected cases of avian respiratory and nervous syndrome in wild animals from July 2023 to June 2024

It was observed that there was a broader pattern of the geographical distribution of probable cases among wild birds along the Atlantic seaboard of Brazil (Figure 12), also

indicating that most of the investigations were carried out among coastal seabirds in the Atlantic migration route. Additionally, the States performing the most investigations of probable cases in wild birds were SP and SC (Figure 13).

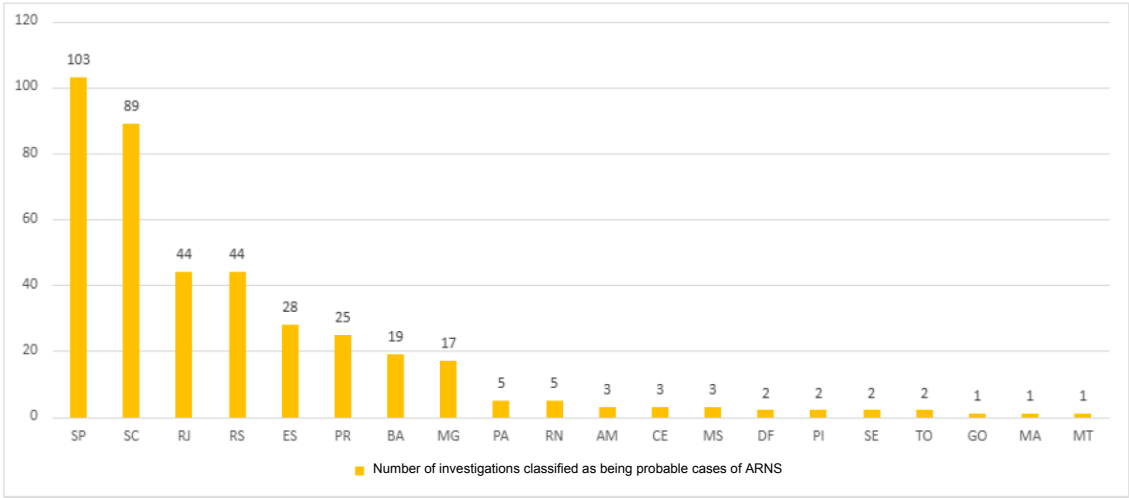


Figure 13. Number of investigations involving probable cases of ARNS in wild animals, by State.

A marked fall in the total number of investigations of wild animals was observed in Brazil as of November 2023 (Figure 14), possibly as a result of the fact that viral circulation was not maintained in the continent.

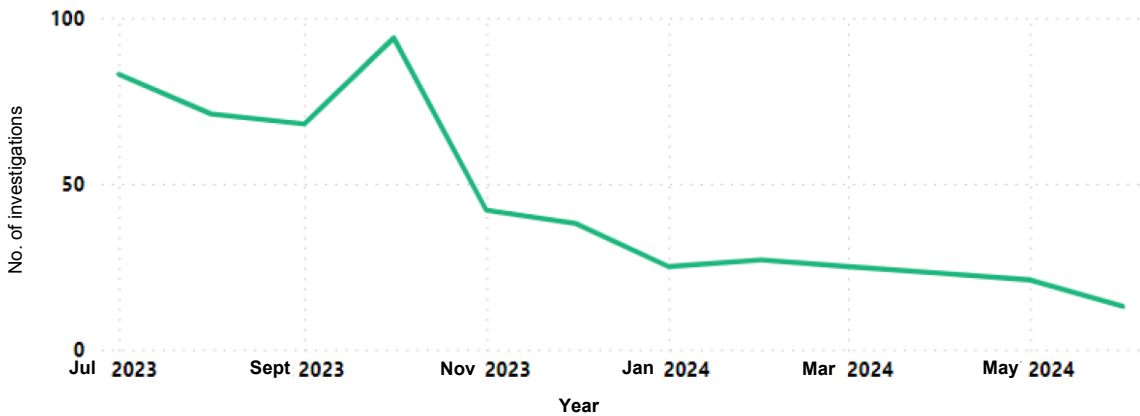


Figure 14. Temporal distribution of ARNS investigations in wild animals from July 2022 to June 2024

2.4.2 Animal species sampled

Data for passive surveillance for the period from July 2023 to June 2024 show that clinical and epidemiological investigations were carried out on approximately 140 species listed in ANNEXES.

The wild bird species with the largest number of ARNS investigations was *Thalasseus acutiflavus* – Cabot’s tern, with 48 call-outs for the OVS in the period under assessment.

This is a species of coastal seabird that nests on islets and islands off the coast of Brazil from Espírito 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 (*Sterna hirundinacea*) and Royal tern (*Thalasseus maximus*), accounting respectively for 20 and 37 investigations. Breeding behaviors in mixed colonies were deemed a relevant factor for the transmission of HPAI among the most widely-affected species.

During that period team responses to attend marine mammals stand out, due to the appearance of such animals on the coastlines of RS, SC and SP displaying neurological clinical signs. Owing to the challenge of handling such animals and harvesting samples from them, most of the cases among these species were confirmed by epidemiological and clinical criteria.

It should be emphasized that at that time, sample-taking from marine mammals was a task shared with the environmental sector; therefore the OVS provided technical and operational support to sample-taking in that situation.

2.4.3 Outbreaks of Highly Pathogenic Avian Influenza and PPMV-1 in wild birds and animals

In the period under assessment 100 HPAI outbreaks were reported in free-living wild birds, and five outbreaks among free-living aquatic mammals. Occurrences peaked in September and October 2023, both months having 27 outbreaks. After that, there was a drop in the number of outbreaks and the situation stabilized (Figure 15). This fall may be related to the dwindling numbers of these birds as migratory species returned to other continents, without disseminating or maintaining viral circulation in Brazil.

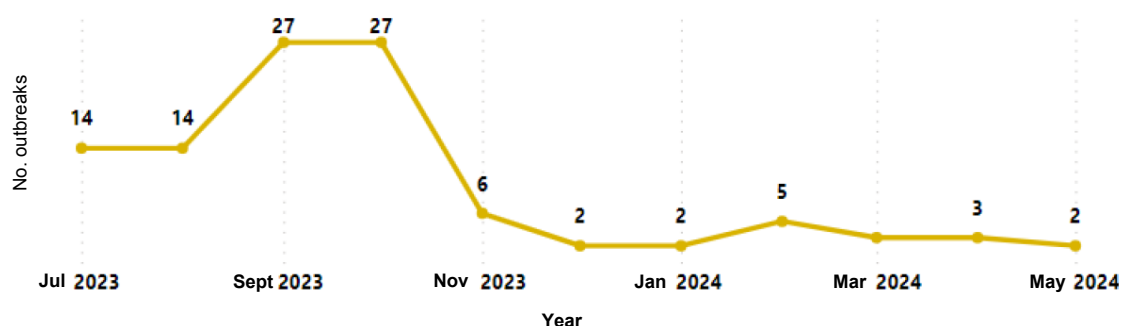


Figure 15. Number of HPAI outbreaks in wild animals.

It should be emphasized that the number of outbreaks reported does not reflect the real impact of the infection **in wild animals**, since the OVS does not require investigation of new cases in any given municipality for species in which the disease has already been confirmed in the last 30 days. This measure optimizes both human and laboratory resources, saving them in order to ensure response to suspected cases in domestic birds, when the presence and spread of the virus is detected over time and within the space of the other species.

The wild bird species hit hardest by HPAI were the Royal tern (*Thalasseus maximus*), Cabot's tern (*Thalasseus acutirostris*), and the Common tern (*Sterna hirundo*). For marine mammals the most hard-hit species were the South American sea lion (*Otaria flavescens*) and the South American fur seal (*Arctocephalus australis*). Table 6 shows the total numbers of cases per species.

Table 6. Number of cases and outbreaks of HPAI by major wild-animal species.

Species	Cases	Outbreaks*
<i>Otaria flavescens</i> (South American sea lion)	578	5
<i>Arctocephalus australis</i> (South American fur seal)	439	3
<i>Thalasseus maximus</i> (Royal tern)	61	33
<i>Thalasseus acutiflavus</i> (Cabot's tern)	57	36
<i>Sterna hirundo</i> (Common tern)	12	11
<i>Plegadis chihi</i> (White-faced ibis)	11	1
<i>Sterna hirundinacea</i> (South American tern)	7	6
<i>Puffinus puffinus</i> (Manx shearwater)	4	4
<i>Procellaria aequinoctialis</i> (White-chinned petrel)	2	2
<i>Sula leucogaster</i> (Brown booby)	2	2
<i>Ardea alba</i> (Great Egret)	1	1
<i>Ardea cocoi</i> (Cocoi heron)	1	1
<i>Egretta thula</i> (Snowy egret)	1	1
<i>Nannopterum brasilianum</i> (Neotropic Cormorant)	1	1
<i>Pachyptila desolata</i> (Antarctic prion)	1	1
<i>Pluvialis dominica</i> (American golden-plover)	1	1
<i>Vanellus chilensis</i> (Southern lapwing)	1	1
Total	1180	110

*Outbreak: an epidemiological unit where at least one confirmed case of the disease in any species has been found.

HPAI outbreaks occurred in: SP (49), SC (19), RJ (18), ES (7), PR (7), RS (5).

