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TECHNICAL REPORT

The Integration of Epidemiological Surveillance and Genomic Surveillance for Highly Pathogenic Avian Influenza (HPAI) in Brazil



Ministry of Agriculture and Livestock

Secretariat of Animal and Plant Health

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The Integration of Epidemiological Surveillance and Genomic Surveillance for Highly Pathogenic Avian Influenza (HPAI) in Brazil

MISSION OF MAPA:

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

Brasilia

MAPA

2026

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GLOSSARY

Bootstrap value	A statistical measurement used in order to evaluate the support for groupings observed in a phylogenetic tree. Higher values indicate greater support for a given grouping.
Bridging host	A species able to establish an epidemiological connection between populations or environments that normally have only limited contact, favoring opportunities to transmit the agent.
Clade	A genetically-related group of viruses sharing a common ancestor. In the present report, the H5N1 viruses that were detected belong to clade 2.3.4.4b.
Complete genome	Complete set of genetic material of an organism or virus. For the influenza A virus it corresponds to the eight segments of viral RNA.
Ecological Interface	A situation or environment in which different species or populations share space, resources or other conditions that enable direct or indirect exposure to the infectious agent.
Epidemiological intelligence	A process of integrating, analyzing and interpreting distinct data sources to produce information able to help the OVS carry out risk assessment and decision making.
Genetic distance	A measure of the differences between genetic sequences, used in order to assess the degree of similarity or divergence of the viruses.
Genetic reassortment	A characteristic process of segmented genome viruses, such as influenza A, in which genomic segments from distinct viruses can be combined when they simultaneously infect the same host, resulting in a new genomic constellation.
Genetic similarity	Degree of similarity between the genetic sequences of two or more viruses. A high level of similarity may support epidemiological hypotheses but alone does not demonstrate direct transmission.

Genomic sequencing	The process of determining the sequence of a virus's genetic material, enabling genome segments to be characterized and comparative, phylogenetic, and mutation analyses to be performed.
Genotype	A classification based on the combination of genomic segments making up a given influenza virus. Viruses belonging to the same clade may present distinct genotypes.
Molecular marker	An identifiable genetic change or trait that may be associated with a given biological characteristic, such as adaptation to hosts, pathogenicity, or sensitivity to antivirals.
Mutation	A change in the genetic sequence of the virus which may or may not lead to a change in its biological characteristics.
NGS	Next-generation sequencing: a set of technologies that enable a large number of genetic sequences to be determined simultaneously and, among other applications, obtain the complete genome of the virus.
Phylogenetic analysis	Analysis of evolutionary relations among genetic sequences, used in order to assess the degree of proximity between viruses and identify genetically related groupings.
Phylogenetic tree	A graphic representation of evolutionary relationships among genetic sequences, allowing clusters and ancestries shared by the viruses under analysis to be visualized.
Spillover	Event of transmission of an infectious agent from one host species or population to another species.
Spillover transmission	The process whereby after an infectious agent has been introduced into a given region or population, it circulates more widely among different species or communities that share environments and resources, creating more opportunities for transmission and exposure.
Viral lineage	A genetically-related group of viruses sharing a common evolution history.

LIST OF ACRONYMS

Acronym	Meaning
AGID	Agar Gel Immunodiffusion
AI	Avian Influenza
CVP	Permanent Veterinary Committee of the Southern Cone
DSA	Department of Animal Health
FAO	Food and Agriculture Organization of the United Nations
HPAI	Highly pathogenic avian influenza (HPAI)
LFDA	Federal Animal and Plant Health Laboratories
MAPA	Ministry of Agriculture and Livestock
MMA	Ministry of the Environment and Climate Change
OFFLU	FAO/OMSA Joint Network of Animal Influenza Experts
OVS	Official Veterinary Service
PANAFTOSA	Pan-American Center for Foot and Mouth Disease and Veterinary Public Health
PNSA	National Poultry Health Program
RT-PCR	Real-Time Reverse Transcription Polymerase Chain Reaction
SDA	Secretariat of Animal and Plant Health
SVS	State Veterinary Service (SVE - Serviço Veterinário Estadual)
WOAH	World Organisation for Animal Health

INTRODUCTION

Highly pathogenic avian influenza (HPAI) is one of the main health threats to global poultry production, requiring surveillance systems capable of early detection of viral circulation, of understanding its epidemiological dynamics, and of providing rapid, evidence-based responses.

Since 2020, given the intensification of the global circulation of highly pathogenic avian influenza viruses (HPAI), driven by the dissemination of the H5 virus of clade 2.3.4.4b, and the resulting increased infection pressure on wild bird populations, the Ministry of Agriculture and Livestock (MAPA) has strengthened epidemiological surveillance, laboratories' diagnostic capacity, the investigation of suspected cases and outbreaks, and Brazil's preparedness for animal health emergencies.

At the same time, the incorporation of genomics into the surveillance of avian influenza viruses has enhanced understanding of the origin, evolution, and genetic diversity of circulating viruses. Beyond characterizing the viruses genetically, the Brazilian experience has demonstrated the potential of integrating epidemiology and genomics so as to support risk assessment, guide risk-based surveillance strategies, bolster preparedness for health emergencies, and act as an important tool to aid decision-making by the Official Veterinary Service (OVS).

The present report consolidates the Brazilian experience and provides the key official surveillance findings resulting from the integration of epidemiological and genomic data from May 2023 to July 2026. It presents the results of this approach, its applications, and the potential gains from the understanding of the dynamics of HPAI in order to strengthen OVS actions. Additionally, it discusses prospects for enhancing surveillance in line with One Health principles.

Furthermore, the data presented here underscore MAPA's commitment to innovation, to technical excellence, and to the continuous strengthening of the OVS. It is expected that the consolidated information will contribute not only to the enhancement of national actions but also to inter-institutional exchanges of knowledge and the consolidation of international cooperation in combating avian influenza and other cross-border animal diseases.

CHAPTER 1

The avian influenza surveillance system

1.1. Introduction

Brazil has one of the largest poultry production systems in the world, and ranks very high in the production and export of chicken meat and poultry products. Maintaining the sanitary status of domestic poultry farming depends on a surveillance system capable of the early detection of economically significant diseases and public health concerns, implementing timely response measures, and continuously demonstrating the non-circulation in commercial production of reportable pathogens.

Among these diseases, HPAI poses one of the main challenges for the OVS owing to its high potential for transmission, the broad spectrum of susceptible hosts, and the sanitary, economic, environmental, and trade impacts resulting whenever it occurs.

Beyond protecting poultry farming, HPAI surveillance is strategically important for public health, the preservation of biodiversity, environmental protection, and maintaining international trade. In this context, avian influenza surveillance is one of the major domestic instruments for prevention, preparedness, and response, and is based on the integration of epidemiological, laboratory, genomic, and ecological surveillance.

Within the Department of Animal Health (DSA), the National Poultry Health Program (PNSA) is structured around complementary strategies of risk-based surveillance, laboratory diagnosis, epidemiological investigation, biosafety implementation, and rapid response to zoonotic emergencies. Coordinated action involving MAPA, State-level Veterinary Services (SVSs), the official laboratory network, and the poultry-production industry, all form one of the pillars for maintaining the sanitary status of Brazilian poultry farming and preserving the country's credibility in international markets.

The topics below present the organization of the avian influenza surveillance system, encompassing its institutional structure, passive and active surveillance strategies, the official laboratory network, and the incorporation of genomics into surveillance actions. The integrated operation of these components strengthens the capacity of the OVS to detect viral circulation early, monitor the epidemiological situation, investigate health events, characterize circulating viruses, and produce evidence to support risk assessment, decision-making, and the formulation of public policies aimed at prevention, preparedness, and response to zoosanitary emergencies.

1.2 Brazil's National Poultry Health Program

Set up in 1994, the PNSA is one of Brazil's most important instruments for domestic animal health policy, laying down guidelines for the prevention, surveillance, control, and response to the main diseases affecting Brazilian poultry farming.

Since its inception, the program has been continuously improved, consolidating an integrated structure of epidemiological surveillance, health monitoring, laboratory diagnosis, health certification, biosafety measures, and preparedness for zoosanitary emergencies. Along the way, this has strengthened the capacity of the OVS to prevent the introduction and spread of officially-controlled diseases, preserve the value of domestic poultry raising, and ensure Brazil's animal health credibility in international markets.

Key objectives include:

- To prevent and control diseases of concern to poultry-raising and public health;
- To define actions to enable the sanitary certification of Brazil's poultry flock;
- To favor the preparation of healthy poultry products to be offered to the domestic and external markets.

The most significant notifiable diseases controlled by the PNSA are:

- Avian influenza – HPAI-free country, only one outbreak in commercial poultry in May 2025;
- Newcastle Disease - ND-free country, only one outbreak in commercial poultry in June 2024;
- Salmonellas – *Salmonella gallinarum, Salmonella pullorum, Salmonella enteritidis, Salmonella typhimurium* and monophasic Salmonellas;
- Mycoplasma – *Mycoplasma gallisepticum, Mycoplasma* and *Mycoplasma melleagridis* (in turkeys).

The activities of the PNSA involve integrated efforts between MAPA, responsible for coordinating national policies and technical guidelines, and the SVSs, responsible for carrying out surveillance activities, responding to suspected cases, conducting epidemiological investigations, taking samples, implementing animal health measures, and performing inspection in the field.

1.3 The organization of the National Surveillance System

Avian influenza surveillance is organized in a decentralized and integrated manner, coordinating actions involving MAPA and the SVSs across the different tiers of jurisdiction of execution by the OVS.

At the federal level, it is the responsibility of the Department of Animal Health (DSA) to technically coordinate national actions, create standards and guidelines, carry out risk

assessments, coordinate responses to health emergencies, and represent the country before international organizations.

The Offices of the Federal Superintendents of Agriculture and Livestock provide regional coordination by monitoring activities executed by States and facilitating communication between the central level and the SVSs

The SVSs form the operational structure of the system and are tasked with carrying such field-level actions as:

- passive surveillance;
- active surveillance;
- epidemiological investigation;
- sample-taking;
- inspection;
- the execution of sanitary measures;
- responding to notifications;
- animal health education.

The integration between these components enables the rapid detection of animal health events and the coordinated implementation of response measures.

1.4 Brazil's National Avian Influenza Surveillance Plan

In 2022 DSA, as part of the structure of MAPA, conducted a technical and epidemiological review of national surveillance strategies for avian influenza and Newcastle Disease (ND). As a result, the new National Surveillance Plan was set up in order to enhance the capacity for early detection, continuous monitoring, prevention of the introduction of diseases, and the response to the spread of these diseases in Brazil.

The National Plan is organized into distinct surveillance components, covering commercial domestic birds, backyard poultry, wild birds, and certified poultry compartments, allowing targeted surveillance strategies to adjust to the epidemiological risk of each target population.

The surveillance of avian influenza is founded upon the integration of passive and active surveillance, laboratory diagnosis, genomic surveillance, and risk assessment. This approach enables a complementary analysis of information from different sources, broadening the understanding of the disease's epidemiological dynamics and informing evidence-based decision-making.

1.4.1 Passive surveillance

Passive surveillance is the principal mechanism for the early detection of HPAI in Brazil and comprises **Component 1 – Surveillance in domestic birds** and **Component 2 – Surveillance in wild birds** of the National Avian Influenza and ND Surveillance Plan.

Passive surveillance is an event-based surveillance strategy, focusing on identifying and investigating unusual health events, such as raised mortality, clinical changes, or other occurrences compatible with avian influenza.

This strategy relies on the immediate declaration of clinically or epidemiologically suspect cases by growers, veterinarians, environmental agencies, research institutions, laboratories, and other players in the production chain and in wildlife surveillance.

Every suspected case is investigated by the OVS, following standardized procedures that include epidemiological investigation, clinical inspection, sample-taking, referral to the official laboratory, and the implementation of necessary sanitary measures.

Covering as it does both domestic and wild birds, passive surveillance is the component with the greatest degree of sensitivity for identifying unexpected health events, above all those associated with increased mortality or the occurrence of clinical signs compatible with Avian Respiratory and Neurological Syndrome (ARNS). Taking into consideration the syndromic approach adopted, notifications meeting the criteria for suspected cases are investigated for avian influenza and ND, with simultaneous laboratory diagnosis for both diseases, enabling them to be detected or ruled out by the same investigation.

Procedures for responding to suspected cases have been standardized and are given in the **Technical specifications on Avian Influenza**, available at https://sisbravet.agro.gov.br/ficha-tecnica/publica/consultar.

In order to promote transparency and the dissemination of information obtained from passive surveillance, MAPA has set up the **Avian Influenza and Newcastle Disease Surveillance Panel** **https://mapa-indicadores.agricultura.gov.br/publico/extensions/SRN/SRN.html**, which gathers and presents updated data on investigations and confirmed outbreaks of these diseases in Brazil, enabling the temporal and geographical distribution of the diseases to be tracked as well as the populations and species affected. The information available on this panel is an official public consultation source concerning the occurrence of avian influenza in Brazil and was used as one of the data sources to underpin the epidemiological and genomic analyses contained in the present report.

1.4.2 Active surveillance

Active surveillance complements passive surveillance by systematically sampling populations defined in advance, enabling continuous monitoring of viral circulation even in the absence of clinical signs.

In Brazil, actions include the animal health monitoring of small-scale commercial and industrial poultry establishments, backyard flocks, and certified poultry compartments, using epidemiological criteria to select properties and sampling units.

By adopting risk-based surveillance methodologies, efforts can be targeted towards geographic areas, production systems, and populations that are more at risk of exposure to the virus, increasing operational efficiency and the sensitivity of the system, thus enabling early detection.

From 2022 to 2026, surveillance resulted in over 320,000 laboratory tests for HPAI and ND, demonstrating the robustness of official surveillance and its ability continuously to monitor the sanitary situation of Brazilian poultry farming.

1.5 Official Laboratory Network

The official laboratory network for diagnosing avian influenza is formed of Federal Animal and Plant Health Laboratories (LFDAs) and MAPA-accredited laboratories which work jointly to support surveillance, monitoring, and responses to zoosanitary emergencies.

LFDAs carry out official diagnostic testing, confirmation, and laboratory-based characterization of etiological agents, including molecular assays, viral isolation, antigenic and genetic characterization, as well as the genomic sequencing of avian influenza viruses within the scope of passive and active surveillance. In turn, the accredited laboratories only conduct screening tests within the active surveillance framework (confirmatory tests are performed in the LFDAs), thereby playing a vital role in the certification of poultry compartments and compliance with sanitary requirements for export.

The São Paulo State LFDA (LFDA-SP) is a Reference Center for the Food and Agriculture Organization of the United Nations (FAO) and is also a Reference Laboratory for avian influenza for the World Organisation for Animal Health (WOAH). LFDA-SP therefore plays a strategic role in surveillance, since it performs diagnostic confirmation, the characterization of circulating viruses, genomic sequencing, and provides technical/scientific support for epidemiological investigations.

Within the scope of genomic surveillance, the Goiás State LFDA (LFDA-GO) also participated during the first year of the health emergency in the execution of stages of the genomic sequencing protocol in liaison with the official laboratory network. With the expansion and consolidation of their installed capacity, the Federal Animal and Plant Health Laboratories LFDA-SP and, in Minas Gerais, LFDA-MG currently carry out the complete viral

genome sequencing process with bioinformatics, enabling complete genomes to be obtained and the molecular characterization of detected viruses.

The expansion of the capacity for diagnosis and genomic analysis that has taken place within the official laboratory network in recent years has increased the generation of strategic information for the Official Veterinary Service (OVS), contributing to epidemiological investigation, the characterization of viruses that have been introduced, an understanding of the circulation and dispersion patterns of the virus, and risk assessments.

With the incorporation and advance of diagnostic and molecular characterization tools within the official routine, the complete sequencing of the viral genome has consolidated itself as a supplementary tool for epidemiological investigation. The structure of genomic surveillance and its applications within the national avian influenza surveillance framework are presented in detail in Chapter 3.