Nine outbreaks of APMV-1, PPMV-1 variant, were reported in the period under analysis: five in RS, three in PR and one in MG (Figure 16). All the affected birds were pigeons, except for three outbreaks in RS, where the affected species were eared doves and black swans.

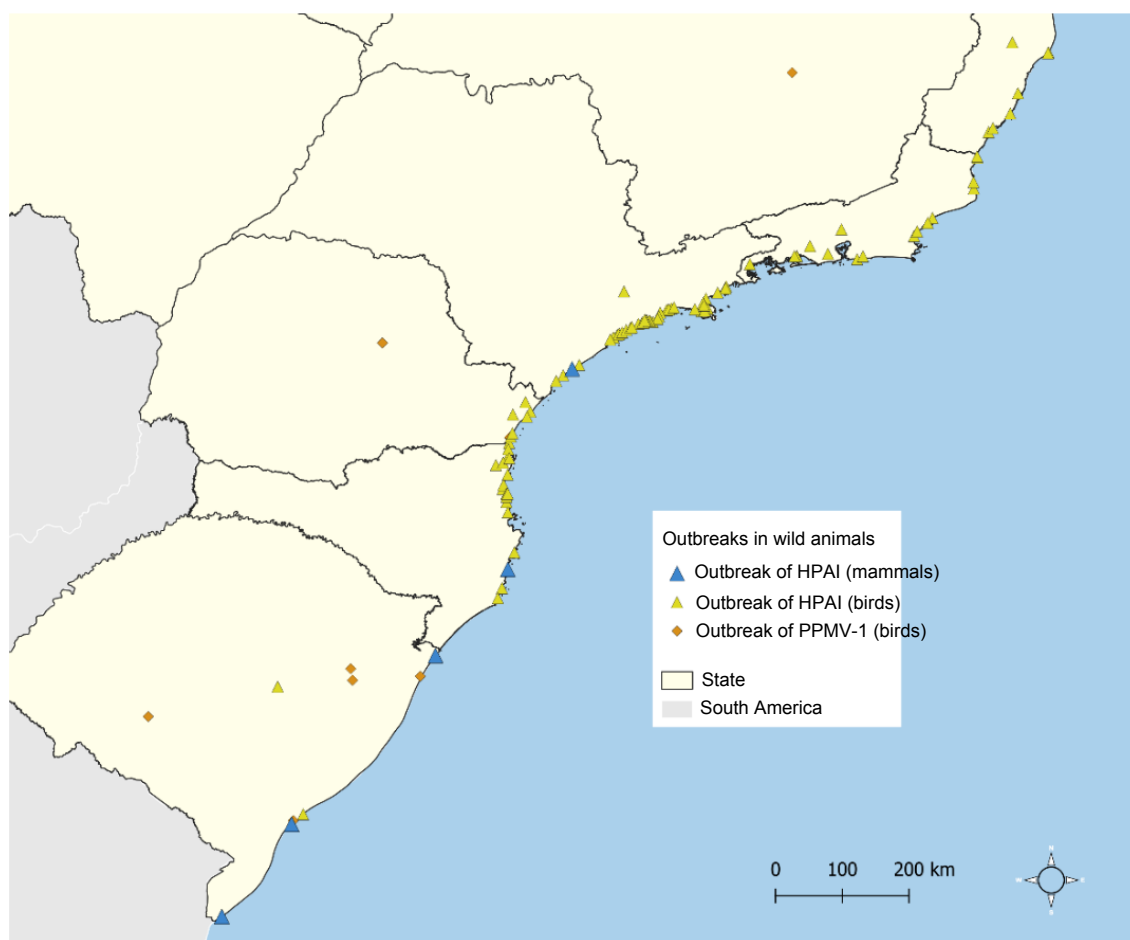


Figure 16. Number of HPAI and PPMV-1 outbreaks in wild animals.

2.5 CONCLUSIONS OF PASSIVE SURVEILLANCE IN WILD BIRDS AND ANIMALS

Passive surveillance in wild birds aims to enable the monitoring of changes in ARNS in the wild environment, so as to detect and rapidly control the possible introduction of influenza A and APMV-1 into backyard or commercial domestic poultry.

MAPA, aided by the environmental agencies, 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.

Most of the HPAI outbreaks occurred in terns in the States of SP and SC, above all in September and October 2023. After the peak, the number of outbreaks diminished and remained stable in a range from two to six outbreaks per month.

The HPAI outbreaks occurred in bird species in which they had not been detected previously: American golden plover; Manx shearwater; White-faced ibis; Antarctic prion; Great egret; Snowy egret; Cocoi heron; White-chinned petrel; Southern lapwing, and among marine mammals (South American sea lion and South American fur seal).

MAPA integrated genomic analysis of the virus with the AI surveillance system as a strategy to elucidate the origin, dynamics of transmission and the evolution of the

circulating viruses. As a result, phylogenetic tests for virus H5N1 clade 2.3.4.4b, which is found in Brazil, confirmed proximity to the virus circulating in Chile and Peru, suggesting that the likely source for the introduction of the virus into Brazil was through the migration of birds in the Pacific route.

3. COMPONENT 3 - ACTIVE SURVEILLANCE IN INDUSTRIAL POULTRY PRODUCTION

3.1 OBJECTIVE, APPROACH AND SOURCE OF DATA

Component 3 of the surveillance system had as its objective to demonstrate that Brazil's industrial poultry production is HPAI virus-free 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 in order to record, consolidate and share data from the study activities.

3.2 RISK INDICATOR

The types of establishment, 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, WOAHA), 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 line animals)
- MODERATE RISK □ Laying hen farms
- HIGH RISK □ Duck, turkey and quail farms

3.3 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 17) and with a housing capacity greater than 1,000 birds. Component 3 excludes backyard operations, breeders 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.

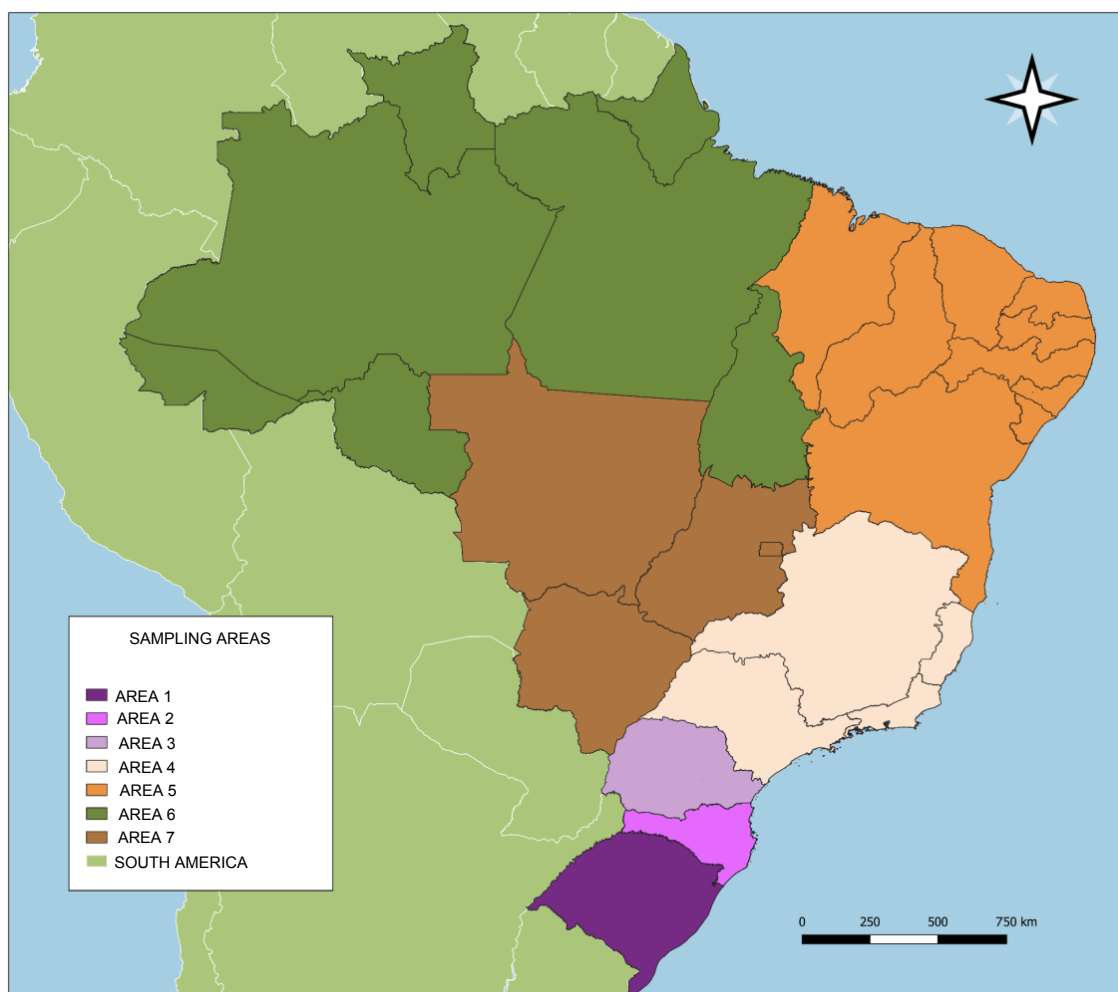


Figure 17. Sampling areas for component 3

3.4 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, and can be 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, farms would be selected at random, and in the second step, for each selected farm, birds would be sampled in order to classify the farm according 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 carried out, 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 an estimated prevalence on farms of 30% and confidence level of 95%.