1.6 The integration of surveillance

Although described separately in the present document for didactic purposes, passive and active surveillance of avian influenza are components that work together to boost early detection. The detection of suspected cases in the field, epidemiological investigation, laboratory diagnosis, genomic characterization, and risk assessment are complementary stages of a single process of generating epidemiological intelligence. This integration boosts the OVS's capacity to understand the dynamics of the disease, mitigate uncertainties in investigations, and support technical and strategic decision-making in order to enable prevention, preparedness, and response to zoonotic emergencies.

CHAPTER 2

Genomic Surveillance of Avian Influenza in Brazil: Implementation and Applications

2.1. Introduction

The confirmation of the first outbreaks of HPAI in Brazil in May 2023 was a landmark for animal health surveillance and demonstrated the need to enhance the OVS's capability to understand the epidemiological dynamics of the virus introduced in the country. Although traditional epidemiological investigation can and does provide essential information about the occurrence of outbreaks, the species affected, geographical locations, and potential risk factors, there remain several questions that can only be answered through genomic surveillance: what were the origins of the viruses, the events that led to their introduction, and the genetic evolution of the viruses.

Unlike the use of genetic sequencing solely for molecular characterization purposes, the strategy adopted by MAPA was based on the integration of genomics and epidemiology, aiming to incorporate laboratory findings into the interpretation of sanitary events and expand its understanding of the possible origins, epidemiological relationships, and dynamics of the dispersion of viruses detected in the country.

2.2 Justification for Implementing Genomic Surveillance

The current H5N1 virus clade 2.3.4.4b panzootic is characterized by high genetic diversity, multiple independent introduction events across continents, and frequent genetic reassortment processes, making the exclusive use of traditional epidemiological information insufficient for understanding the dynamics of its circulation.

The epidemiological complexity of the disease owing to the wide dispersion of the virus among migratory birds, the virus's ability to adapt to different host species, and the occurrence of events in wild and domestic mammals, all necessitate using genomic and bioinformatics tools to pursue such strategic questions for the OVS as:

- Are the detected viruses related to the same introduction event or do they represent independent events?
- Is there evidence of secondary dissemination among establishments or regions?
- What is the probable origin of the detected viruses?
- How do these viruses evolve over time?

- Are there new lineages circulating in South America?
- Are the detected viruses associated with different host species?
- How can this information support risk assessments and guide surveillance?

Genomic surveillance has been incorporated specifically to complement these answers, providing additional evidence to epidemiological investigations.

Chart 1. Applications of genomic surveillance to support epidemiological investigation and decision-making by the Official Veterinary Service.

Epidemiological question	How Genomic Surveillance contributes	How it applies to the Official Veterinary Service
Where did the virus come from?	Phylogenetic analyses and comparisons with domestic and international sequences allow identification of the probable origin of the virus and its relationship with circulating lineages in other countries.	Supports the identification of possible introduction pathways and informs risk assessments.
Has the virus undergone significant mutations?	Comparisons among complete genomes enable the identification of mutations, deletions, insertions, and other evolutionary events.	Monitors viral evolution, identifies changes that might impact adaptation to new hosts, pathogenicity, transmission, and sensitivity to antivirals, supporting risk assessment in the context of One Health.
Does the outbreak represent a new introduction or is it related to another outbreak?	Comparison of genotypes, phylogenetic trees, and genetic distance among viruses.	Helps differentiate between independent introductions and possible secondary dissemination events.
May there have been a spread between establishments?	High genetic similarity among viruses, interpreted alongside epidemiological, temporal, and spatial information.	Complements epidemiological investigation and strengthens conclusions about possible transmission chains.
How is the virus evolving over time?	Complete genome sequencing, the analysis of variants and phylogenetics, and the continuous monitoring of circulating lineages.	Enables the tracking of evolutionary changes and updates risk assessments.
Are new lineages or genotypes circulating?	Continuous genomic monitoring and comparison with international databases.	Detects new introductions early on and informs surveillance and preparedness strategies.
Are different outbreaks epidemiologically related?	Integration of genetic distance, phylogenetic positioning, event chronology, and geographical location.	Supports the reconstruction of the dynamics of dissemination and the interpretation of investigations in the field.

Do the surveillance strategies need adjustment?	The integration of genomic, epidemiological, and ecological information allows surveillance to adopt a risk-based approach, along spatial dissemination routes, and viral circulation patterns.	Guides risk-based surveillance, area selection, and, if necessary, the prioritization of species.

2.3 The objectives of Genomic Surveillance

Genomic surveillance had the following objectives:

- to complement epidemiological surveillance through virus characterization and to reconstruct the dynamics of dissemination, in order to guide a response to the event;
- genetically to characterize the viruses detected in Brazil;
- to identify the possible origins and pathways whereby the virus was introduced;
- to assess the relationship between different outbreaks and infection events;
- to monitor the genetic evolution of circulating lineages as well as the introduction of new viral variants;
- to identify genetic changes associated with adaptation to different hosts;
- to detect genetic reassortment events and the emergence of new genotypes;
- to integrate genomic information with epidemiological investigations;
- to support risk assessments;
- to strengthen evidence-based surveillance integrated into a One Health perspective.

2.4 Operational Flow of Genomic Surveillance

Genomic surveillance has been structured as an extension of the epidemiological investigation process. Thus, in the operational flow, after a laboratory confirmation of infection with the HPAI virus, samples that meet quality criteria undergo complete viral genome sequencing.

The data generated from sequencing are processed through bioinformatics analyses, such as genome assembly, identification of viral segments, determination of genotype, construction of phylogenetic analyses, and comparison with sequences available in international databases.

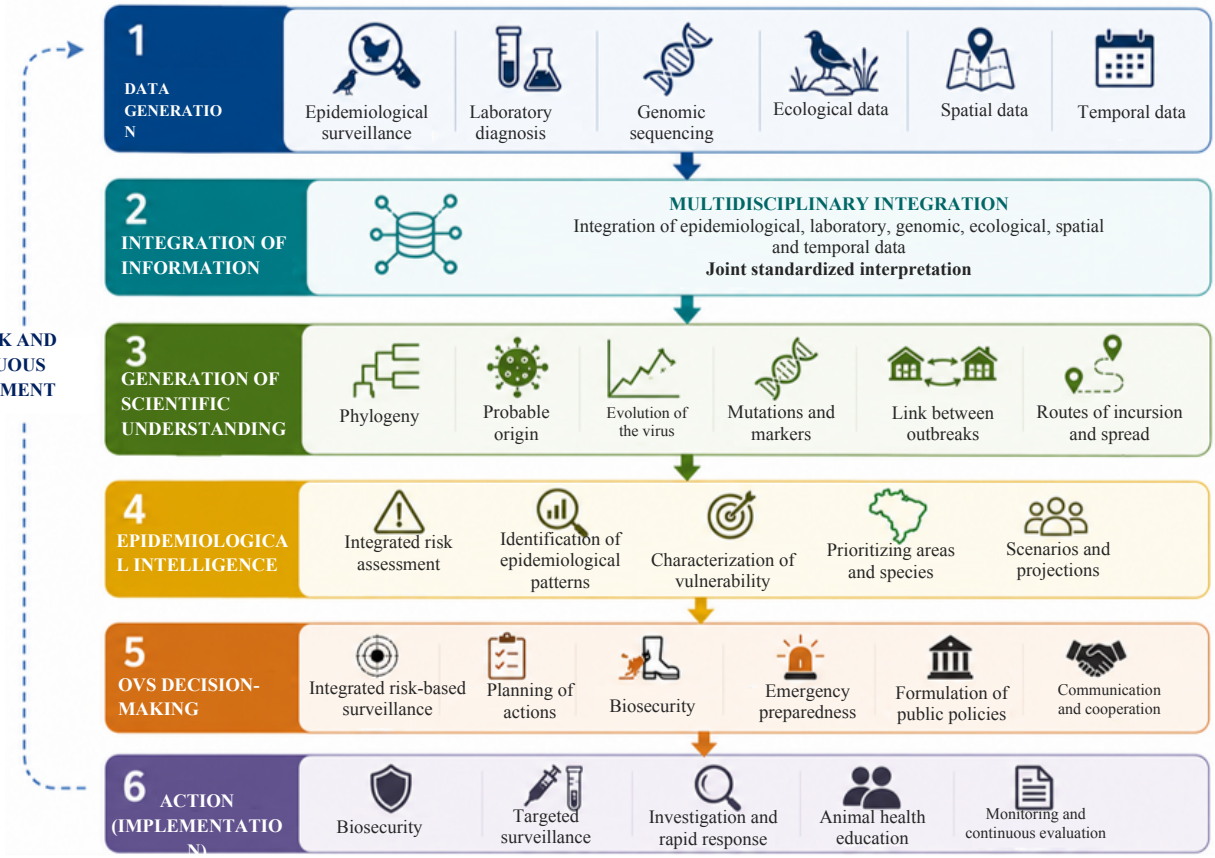
After this, genomic results are integrated into the epidemiological information, including:

- host species;
- geographical location-finding;
- date of detection;
- characteristics of the outbreak;

- any risk factors identified;
- epidemiological history.

Integration enables broader interpretation of the dynamics behind the introduction, circulation and evolution of the virus.

Figure 1. Conceptual model of epidemiological surveillance and genomic surveillance integration



CHAPTER 3

The epidemiological surveillance of Avian Influenza in Brazil: Results

3.1. Introduction

The confirmation of the first outbreaks of Highly Pathogenic Avian Influenza (HPAI) in Brazil was the beginning of a new phase for the OVS, marked by the need for continuous monitoring of viral circulation in a vast territory that is home to one of the largest poultry populations in the world. Since then, surveillance has consolidated a wide array of information

from notifications and investigations conducted in both wild and domestic birds. These data have enabled the temporal and spatial evolution of the disease to be tracked, changes in the profile of affected species and environments to be identified, and the ecological and epidemiological dynamics of the virus in Brazil to be better understood.

Throughout the period analyzed, it was observed that the epidemiological pattern of HPAI in Brazil did not remain static. The initial phase of the emergency was predominantly characterized by the occurrence of outbreaks among migratory aquatic birds and other wild birds associated with coastal environments. Then there was an expansion in the distribution of occurrences, with increased detections in non-marine birds, rural areas, and domestic birds, resulting in an increasingly complex epidemiological scenario.

3.2 The introduction of the virus into the country

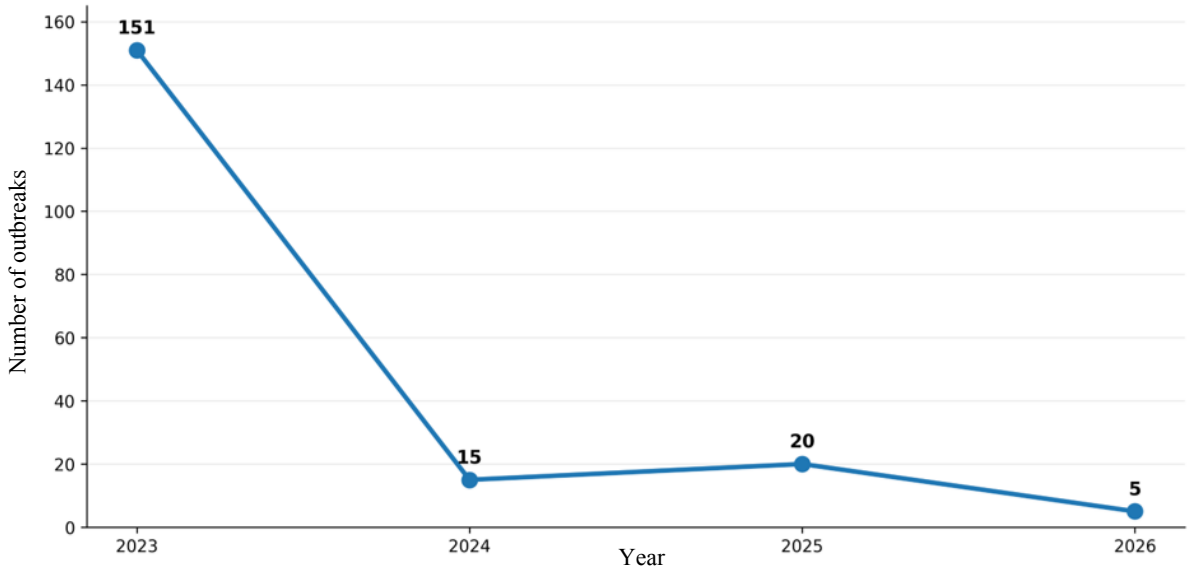
HPAI was first confirmed in Brazil on May 15, 2023, marking the introduction of highly pathogenic influenza A(H5N1) virus into the country. Until that moment the disease had been deemed to be exotic to Brazil.

The first detections occurred in two individuals of the species *Thalasseus acuflavidus* (Royal Tern) and one individual of the species *Sula leucogaster* (Brown Booby), found near the coast of the State of Espírito Santo. These records were consistent with the pattern observed during the spread of H5N1 virus through South America, associated above all with the movement of wild birds and viral circulation in natural and coastal environments.

3.3 Chronology of the Outbreaks

The annual distribution of outbreaks demonstrates that the greatest epidemiological impact occurred during the first year after the detection of the virus in the country, as presented in Figure 2.

Figure 2. Annual development in the number of new outbreaks of highly pathogenic avian influenza in Brazil



*Partial data for 2026

In 2023, 151 outbreaks of HPAI were confirmed. Following 2023, there were 15 outbreaks in 2024; 20 in 2025; and 5 from January to July 2026.

These data show a significant reduction in the annual number of new outbreaks compared to the initial period of the health emergency. The reduction, however, does not eliminate the risk of new introductions or viral dissemination, given its spread in wild bird populations, its wide geographical distribution globally, and the possibility of recurrent exposure of domestic birds.

3.4 The epidemiological situation during the period under analysis

From May 15, 2023, to the end of July 2026*, 191 HPAI outbreaks were confirmed in Brazil, spread across 14 States and 91 municipalities.

The outbreaks involved 35 distinct species: free-living wild birds, captive wild birds, domestic backyard flocks, commercially farmed poultry, ornamental birds, and wild mammals. This diversity underscores the great variety of susceptible hosts and epidemiological contexts in which exposure to the virus occurred during the period under analysis.

In this period, there were 4,346 investigations of suspected cases of avian respiratory and neurological syndrome (ARNS), of which 1,333 were classified as probable cases, which underwent the epidemiological investigation and laboratory diagnosis procedures outlined by the National Surveillance System.

The majority of outbreaks occurred in free-living wild birds, accounting for 172 records, or 90.1% of the total. This reflects the central role of these populations in the introduction, spread, and maintenance of viral circulation in natural environments.

Albeit less frequent in backyard flocks, 16 outbreaks were recorded. Domestic systems present a greater potential for contact with wild birds due to frequent access to the external environment and the possibility of sharing water, food, and other resources — an important interface to be taken into account when assessing possible pathways for the introduction of the virus.

On May 15, 2025, the first outbreak of HPAI was confirmed among commercial production birds in a breeding establishment located in Montenegro, Rio Grande do Sul. The episode was deemed to be a sporadic case and immediately underwent the containment and eradication measures outlined in the National Contingency Plan, including blocking of the farm, the culling of birds, clean-up and disinfection, surveillance in established areas, and investigation of possible epidemiological links. After the sanitary measures were concluded, the absence of new outbreaks in commercial birds and compliance with applicable requirements allowed Brazil to recover its status as a country free from HPAI in commercial production birds, through a self-declaration submitted to the World Organization for Animal Health (WOAH), on June 18, 2025.

Together, these results demonstrate that the epidemiology of HPAI in Brazil evolved over the period under analysis. After an initial phase characterized by a high occurrence of outbreaks linked to the introduction of the virus in wild birds, there was a reduction in the annual number of new outbreaks and a gradual change in the epidemiological profile of the events detected. Understanding this dynamic requires the integration of temporal, spatial, ecological, and genomic analyses, presented in the subsequent sections, which enable patterns of introduction, dispersion, and viral evolution to be identified.