3.5 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 farms to be examined, the farms 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, 11 animals were sampled in each production center (epidemiological unit or “nucleus” in Portuguese) as of 30 days of age. In the case of production centers possessing several sheds, the prevalence among sheds was deemed to be 30%, and therefore in those production centers the sampled animals would have to be distributed among the several poultry houses.

In establishments comprising more than one production center, the guidance given was to sample all the eligible production centers, respecting the minimum age of the chickens and the maximum number of five production centers per establishment.

Additionally, an incremental sample-taking was performed, targeting exclusively those municipalities located in areas close to areas where Nearctic migratory birds gathered. Given the diversity and importance of these areas in the country, above all for the presence of the orders *Charadriiformes* and *Anseriformes*, that strategy aimed to increase the sensitivity of that component of surveillance. The increase represented 10% of the expected sampling at outset.

The final size of sampling was thus 2,391 establishments, ranging from 305 to 351 by sampling area.

3.6 TYPE OF MATERIAL HARVESTED

In order to obtain serum, blood samples were harvested on an individual basis by venipuncture from 11 live birds. Additionally, tracheal and cloacal swabs were taken from each selected bird.

As recommended in the surveillance plan, swabs are to be packed in pools. For hens, the pools can be formed as follows:

- One pool of 11 tracheal swabs and another pool of 11 cloacal swabs; or
- Eleven cloacal swabs divided into two pools, one pool being made up of five swabs and the other pool containing 6 swabs.

The same logic applies to tracheal swabs.

It should be emphasized that when sampling involved other species (turkeys, ducks, quail and teal), the 11 samples of tracheal and cloacal swabs from each production center were necessarily split into four pools.

3.7 THE INDIVIDUALS THAT TOOK THE SAMPLES

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.

3.8 ACTIVITIES PERFORMED

3.8.1 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 (UF).

3.8.2 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.

The minimal script for information audited during inspections of the breeding establishments and commercial poultry farms is shown in ANNEXES.

3.9 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, 2,391 commercial raising establishments were visited 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 RS (Area 1), SC (Area 2), and PR (Area 3), had the largest number of establishments sampled, on average totaling 350 establishments each (Figure 18). The total number of sampled establishments by sampling area is shown in Figure 18.

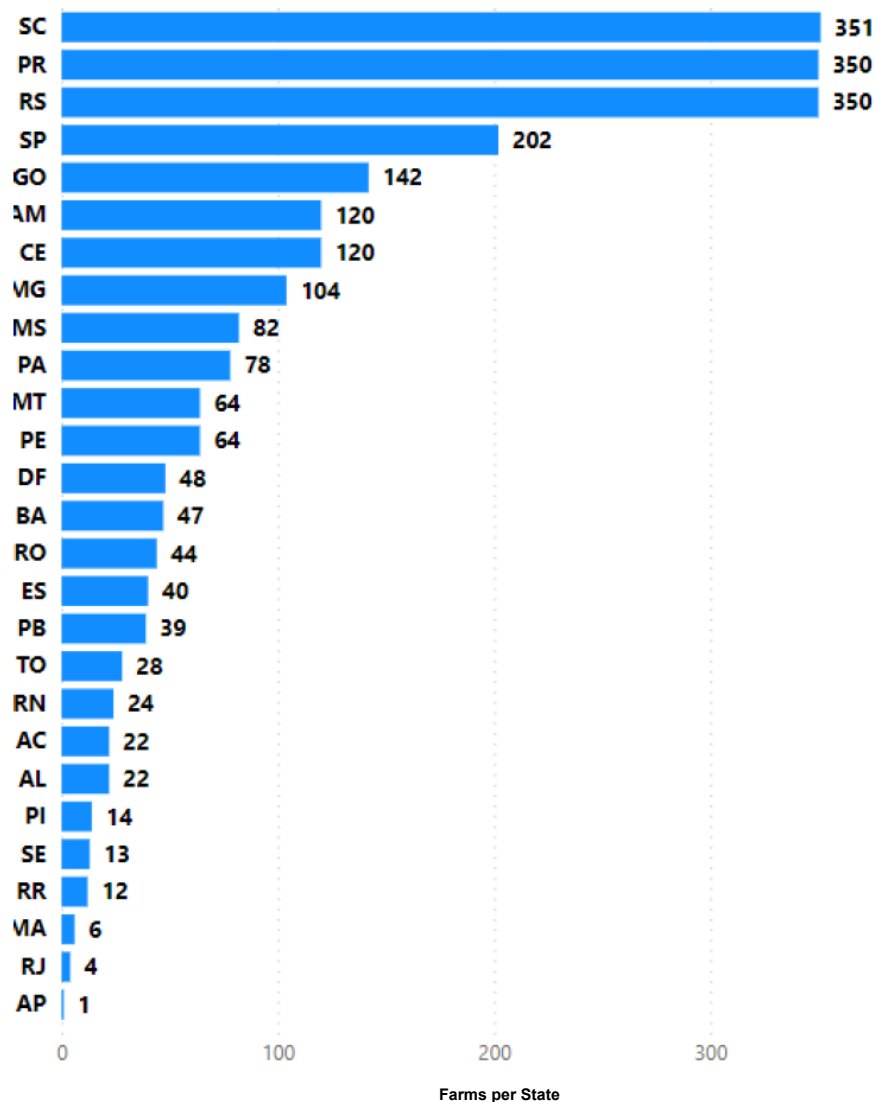


Figure 18. Distribution of the number of sampled poultry establishments, by State, for component 3

Figure 19 presents the geographical distribution of the poultry establishments sampled in the distinct sampling areas of component 3, in accordance with the sampling plan.

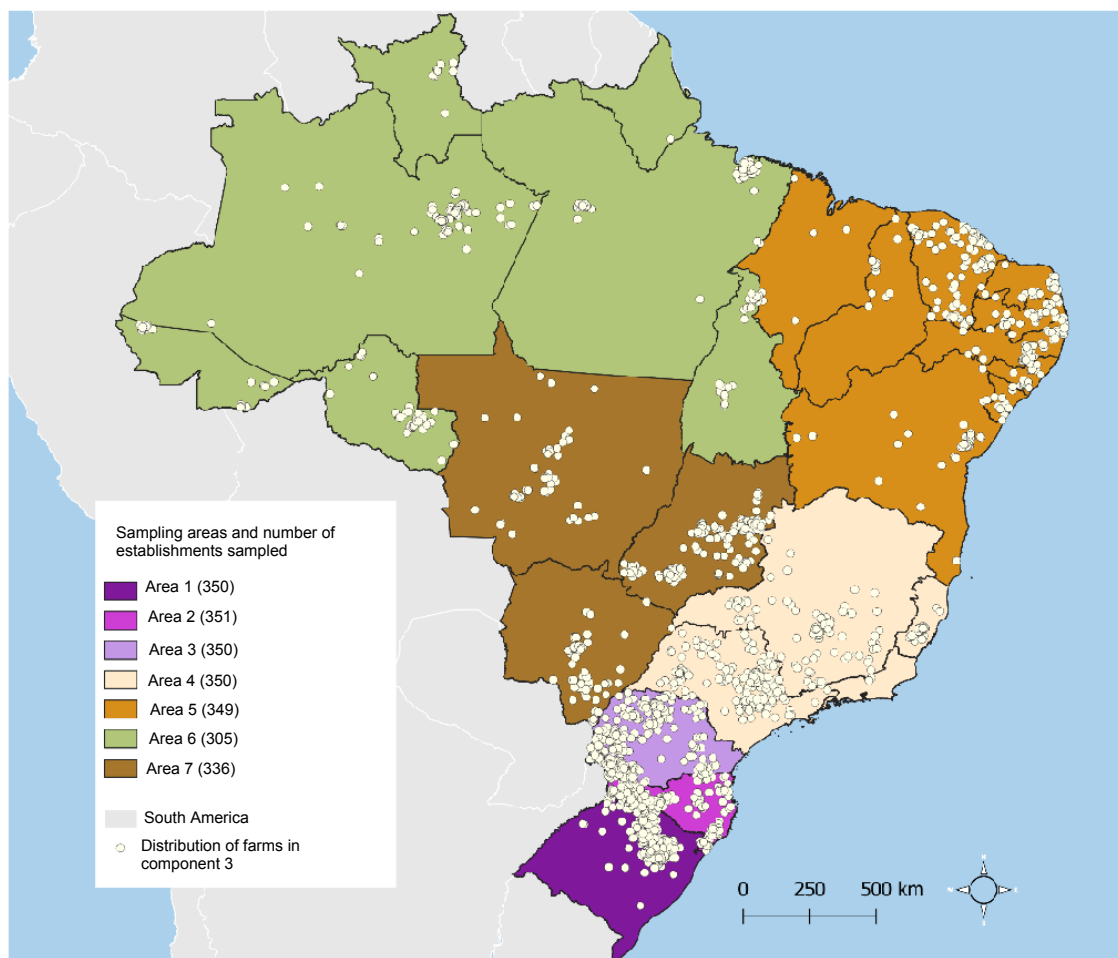


Figure 19. Geographical distribution of poultry establishments sampled in the distinct sampling areas of component 3

3.9.4 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 commercial poultry-raising establishments, to wit: broilers, commercial egg-laying, parent birds and grandparent birds, as shown in figures 20 and 21.