*reference date: July 27, 2026

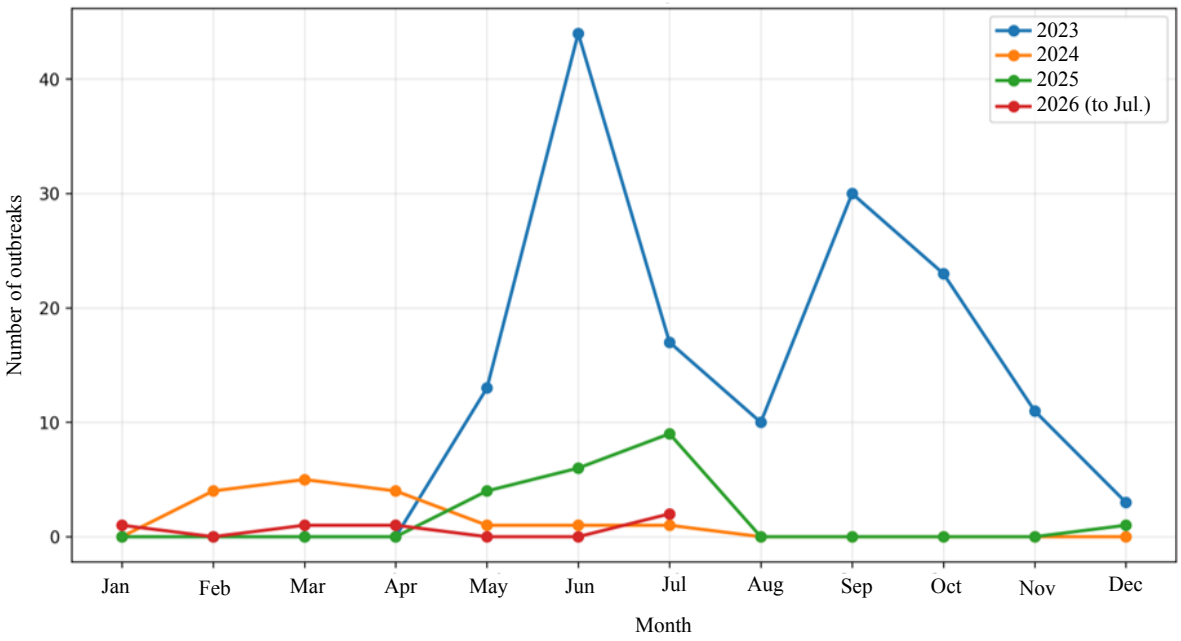
3.5 Changes over Time

The temporal distribution of outbreaks shows that the introduction of HPAI in Brazil occurred during a period consistent with the arrival of migratory birds along the main migratory routes of South America, corroborating signs that these species played a fundamental role in introducing the virus to the continent. However, as noted by WOAH and OFFLU, the occurrence of outbreaks in subsequent years shows that the epidemiology of the disease cannot be explained solely by long-distance migratory movements. While the temporal distribution of outbreaks continues to be associated with periods of more intensive migratory bird movements, reports were observed at different times of the year, indicating that the risk of the occurrence of the disease results from the interaction of several ecological and epidemiological factors.

This scenario reinforces the need to maintain continuous and sensitive epidemiological surveillance systems that can detect alterations in the pattern of occurrence over time of the disease in a timely manner and identify changes in the epidemiological profile of outbreaks in order to support regularly updated risk assessments.

Figure 3 presents the monthly distribution of confirmed HPAI outbreaks in Brazil from May 2023 to July 2026.

Figure 3 - Monthly distribution of highly pathogenic avian influenza outbreaks in Brazil



In the first year of the animal health emergency (2023), a significant increase in the number of outbreaks was observed between June and October, corresponding to the introduction of the virus into the country and the occurrence of the first records in wild birds, predominantly along Brazil's coastline.

In 2024, there was a sharp reduction in the number of outbreaks, with reports concentrated between February and July, indicating an epidemiological scenario distinct from that observed during the initial phase of the introduction of the virus.

In 2025, there was another increase in the number of outbreaks between May and July, during which the first outbreak in commercial production birds in the country was confirmed, along with records in subsistence domestic birds, wild birds, and wild mammals.

Up to July 2026, the outbreaks exhibited a temporal distribution compatible with the period of heightened migratory bird movement, demonstrating that the risk of new introductions remains higher during this period.

Overall, the data highlight the strong influence of a seasonal pattern driven by the movements of wild migratory birds, without excluding the contribution of other ecological and epidemiological factors to the occurrence of registered outbreaks. These results reinforce the importance of maintaining surveillance activities throughout the year, intensifying actions during periods of higher epidemiological risk.

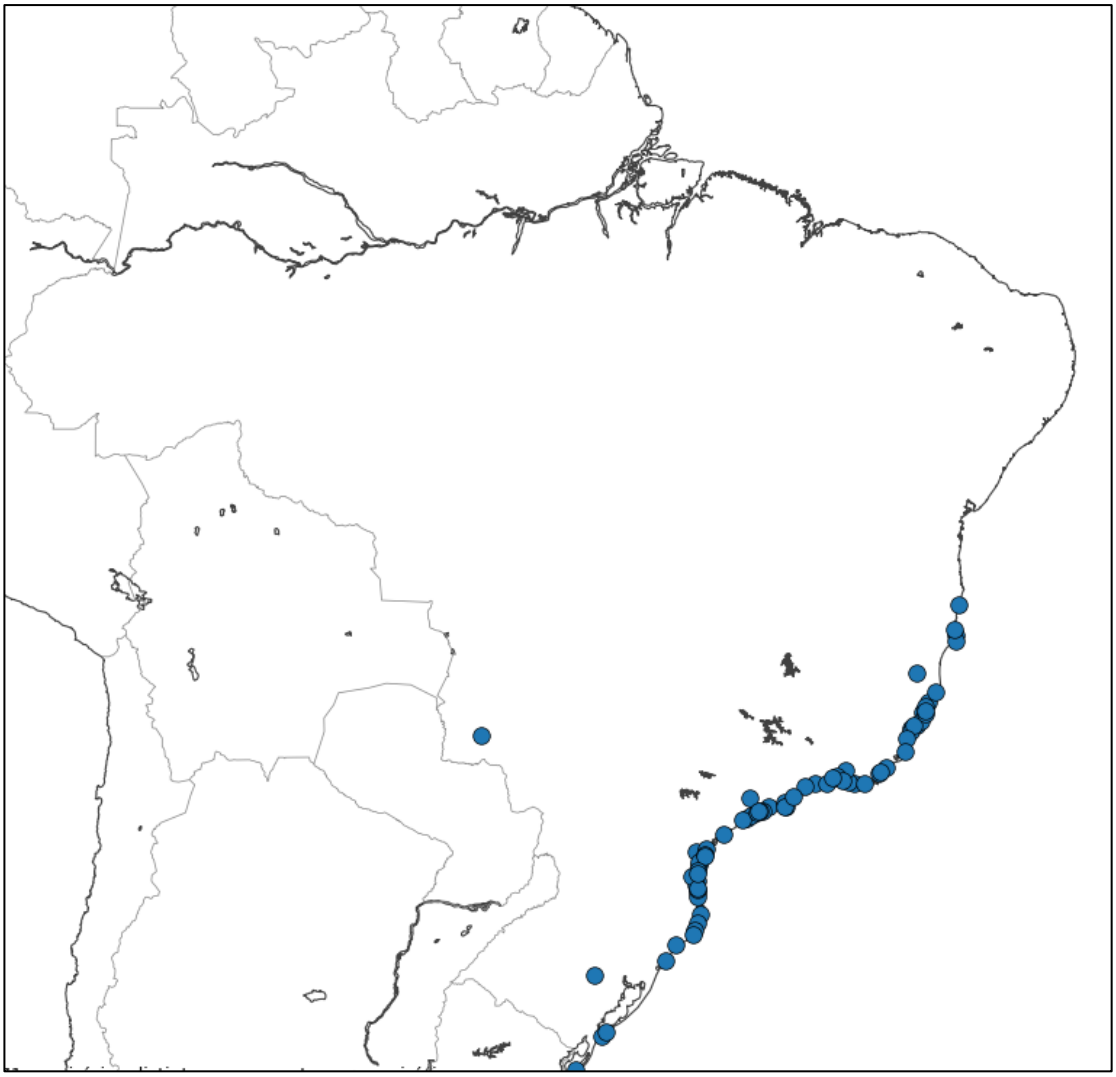
3.6 Geographical distribution

The geographical distribution of HPAI outbreaks in Brazil demonstrates a gradual change in the spatial pattern observed since the virus's introduction into the country. From May 2023 to July 2026, outbreaks were confirmed in 14 States and 91 municipalities, covering several distinct biomes, ecosystems, and production systems. The spatial analysis shows that outbreaks occurred in a range of epidemiological contexts over the period, reflecting the diversity of susceptible species, ecological environments, and interfaces between wild and domestic birds.

The first outbreaks were mainly reported in along the coastal areas of the Southeast and South regions, involving marine and coastal birds, particularly terns (*Thalasseus* spp. and *Sterna* spp.), boobies (*Sula leucogaster*), and other species associated with coastal environments. The concentration of the first outbreaks of HPAI in Brazil's coastal region coincides with areas recognized as important for migratory birds, routinely used for routes, resting, feeding, and breeding. These areas concentrate individuals from different migratory populations and favor interactions between migratory and resident birds, increasing opportunities for exposure to the virus and its spread along migratory routes.

The geographical distribution of the outbreaks reported in 2023 and 2024 showed a marked concentration along Brazil's coastline (Figure 4) which is compatible with the predominance of findings in wild birds associated with coastal and environments in this period.

Figure 4 - Geographical distribution of HPAI outbreaks in 2023 - 2024



This matches the assessments of WOAH, FAO/OFFLU and the European Food Safety Authority (EFSA), all of whom acknowledge the role played by migratory birds in introducing the virus into new territories while highlighting that the observed spatial distribution after introduction begins to reflect a more complex ecological dynamic involving several wild species, natural environments and ecological interfaces and can therefore not be explained exclusively by long-distance migratory movements.

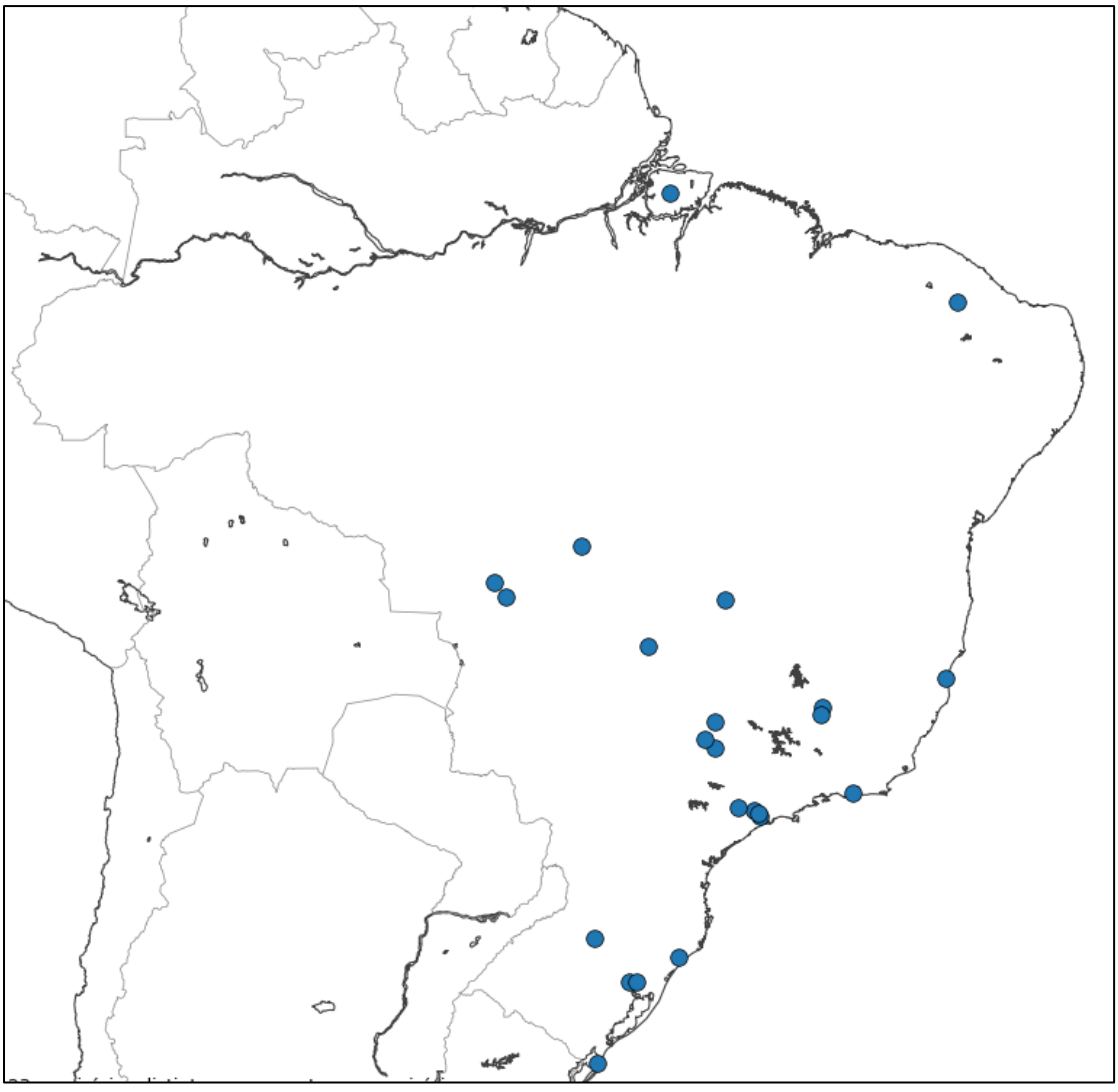
In 2023 there was a virtually constant distribution of outbreaks along the coastline of the Brazilian States of Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul and the south of Bahia. The intense concentration of reports in those areas coincides with significant areas for feeding, resting and breeding for aquatic and marine birds, as well as extensive coastal environments recognized internationally as locations at greater risk for the introduction of the avian influenza virus.

From an epidemiological standpoint, these environments may represent significant ecological interfaces for the introduction and spread of the virus because of the high density of birds, the sharing of feeding and resting places and the temporary presence of infected individuals, all favoring transmission among distinct wild species. The process may also

contribute to subsequent spillover events targeting resident bird species and possibly domestic birds when there are favorable epidemiological interfaces.

In subsequent years, above all in 2025, a greater geographical dispersion of detected cases of HPAI was seen, with records from more inland areas, including resident aquatic birds and backyard poultry. This pattern was also described in countries of Europe and in the Americas, where, after an initial incursion of the virus, a greater diversity of affected species and environments in which there was detection, were observed.

Figure 5 - Geographical distribution of HPAI outbreaks in 2025 - July 2026



Outbreaks in domestic backyard flocks showed a more widespread distribution, and were reported in several States in the Center-West, Southeast, South, Northeast and North regions, including the States of Mato Grosso, Mato Grosso do Sul, Goiás, Espírito Santo, Minas Gerais, São Paulo, Santa Catarina, Rio Grande do Sul, Ceará and Pará. This pattern shows that

there can be interfaces between wild birds and backyard flocks in a whole range of ecological and production contexts regardless of proximity to the coastline.

In contrast to this, throughout the period under analysis, only one outbreak was confirmed in a commercial poultry-raising establishment, in the State of Rio Grande do Sul, and was quickly controlled by the OVS. The fact that there was only one outbreak in commercial poultry-raising compared with a range of detected cases in wild birds, shows that viral circulation among wildlife does not necessarily lead to introduction of the disease into commercial systems, above all when effective biosecurity and prevention measures are kept up.

In general terms, the overall evolution of geographical distribution of outbreaks in Brazil shows a pattern compatible with what has been internationally described for HPAI: an initial incursion through wild birds associated with coastal environments, followed by the identification of outbreaks in a greater range of species and environments. This spatial expansion can be interpreted as the result of the diversity of susceptible hosts, the ecological characteristics of distinct environments, and the intensification of OVS surveillance.

These results underscore the importance of maintaining surveillance systems evenly distributed throughout a national territory, encompassing coastal areas and wetlands visited by migratory birds, as well as more inland environments where there is an interface between wildlife and domestic poultry, enabling early detection of any new introduction and the rapid execution of animal health response measures.