It is worth emphasizing that in areas 1, 2, 3 and 7, the majority of sampled chicken establishments were intended for the production of broilers, whereas in areas 4, 5 and 6, commercial egg-laying establishments predominated. The only four grand-parent stock establishments sampled were in areas 3 (PR) and 7 (DF, GO and MS).

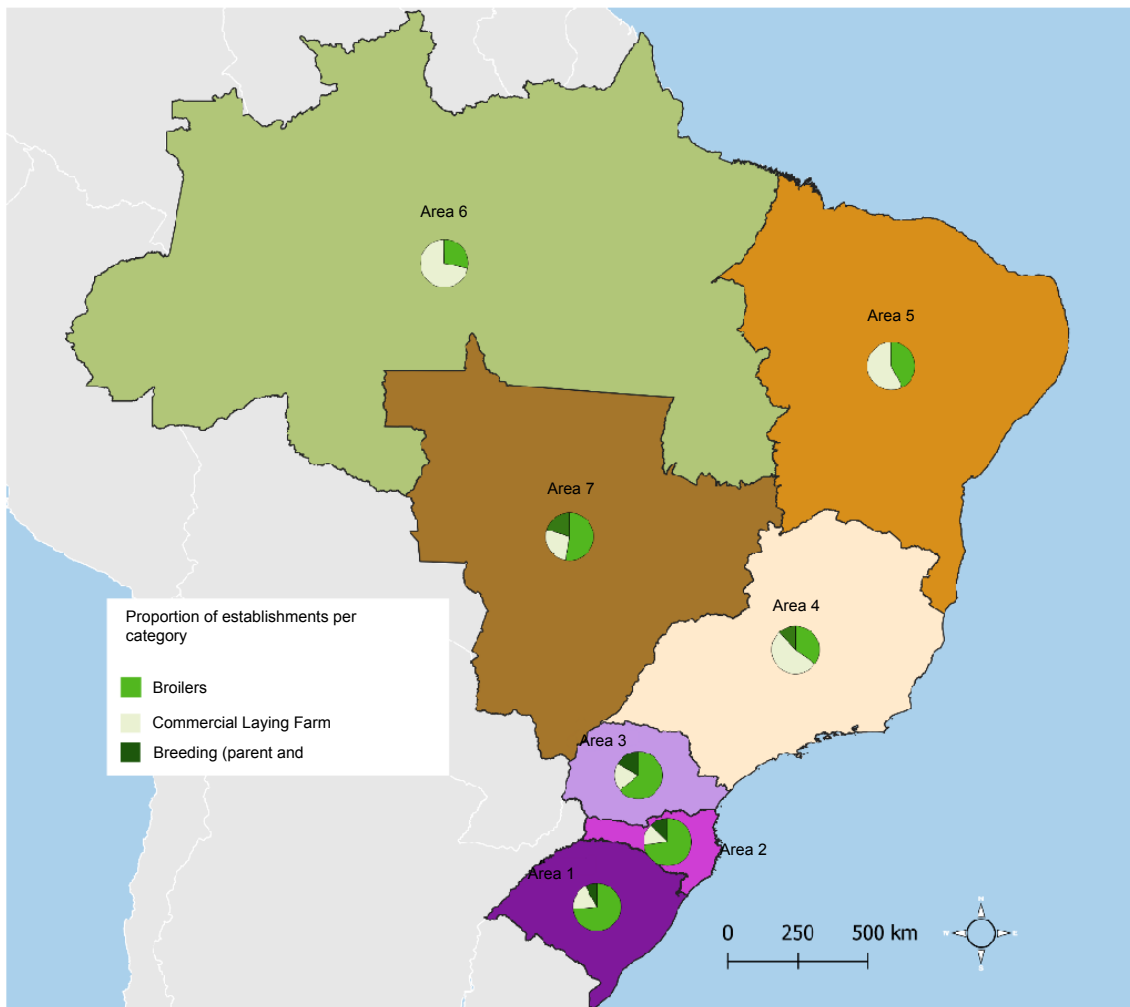


Figure 20. Ratio of the several types of poultry establishment sampled in component 3

Overall, the poultry production category most highly-represented among sampled establishments was commercial broiler production, accounting for 53.2% of the total. Figure 21 shows the distribution of the sampled establishments in component 3 by distinct category of poultry production.

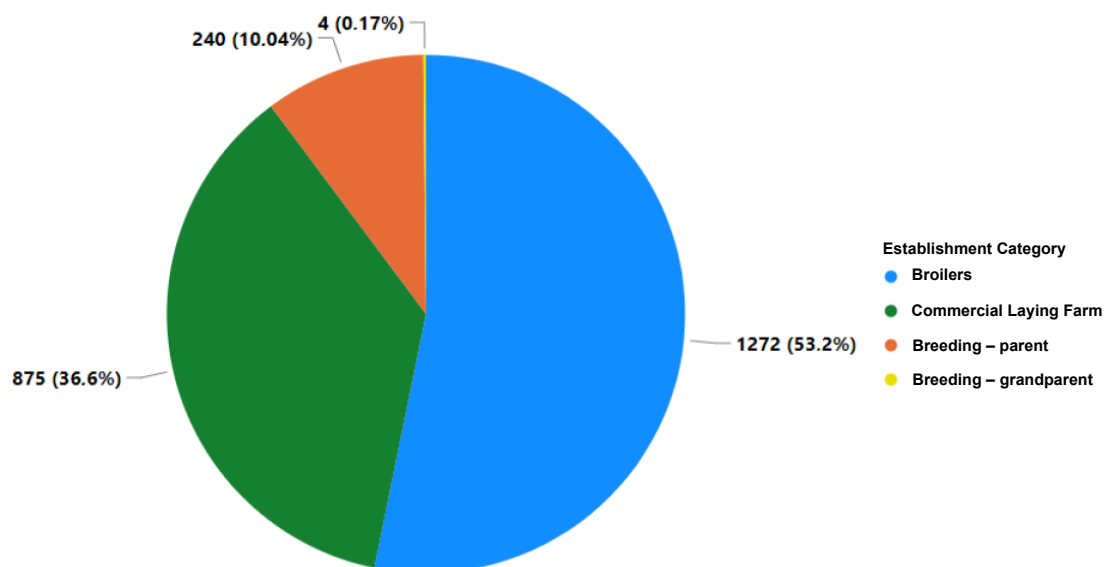


Figure 21. Frequency of the number of sampled establishments in component 3 by distinct category of poultry production

As to sampled species, hens stood out as the most representative with 2,107 sampled producing establishments, accounting for 88.12% of the total. Turkeys were the second-most frequent species with 224 establishments (9.36%), as shown in Figure 22. Other species like quail, ducks and teal were also included in the samples for component 3. Figure 22 shows the distribution among species in accordance with the number of sampled poultry establishments.

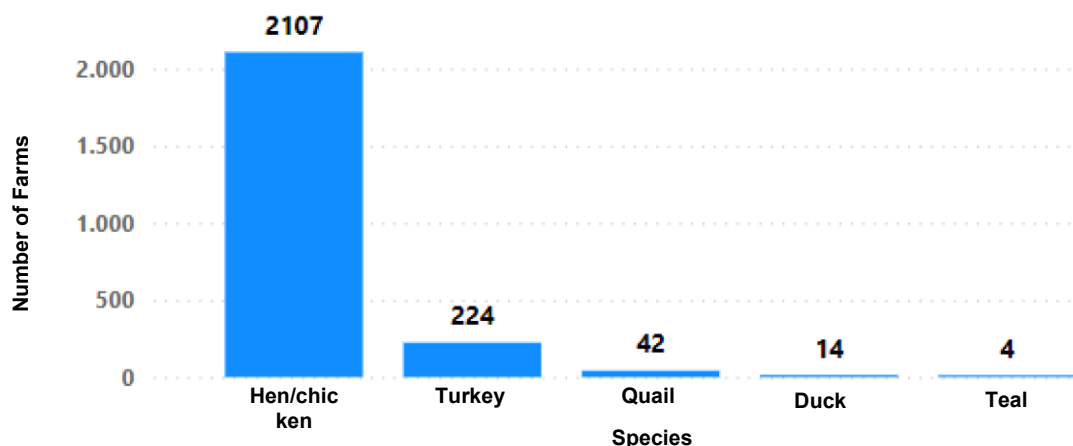


Figure 22. Frequency of the number of establishments housing distinct species of poultry and sampled in component 3.

Sample-taking in hens/chickens encompassed all 27 States of Brazil. Sample-taking among commercial quail and ducks was more frequent in SC (Figure 24). Turkeys were sampled only in the states of the South of Brazil (RS, SC and PR).

For teal, samples were taken on 4 farms in the State of SC (Figure 25).

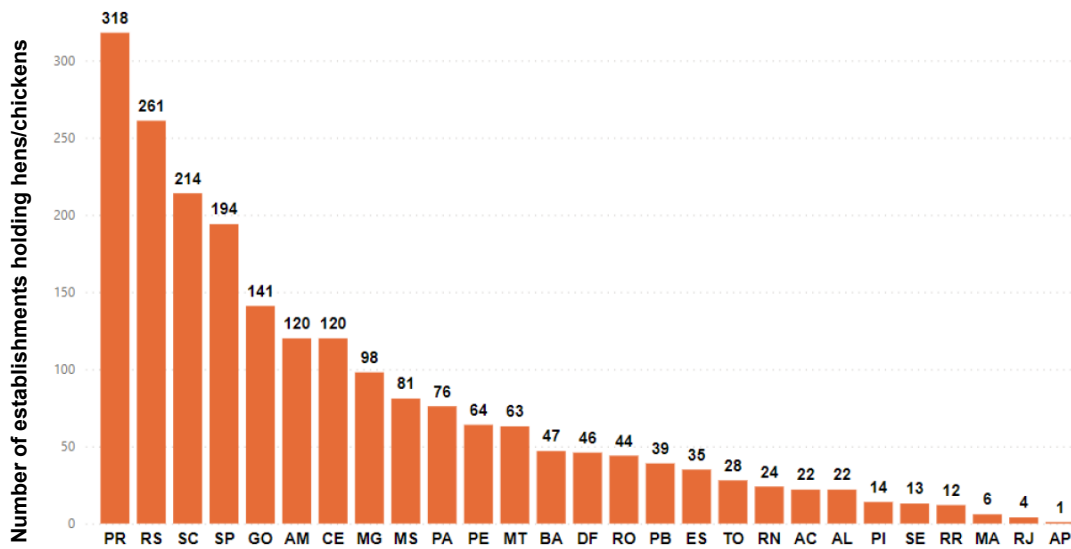


Figure 23. Distribution of poultry establishments holding hens/chickens, by State, and sampled for component 3

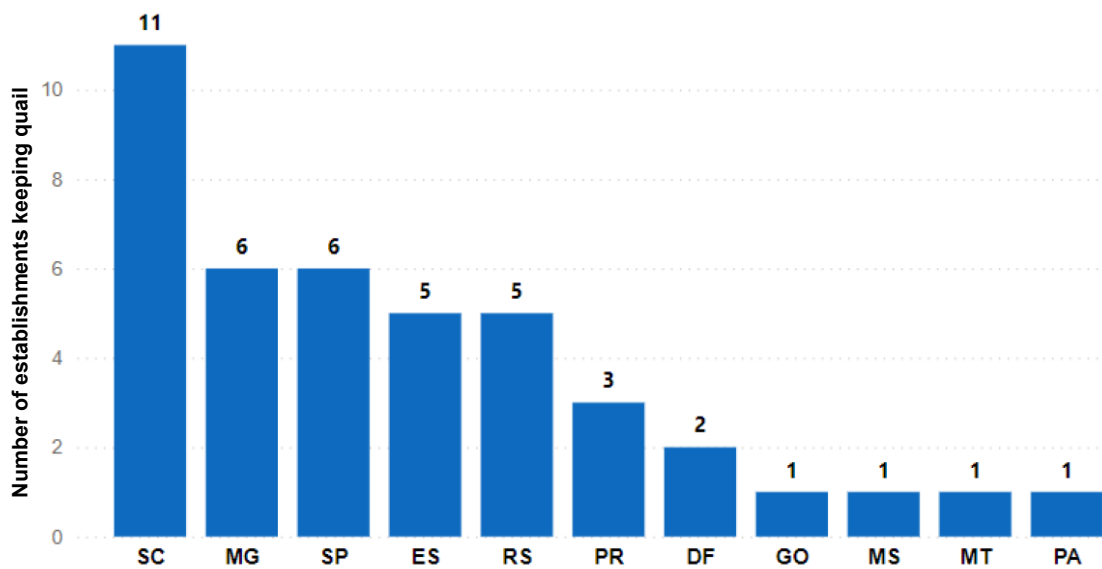


Figure 24. Distribution of establishments holding quail, by State, and sampled for component 3

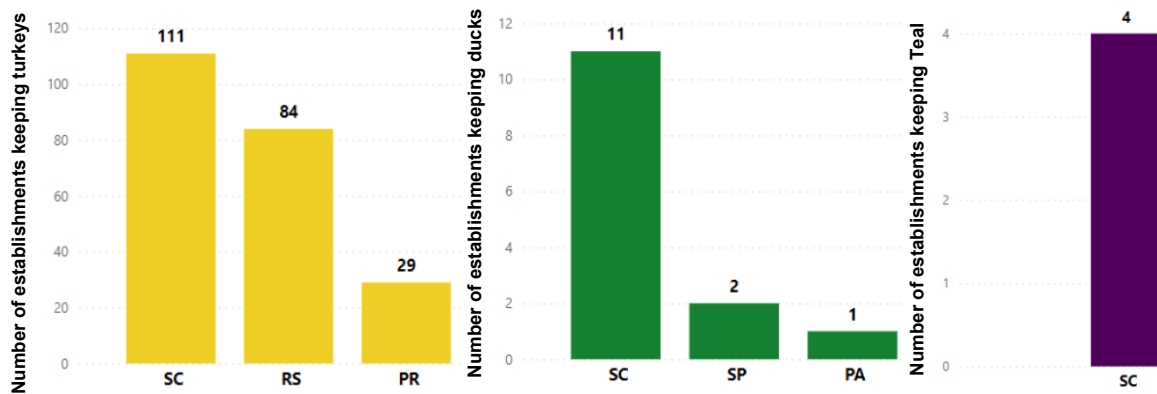


Figure 25. Distribution of establishments holding turkeys, ducks and teal by State, and sampled for component 3

3.9.5 Laboratory tests

The total number of samples taken for component 3 led to 47,568 laboratory tests being performed. In order to meet the scheduled provisions of the plan, the set of 11 animals sampled in each production center of a poultry farm was to generate at least 15 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 varying numbers of production centers on the sampled farms, and of the sampled species, as well as samples being rejected and laboratory losses.

The distribution of samples analyzed per state in component 3 is shown in Table 8. Santa Catarina (Area 2) accounted for the largest number of laboratory tests performed, with a total of 7,347.

Table 8. Frequency of laboratory testing, by State

State	Number of laboratory tests
SC	7347
RS	7008
PR	6729
SP	3623
CE	2676
GO	2410
AM	2007
MG	1818
MS	1686
PA	1646
PE	1533
RN	1434
PB	1310
ES	1046
MT	987
DF	923
RO	773
BA	613
SE	513
AL	414

AC	346
TO	272
PI	195
MA	130
RJ	60
RR	49
AP	20
Total	47568

3.9.5.6 Serological tests

No samples reacting to HI were found in the serological monitoring of AI to detect antibodies to the distinct subtypes of influenza virus A hemagglutinin (H1– H16).

3.9.5.7 Molecular tests

In confirmatory molecular tests for AI and ND, no positive samples were detected.

3.10 CONCLUSIONS OF ACTIVE SURVEILLANCE IN INDUSTRIAL POULTRY

Active surveillance was carried out in order to monitor for, and detect, possible AI and ND viral circulation and infection in industrial-production bird populations.

In serological monitoring for AI, no antibodies were identified in the industrial populations assessed.

Regarding molecular tests, the absence of any positive results for influenza virus A, along with the information resulting from epidemiological surveillance, showed that AI or ND infection were absent in the industrial-production bird populations that were sampled.

4. COMPONENT 4 - ACTIVE SURVEILLANCE IN BACKYARD POULTRY AND SMALL-SCALE PRODUCTION

4.1 OBJECTIVE, APPROACH AND SOURCE OF DATA

Component 4 aims to seek to detect AI and ND in backyard poultry populations or small-scale production flocks intended for local trade in areas of greater risk; in other words, areas where there is a greater likelihood of exposure to migratory wild birds. Surveillance of these populations is warranted by the lower level of biosecurity implemented by these establishments, signifying a raised risk of the introduction of the virus.

Epidemiological surveillance by means of the inspection of backyard poultry-raising facilities provides an opportunity to detect susceptible animals showing clinical signs or changes in animal husbandry rates that can lead one to suspect that the target diseases of the present study are present. 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 poultry or small-scale production” have been recorded in the Epicollect5 application software.

4.2 TYPE OF RISK INDICATOR

The concept of risk-based surveillance is built into the study design, whereby sampling targets those farms and birds that are 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 the risk-based surveillance adopted during the period covered by the present report, criteria provided for in the AI and ND surveillance plan were taken into account. An extensive database on locations where migratory birds had been reported present either by direct sighting 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. Municipalities in which there are commercial farms;
2. Municipalities in which there are 530+ registered small-scale farms;
3. Any overlap with municipalities in which there were sightings of Northern Hemisphere birds; and
4. Located within the zone of influence (100km) of the three major routes for migratory birds passing through Brazil.

4.3 TARGET POPULATION

To meet the objectives of this component, the target population comprised backyard poultry or small-scale production located in areas identified as being at risk for the introduction of the viruses.

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

In addition to the most common species of gallinaceous poultry, 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 domestic species that are susceptible to AI and ND and are found within Brazil.

4.4 SAMPLE DESIGN

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

Backyard poultry populations and small-scale production flocks were taken into consideration for estimating the sample size.

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 was 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 which contains 7 areas delimited by State borders, component 4 considered regions linked to the three main migratory routes: Central Brazil (BR-CE), Atlantic/Northeast (NE-ATL) and the Amazon route that cross Brazil; the political limits (borders) of the ‘federative units’ (States) were therefore not taken into account.