3.7 Distribution by species

The distribution by species of the outbreaks also shows a change in the epidemiological profile of HPAI in Brazil. In the period under analysis, 41 species of bird and 2 species of mammal affected by HPAI were identified. As mentioned above, in the first two years of the animal health emergency, more than 90% of detected cases occurred in aquatic birds, above all in migratory species associated with coastal environments, so that terns (*Thalasseus acuflavidus* and *Thalasseus maximus)*, predominated, concentrating the majority of confirmed report. Outbreaks were also identified in other marine and coastal birds: terns (*Sterna* spp.), boobies (*Sula leucogaster*), gulls, frigates and cormorants.

However, over time, occurrences revealed significant changes in the diversity of affected species. An increased number of infections were detected in resident aquatic birds, free-living land birds, backyard flocks and, eventually, commercial production flocks. Outbreaks were reported in such species as: White-faced whistling duck (*Dendrocygna viduata*); Black-bellied whistling duck (*Dendrocygna autumnalis*); Fulvous whistling duck (*Dendrocygna bicolor*); Rufous hornero (*Furnarius rufus*), herons, Crested caracara (C*aracara plancus*), hawks, domestic chickens (*Gallus gallus domesticus*), Guinea fowl (*Numida meleagris*), turkeys (*Meleagris gallopavo*), to name but a few, as well as marine mammals such as the South American sea lion (*Otaria flavescens*).

Table 1 shows the distribution of outbreaks by Order and species for the period under analysis.

Table 1: Distribution of highly pathogenic avian influenza by affected Order and species

Order	Species	Number of outbreaks
Accipitriformes	*Rupornis magnirostris* (Roadside Hawk)	1
Accipitriformes	*Urubitinga urubitinga* (Great-black hawk)	1
Anseriformes	*Dendrocygna viduata* (White-faced whistling duck)	4
Anseriformes	*Cygnus atratus* (Black swan)	2
Anseriformes	Anatidae (waterfowl)	1
Anseriformes	*Coscoroba coscoroba* (Coscoroba swan)	1
Anseriformes	*Cygnus melancoryphus* (Black-necked Swan)	1
Anseriformes	*Dendrocygna autumnalis* (Black-bellied whistling duck)	1
Anseriformes	*Dendrocygna bicolor* (Fulvous whistling duck)	1
Carnivora	*Otaria flavescens* (South American sea lion)	5
Charadriiformes	*Thalasseus acuflavidus* (Cabot's tern)	61
Charadriiformes	*Thalasseus maximus* (Royal tern)	51
Charadriiformes	*Sterna hirundo* (Common tern)	12
Charadriiformes	*Sterna hirundinacea* (South American tern)	8
Charadriiformes	*Chroicocephalus cirrocephalus* (Gray-hooded gull)	1
Charadriiformes	*Chroicocephalus maculipennis* (Brown-hooded gull)	1
Charadriiformes	*Pluvialis dominica* (American golden-plover)	1
Charadriiformes	*Vanellus chilensis* (Southern lapwing)	1
Falconiformes	*Carcara plancus* (Crested caracara)	1
Galliformes	*Meleagris gallopavo* (turkey)	1
	Numida meleagris (Guinea fowl)	1
	Gallus gallus (Domestic chicken)	15
Passeriformes	*Furnarius rufus* (Rufous hornero)	1
Pelecaniformes	*Ardea alba* (Great Egret)	1
Pelecaniformes	*Ardea cocoi* (Cocoi heron)	1
Pelecaniformes	*Egretta thula* (snowy egret)	1
Pelecaniformes	*Nycticorax nycticorax* (Black-crowned night heron)	1
Pelecaniformes	*Plegadis chihi* (White-faced ibis)	1
Procellariiformes	*Puffinus puffinus* (Manx shearwater)	4
Procellariiformes	*Procellaria aequinoctialis* (White-chinned petrel)	2
Procellariiformes	*Pachyptila desolata* (Antarctic prion)	1
Strigiformes	*Megascops choliba* (Tropical screech owl)	1
Suliformes	*Sula leucogaster* (Brown booby)	3
Suliformes	*Nannopterum brasilianum* (Neotropic Cormorant)	2
Suliformes	*Fregata magnificens* (Magnificent frigate bird)	1

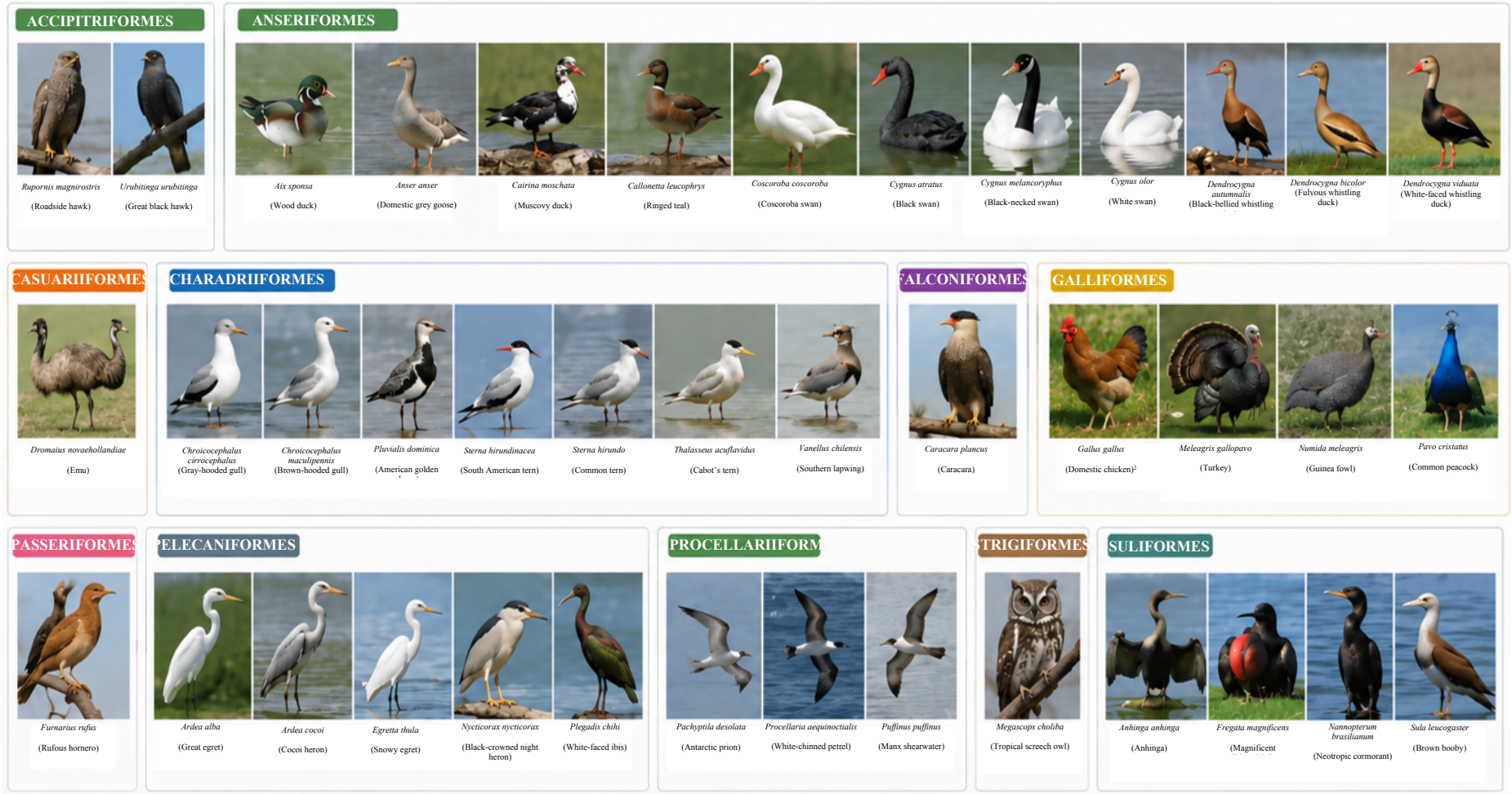
Note: Data obtained from the public avian influenza panel database using only the main species involved in the epidemiological investigation of outbreaks.