Finally, a minimum sampling of 324 units was defined for each region associated with migration routes; the units were distributed as follows: Amazon - 330, BR-CE - 336 and NE-ATL - 424. Samples were taken from 11 birds in each unit, prioritizing *Anseriformes*.

4.5 SAMPLING STRATEGY

The sampling strategy of this component aimed to achieve detection of AI and ND in target populations located in at-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 may also provide warnings of occurrences in locations that can impact Brazil's production systems, enabling measures to reinforce biosecurity and protect industrial poultry production to be taken.

4.6 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 taken 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 poultry 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 4.3, and that each pool of swabs be separated and identified by species.

4.7 THE INDIVIDUALS THAT TOOK THE SAMPLES

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.

4.8 ACTIVITIES PERFORMED

4.8.1 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 (UF). The municipalities that were to be sampled were selected by DSA, but the establishments were selected by the chief veterinarian 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 POULTRY ESTABLISHMENTS AND IN AREAS AT GREATER RISK OF THE INTRODUCTION OF AI – COMPONENT 4. Apart from these characteristics, the priority was to select poultry farms presenting the following features:

- sites 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 poultry-raising establishment.

4.8.2 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 RESULTS

4.9.1 Number and geographical distribution of sampled farms

To assess for the circulation of AI and ND viruses in backyard poultry farms and small-scale farms, samples were taken from 1,090 farms under the three migration routes for serological and molecular testing.

Figures 26 and 27 show the distribution of the sampled poultry-raising establishments located along the wild bird migration routes. On the Atlantic/Northeast Route, 424 establishments were sampled, accounting for 38.89% of the total number of tested establishments. The second-largest number of sampled establishments (336 establishments) was on the Central Brazil route.

The State with the highest number of sampled establishments was Pará with 144 units, accounting for 13.21% of the total, as shown in Figure 26. Pará stands out from the other States for its strategic geographical position and its vast size — it lies under all three migration routes.

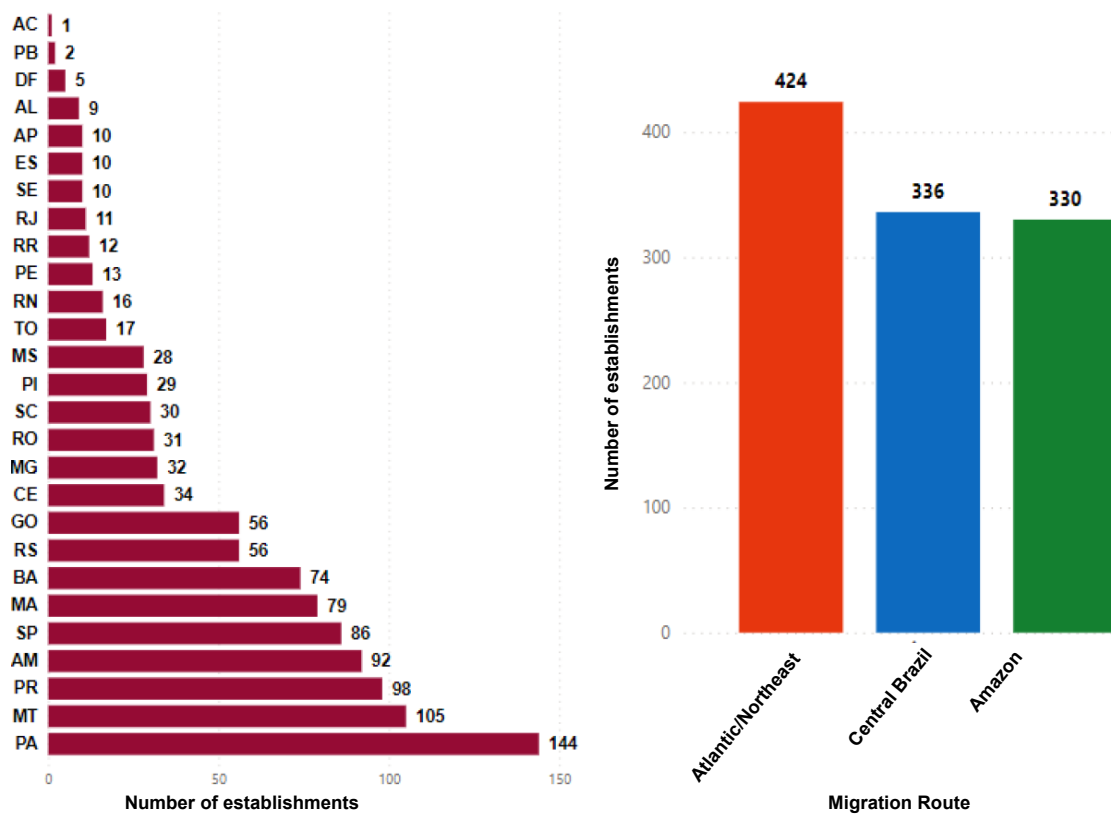


Figure 26. Frequency of the number of establishments in component 4 with backyard poultry populations, by State and migration route

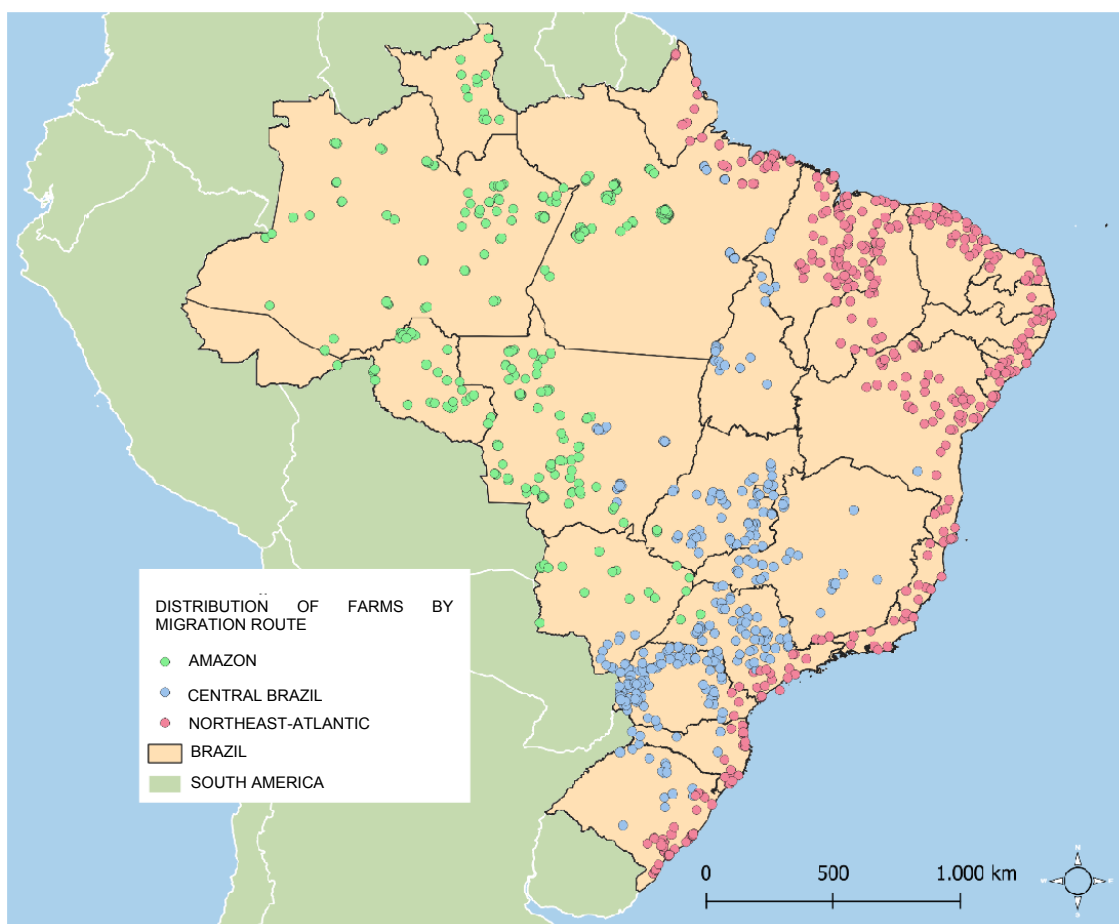


Figure 27. Geographical distribution of establishments in component 4 sampled by route

4.9.2 Bird species sampled

Among backyard operations sampled for component 4, 545 (50%) establishments exclusively raised hens/chickens. A further 61 (5.6%) farms raised mixed populations of hens/chickens and other gallinaceae such as guinea fowl, turkeys and quail.

In turn, 485 establishments (44.4%) held at least one species of *Anseriformes*, among ducks, teal and geese.

Figure 28 shows the frequency of sampled farms housing species of *Anseriformes* by State.

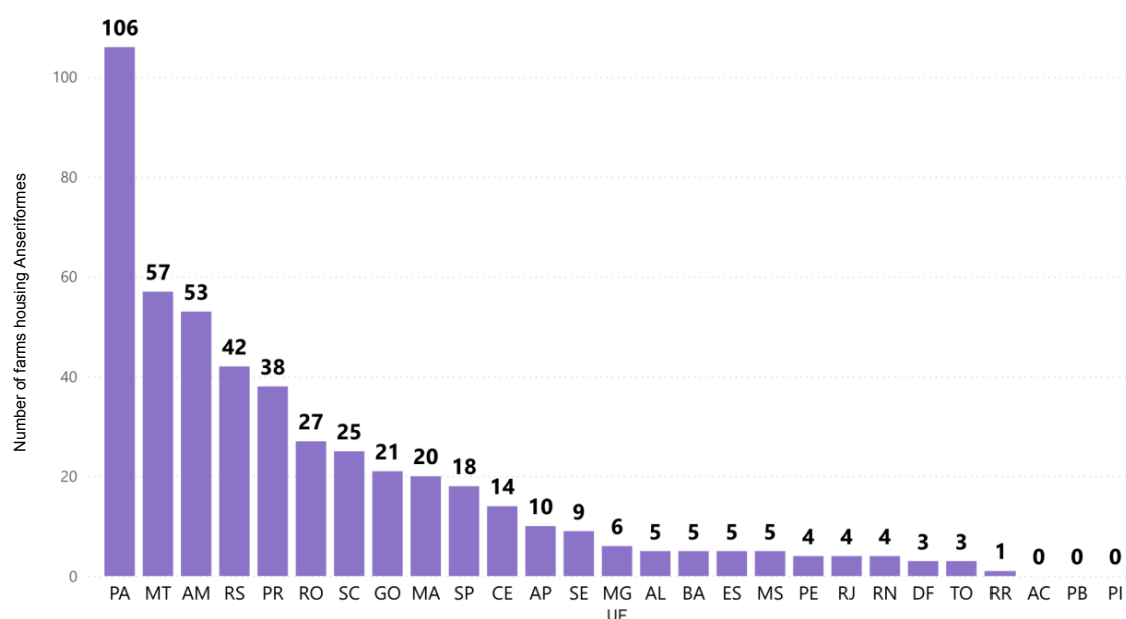


Figure 28. Frequency of farms housing species of Anseriformes, sampled, by State.

4.9.3 Laboratory tests

Epidemiological and laboratory surveillance for component 4 resulted in 31,841 laboratory tests. To comply with the provisions of the plan, the set of 11 animals sampled on each farm was to generate at least 24 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 that were rejected and laboratory losses.

The state of Pará recorded the largest number of laboratory tests, with 4,362 (13.69 %) of the tests, as shown in Table 9.

Table 9. Frequency of the number of laboratory tests, by State, for component 4

State	Number of laboratory tests
PA	4362
MT	2982
PR	2943
AM	2804
SP	2764
MA	2101
BA	1915
RS	1717
GO	1488

SC	1054
CE	1025
RO	919
MG	807
MS	750
PI	742
RN	492
TO	464
PE	398
RJ	334
SE	334
ES	320
RR	312
AP	300
AL	286
DF	150
PB	52
AC	26
	31841

4.9.3.1 Serological tests

All samples testing positive or inconclusive for AI in ELISA underwent HI testing for antibodies against the several subtypes of AI virus (H1 – H16).

Typification of seropositive samples in the ELISA screening test showed only 3 farms testing positive for antibodies to different subtypes of type A influenza virus hemagglutinin. The results showed that samples taken from hens on three backyard establishments in the State of Maranhão presented antibodies to subtypes H3, H12 and H16 of influenza virus A.

Table 10. Farms with samples testing positive in the Hemagglutination Inhibition Test (HI) in component 4

State	HI typification	Number of farms	Component	Affected species
Maranhão	H3, H12, H16	3	4	Hens

For ND, 266 backyard flock-raising establishments were identified as having tested positive in the ELISA serology test for the ND virus.

4.9.3.2 Molecular tests

The M gene of Influenzavirus A was not found in any of the tested samples.

Likewise, all samples tested for ND virus were negative in the confirmation tests, so there was no laboratory evidence of the circulation of the agent in those samples.

4.10 CONCLUSIONS ON ACTIVE SURVEILLANCE IN BACKYARD POULTRY AND SMALL-SCALE COMMERCIAL FLOCKS

Epidemiological surveillance and laboratory testing carried out in Component 4 aimed to detect Influenzavirus A and ND in backyard populations of poultry and in small-scale production flocks, located in areas classified as being at greater epidemiological risk.

Molecular test results showed no detection of the virulent strains of ND virus in these populations, nor was there any laboratory evidence of the circulation of the virus in the period under assessment. The presence of antibodies to ND virus in samples from backyard poultry, along with negative results to confirmatory molecular testing, may show an immune response to the vaccine 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 sampled population.

In serological monitoring for AI, a low frequency of reagent samples to the confirmatory HI test was observed, only on three farms. Serological typification identified that antibodies to subtypes H3, H12 and H16 were present in hens/chickens: these subtypes are compatible with low pathogenicity viruses. It is to be emphasized that corresponding samples tested negative in molecular tests to Influenza A, demonstrating the absence of HPAI virus circulation among the backyard and small-scale production flocks that were sampled.

5. COMPONENT 5 - ACTIVE SURVEILLANCE IN AVIAN INFLUENZA-FREE AND NEWCASTLE DISEASE-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 applies only to 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:

- Breeding compartment: made up of breeding farms and hatcheries as well as associated functional units
- Meat production compartments: made up, as a minimum, of parent breeding farms, their hatcheries, broiler farms and associated functional units.

In the period under assessment, 7 compartments were MAPA-certified, all being involved in breeding. Figure 29 shows the geographical distribution of AI- and ND-free compartments in Brazil.

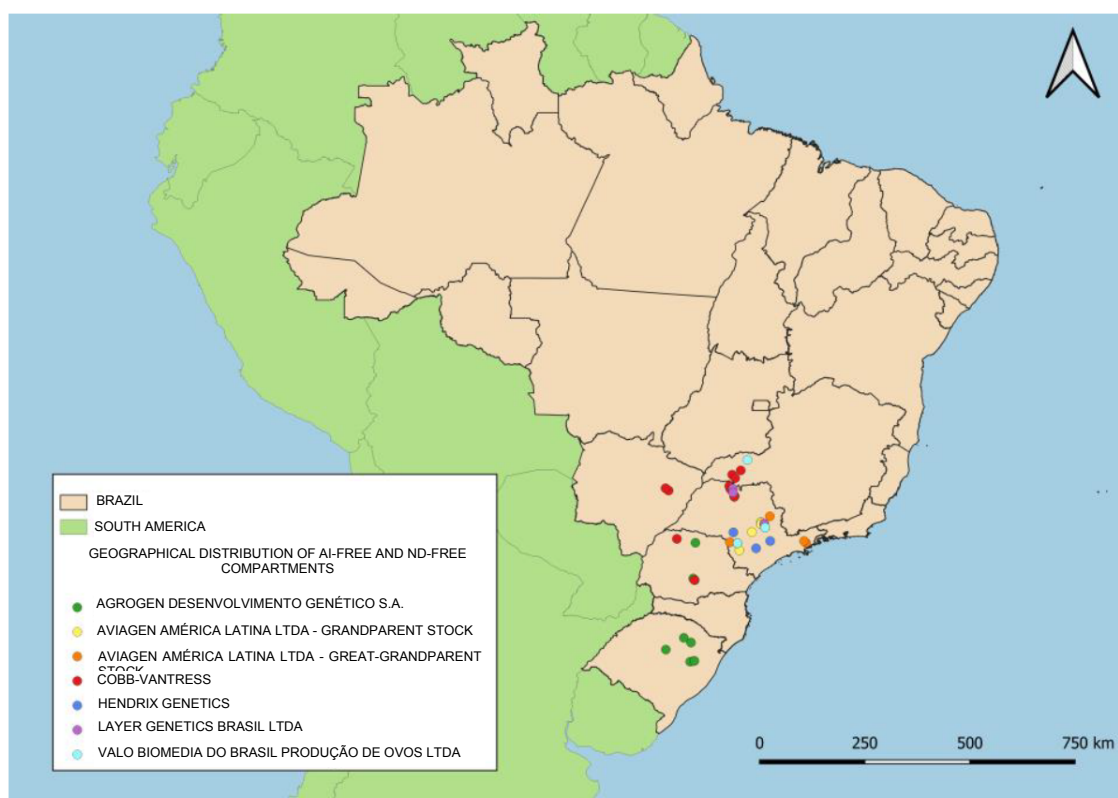


Figure 29. Geographical distribution of epidemiological units (production centers) of establishments with Sanitary Certification of Compartments for the Poultry Production Chain for Avian Influenza and Newcastle Disease in the first cycle of the surveillance plan.

5.1. SURVEILLANCE STRATEGY

Active surveillance in AI and ND-free compartments complies with Chapter 1.4 of the WOAHP Terrestrial Animal Health Code and follows the specific recommendations for the surveillance of compartments as set forth in Article 4.4.5., as well as the articles specifically addressing Avian diseases (Articles 10.4.26. and 10.4.27 for AI and Article 10.9.24 for ND).

5.1.1. Internal Surveillance

All the farms certified as sanitary compartments were included in sampling to detect the presence of AI and ND viruses. Epidemiological and laboratory surveillance of compartments is carried out in accordance with Chapter VI of Normative Instruction no. 21 dated October 21, 2014, which recommends serological and molecular testing for both diseases.

Samples are taken for all production centers on the farm, including those with birds that had been housed for 21 days at the moment of taking the sample. Every six months, blood serum samples, tracheal swabs and cloacal swabs are taken from 10 birds per production center. It should be emphasized that broilers or birds raised for stocking

purposes that may have been given a live ND vaccine are not tested for Newcastle Disease.

The laboratory tests to screen for AI and ND are conducted in public, MAPA-approved laboratories:

- Centro de Diagnóstico Marco Enrietti (CDME), PR;
- Instituto Biológico (IB), SP.

Should positive or inconclusive samples be identified at screening, the OVS is notified and the samples are sent to LFDA-SP for confirmation.

5.1.2 External surveillance

External surveillance of the compartments is carried out for components 1, 2, 3 and 4 of the AI and ND surveillance system, combining active and passive surveillance strategies.

Components 1 and 2 correspond to passive surveillance, which is deemed to be the most effective strategy for the early detection of suspected cases. The approach is based upon immediate mandatory notification of the OVS of a suspected case of AI or ND, so that the OVS can carry out the necessary investigation.

Active surveillance aims to prove the absence of AI and ND infections in industrial poultry-raising and monitor the occurrence of infections in wild migratory birds through the surveillance of backyard poultry populations or small-scale production flocks in regions lying close to areas where wild species gather.

The selection of establishments housing industrial and backyard poultry-raising flocks depends upon a risk analysis for the introduction of AI and ND as shown below in greater detail:

In component 3, the main aspects taken into consideration for the risk categorization of commercial poultry-raising establishments were, in order of importance: the susceptibility of the species that are present; the duration of the production cycle; and the impact of handling, health and biosecurity practices.

In component 4, risk-based sampling targets backyard flocks or small-scale production flocks at greater risk of exposure to AI, encompassing the distinct sampling areas within Brazil. In order to define active surveillance activities in this approach, four criteria defined in the AI and ND surveillance plan were used. It should be emphasized that the criteria include municipalities in which there are industrial poultry-raising operations, ensuring the inclusion of all the compartments in the analysis.

Therefore all nearby commercial poultry establishments, or establishments with a potential connection to compartments, are taken into consideration for random selection. Additionally, all municipalities in which compartments are located are included in the random selection of farms housing backyard flocks or small-scale production flocks in order to enable epidemiological surveillance and sample-taking as set forth in component 4.

External surveillance of the compartments by a combination of passive and active surveillance strategies thus plays a crucial role in identifying possible risks of the introduction of AI and ND. It also enables adjustments to the biosecurity measure to be assessed and introduced, ensuring the maintenance of the sanitary status of avian subpopulations recognized by MAPA as AI and ND-free within the territory of Brazil.

5.2 LABORATORY DIAGNOSIS

On poultry-raising farms in the compartments, the surveillance program is performed periodically through the 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 (production centers) of the sampled compartments during the period of the AI and ND Surveillance Plan under assessment .

Table 11. Number of epidemiological units (production centers) sampled within the period of the surveillance plan under assessment

Compartment/ Purpose	Semester	
	2023/2	2024/1
AGROGEN DESENVOLVIMENTO GENÉTICO S.A./Breeding	15	20
AVIAGEN AMÉRICA LATINA/Breeding	23	28
BRF S.A./Breeding	22	16
COBB-VANTRESS BRASIL/Breeding	27	36
HENDRIX GENETICS/Breeding	5	4
LAYER GENETICS BRASIL LTDA./Breeding	2	3
VALO BIOMEDIA DO BRASIL LTDA/Breeding	2	3

Differences in the number of epidemiological units (production centers) that were sampled in each of the 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, and so forth.

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 sanitary certification of the compartmentalization of the poultry production chain, encompassing breeding farms, broiler-producing farms and hen and turkey hatcheries, aims to acknowledge and validate AI and ND-free avian subpopulations with distinct sanitary statuses.

This condition is ensured by the implementation of strict biosecurity measures and both internal and external epidemiological surveillance of the compartments under continuous supervision and audits.

In the AI and ND surveillance cycle that was analyzed, no samples testing positive for influenza A or the ND etiological agent were detected. Thus the seven MAPA-certified compartments at the time maintained their AI- and ND-free status.

6. RESULTS OF SURVEILLANCE

During the AI and ND surveillance cycle from July 1, 2023 to June 30, 2024, the OVS carried out 1,647 clinical and epidemiological investigations on suspected cases of ARNS throughout Brazil, identifying 572 probable cases of AI and ND among domestic poultry and wild animals.

Within the scope of laboratory surveillance, 47,568 laboratory tests were performed to diagnose AI and ND in Component 3, intended for industrial-production birds; there were also 31,841 tests in Component 4, intended for backyard flocks and small-scale production flocks. Regarding component 5, 206 epidemiological units (production centers) associated with the compartments were sampled.

It should be emphasized that there was no detection of positive samples for AI or ND in commercial flocks during the present cycle of the AI and ND Surveillance Plan. As a consequence of these results, Brazil has maintained its sanitary status before WOA and its trading partners.

On the basis of the actions taken and the results obtained, the AI and ND Surveillance Plan has now consolidated itself as a basic strategic instrument for the protection of Brazil's poultry-production industry. The integration of passive and active surveillance bolsters the country's capacity for the early detection of, rapid response to, and mitigation of epidemiological risks, guaranteeing the sustainability of the poultry chain, Brazil's sanitary safety, and the country's credibility in the domestic and international trade in poultry products.

7. ANNEXES

Table 1. Species investigated* for passive surveillance – Components 1 and 2

Wild		Domestic or Backyard	Domestic Commercial
Atlantic Albatross	yellow-nosed White-rumped sandpiper	Speckled chachalaca	Japanese quail
Black-browed Albatross	Semipalmated Sandpiper	Red-and-green Macaw	Hens
Wilson's Storm-Petrel	Chilean skua	Cockatiel	Helmeted guinea fowl
Grey-breasted martin	Blue-winged macaw	Black swan	Domestic grey goose
Smooth-billed Ani	Brazilian teal	Northern bobwhite/ Virginia quail	Teal
Black-necked Aracari	Masked Duck	Common or European quail	Duck
Hyacinth Macaw	Teal	Japanese quail	Turkey
Blue-and-Yellow Macaw	Great grebe	Buff-necked ibis	
Red-and-green Macaw	White-lined broad-nosed Bat	Emu	
Masked booby	Pygmy Killer-whale	Pheasant	
Brown booby	Vinaceous-breasted Amazon	Silver pheasant	
Eared dove	Blue-fronted Amazon	Hens	
Common Pauraque	Sparrow	Common moorhen	
Southern Right Whale	Audubon's Shearwater	Helmeted guinea fowl	
Semipalmated plover	Great shearwater	Domestic grey goose	
Collared Plover	Sooty Shearwater	Teal	
American Golden Plover	White-chinned petrel	Blue-fronted Amazon	
Great kiskadee	Passeriformes	Passeriformes	
Yellow-fronted Woodpecker	Duck	Duck	
Neotropic cormorant	Muscovy duck	Muscovy duck	
Anhinga	Common Peacock	Common Peacock	
Manx shearwater	Budgerigar	Partridge	
Amazon river dolphin	Budgerigar	Peach-fronted Parakeet	
Ferruginous pygmy owl	Rose-ringed Parakeet	Turkey	
Canine	Peach-fronted Parakeet	Pigeon	
Crab-eating fox	White-backed stilt	Southern lapwing	
Scopoli's shearwater	Turkey		
Cory's shearwater	Southern giant Petrel		
Cockatiel	Campo flicker		
Canary	Magellanic Penguin		
Coscoroba swan	American Oystercatcher		
White-faced ibis	Picazuro pigeon		
Caracara	Pigeon		
Yellow-headed caracara	Psittacidae		
Monk parakeet	Southern lapwing		
Red-browed Amazon	American kestrel		
Black swan	Red-billed tropicbird		
Roseate Spoonbill	Scaled dove		
Green Ibis	Ruddy ground dove		

Burrowing Owl	Rufous-bellied thrush
Barn owl	Creamy-bellied thrush
Striped owl	Black-tufted marmoset
Black-banded owl	Little wood rail
Tropical screech owl	Crab-eating fox
Buff-necked ibis	Mangrove rail
Emu	Rufescent tiger heron
Antarctic prion	Black-crowned night heron
Pheasant	Tropical kingbird
Silver pheasant	Black skimmer
Peregrine falcon	Bare-faced ibis
Magnificent Frigatebird	Green-winged saltator
American purple gallinule	Common tern
Gray-hooded gull	Cabot's tern
Olog's gull	Gull-billed tern
Kelp gull	South American tern
Hens	Brown noddy
Common moorhen	Large-billed tern
Domestic grey goose	Yellow-billed tern
Great egret	Royal tern
Snowy egret	Red-breasted toucan
Cocoi heron	Toco toucan
Cattle egret	Gray river dolphin (Tucuxi)
Rufous-thighed kite	Jabiru stork
Roadside Hawk	American black vulture
Great black hawk	
Pearl kite	
Atlantic petrel	
Soft-plumaged petrel	
White-faced whistling duck	
Wattled Jacana	
Rusty-margined guan	
Rufous-capped motmot	
South American sea lion	
South American fur seal	
Neotropical river otter	
Sanderling	

*A single investigation may encompass more than one species.

Table 2. List of mandatory questions on the Epicollect5 Form

Geographical identification data	Poultry production data	Geolocation	Information on samples taken
State	Selected holding or alternate	Latitude	The ID number of the sampled production center
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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