This diversification of hosts is compatible with the process described by EFSA as ecological amplification, whereby, after the initial introduction of the virus into a region, there is an increase in detections among various communities of wild fauna that share habitats, feeding areas, and resting places.

International literature also highlights the importance of certain species as potential bridge hosts, capable of simultaneously utilizing natural environments and livestock-raising areas. These species can facilitate the epidemiological interface between aquatic birds, which constitute the primary natural reservoir of influenza A viruses, and domestic birds, thus increasing opportunities for exposure. In Brazil, the identification of outbreaks in resident aquatic birds, synanthropic land birds, birds of prey, and domestic birds emphasizes the importance of considering these ecological interfaces in risk-based surveillance strategies.

The diversity of affected species over the period is presented in Figure 6, created from records of main and non-main species associated with HPAI outbreaks (avian influenza panel).

Figure 6. Taxonomic diversity of birds affected by highly pathogenic avian influenza, Brazil, 2023–2026.



Note: To prepare this image, all species records associated with HPAI outbreaks were considered, including those classified in the database (avian influenza panel) as **main and non-main species.** The database comprises 218 records, with 191 from outbreaks containing main species and 27 from non-main species. One investigation may present more than one affected species; therefore, the number of records by species does not necessarily match the number of outbreaks.

Although the diversity of affected species has increased over the period under analysis (Figure 1), aquatic birds remained the primary epidemiological group involved. The expansion of affected species underscores the need for surveillance programs that include not only migratory birds but also resident birds, synanthropic species, predators, scavengers, and other species with potential epidemiological connections between natural environments and production systems.

Table 2 summarizes the major taxonomic groups that have been identified, their ecological traits and the environments in which they mainly occur.

Table 2 – Ecological and environmental characteristics of the main taxonomic groups affected by highly pathogenic avian influenza in Brazil.

Order	Main Ecological Characteristic	Predominant Environment
Charadriiformes	Terns, gulls, sandpipers	Coastal aquatic
Anseriformes	Ducks, teals, swans, whistling ducks	Continental aquatic
Suliformes	Boobies, cormorants	Aquatic
Pelecaniformes	Herons, bitterns	Wetlands
Procellariiformes	Shearwater, petrels	Oceanic marine
Galliformes	Chickens, turkeys, guinea fowl	Terrestrial (domestic)
Accipitriformes	Hawks	Terrestrial (predators)
Falconiformes	Caracara	Terrestrial (predator/opportunistic)
Passeriformes	Rufous hornero	Terrestrial
Strigiformes	Owls	Terrestrial
Carnivora	Sea lions	Marine

Over the period under investigation, HPAI was confirmed in species belonging to 11 taxonomic orders. Initially, outbreaks clustered in orders typically associated with aquatic and coastal environments, particularly Charadriiformes, Suliformes, Procellariiformes, and Anseriformes. Subsequently, there was an expansion to predominantly terrestrial orders, including Accipitriformes, Falconiformes, Passeriformes, Strigiformes, and Galliformes, as well as reports concerning marine mammals (Carnivora). This pattern aligns with the evolution observed in several countries in Europe and the Americas, where the persistence of viral circulation began to evolve towards an increasing number of species and taxonomic orders.

Despite this diversity, a strong concentration of outbreaks was observed in the order Charadriiformes, responsible for 136 records, accounting for approximately 71% of outbreaks, notably banded terns (*Thalasseus acuflavidus*) with the highest number of occurrences.

Table 3 - Summary of the taxonomic diversity of species affected by highly pathogenic avian influenza in Brazil.

Indicator	Value
Total Number of Species Affected	**35**

Number of Taxonomic Orders	**11**
Most Frequent Order	**Charadriiformes (136 outbreaks; ~71%)**
Most Affected Species	*Thalasseus acuflavidus*
Most Commonly-Affected Domestic Species	*Gallus gallus*
First Groups Affected	Aquatic and marine birds

Among domestic species, *Gallus gallus* accounted for the largest number of outbreaks (Table 1). It is emphasized that an increase in the number of outbreaks or of the affected species does not in itself constitute evidence of sustained transmission or persistence of the virus in these populations, as detections may result from independent incursion events associated with exposure to external infection sources, particularly infected wild birds or contaminated environments.

The occurrence of the virus in hosts belonging to different ecological niches and trophic levels demonstrates multiple opportunities for exposure involving such mechanisms as sharing aquatic environments, direct or indirect contact with infected birds, exposure to contaminated environments, predation, and consumption of carcasses. Thus, although occurrences remained numerically concentrated in aquatic and marine birds, the taxonomic diversification observed during the emergence demonstrates the virus's ability to reach various ecological interfaces.

CHAPTER 4

The genomic surveillance of avian influenza in Brazil: results

4.1. Introduction

Since the first detection of HPAI in Brazil in 2023, the sequencing of viruses identified in different bird species and regions of the country has broadened our understanding of their genetic diversity, phylogenetic relationships, and the dynamics of the incursion and spread in the country.

However, genomic analysis should be interpreted in conjunction with epidemiological information. The genetic similarity between viruses does not in itself alone demonstrate direct transmission between hosts or establishments, nor does the identification of mutations or molecular markers necessarily imply phenotypical change or adaptation to a particular host. In this regard, genomic data provide an additional source of evidence for formulating and evaluating epidemiological hypotheses.

This chapter presents the main results of genomic surveillance of HPAI A/H5N1 viruses detected in Brazil from 2023 to 2026, including sequencing and genetic characterization procedures, the genotypes identified, phylogenetic relationships, and the probable origins of the viruses, as well as the occurrence of reassortment and molecular markers of interest. The integration of these results with epidemiological surveillance data contributes to an understanding of the dynamics of HPAI in the country and to enhancing surveillance and risk assessment strategies.

4.2 Sequencing of the viruses detected

The genomic sequencing of avian influenza viruses detected in Brazil was conducted within the official surveillance framework, based on samples from investigations carried out by the OVS. The harvested samples were initially sent to LFDA-SP, which serves as the reference laboratory for OMSA and FAO's reference center for animal influenza in the region. Confirmation of samples positive for influenza A/H5 was performed by RT-qPCR, supplemented by subtyping and partial sequencing using the Sanger method. Samples selected for complete genomic sequencing were subsequently sent to LFDA-MG, where next-generation sequencing (NGS) was conducted on the Illumina MiSeq platform.

Sample selection for NGS took epidemiological and laboratory criteria into account in order to maximize the representativeness of occurrences in the country. Samples were prioritized with a Ct (cycle threshold) below 25, indicating a higher viral load, as well as

samples representative of different species and geographic regions, enabling the epidemiological diversity allowed for in the testing to be expanded.

Sequencing was performed using sequencing-by-synthesis technology, utilizing the Illumina MiSeq platform, following the extraction of viral RNA, cDNA synthesis, and amplification of the eight segments of the influenza A virus genome. The process included specific controls to assess the efficiency of extraction efficiency, the preparation of libraries, and the performance of the sequencing runs. The MS2 bacteriophage served as a control for the extraction process, while the PhiX was included in the runs to monitor sequencing performance.

Bioinformatics processing included the filtering and trimming of readings, quality assessment, removal of contaminants and host sequences, de novo assembly, the inspection of genomes, and the annotation of sequences. The parameters obtained showed high-quality sequences, with an average coverage exceeding 1,000×, Q30 values above 90%, and a proportion of passing filter readings exceeding 85%.

Of the 63 samples from evaluated outbreaks (Annex 1), 50 (79.4%) showed complete genome sequencing, while 13 (20.6%) showed partial sequencing. Therefore, a high proportion of complete genomes among the analyzed samples was observed, above all in more recent years, enhancing the genomic characterization capability of the detected viruses.

4.3 Genetic characterization

The genetic characterization of the viruses that were detected confirmed the occurrence of the highly pathogenic Influenza A/H5N1 subtype (clade 2.3.4.4b), the lineage responsible for the recent expansion of HPAI in the Americas. For samples with complete genome sequencing, the analysis included the eight segments that comprise the influenza A virus genome (PB2, PB1, PA, HA, NP, NA, M, and NS), thereby allowing for the integrated assessment of their genetic composition and phylogenetic relationships among the detected viruses.

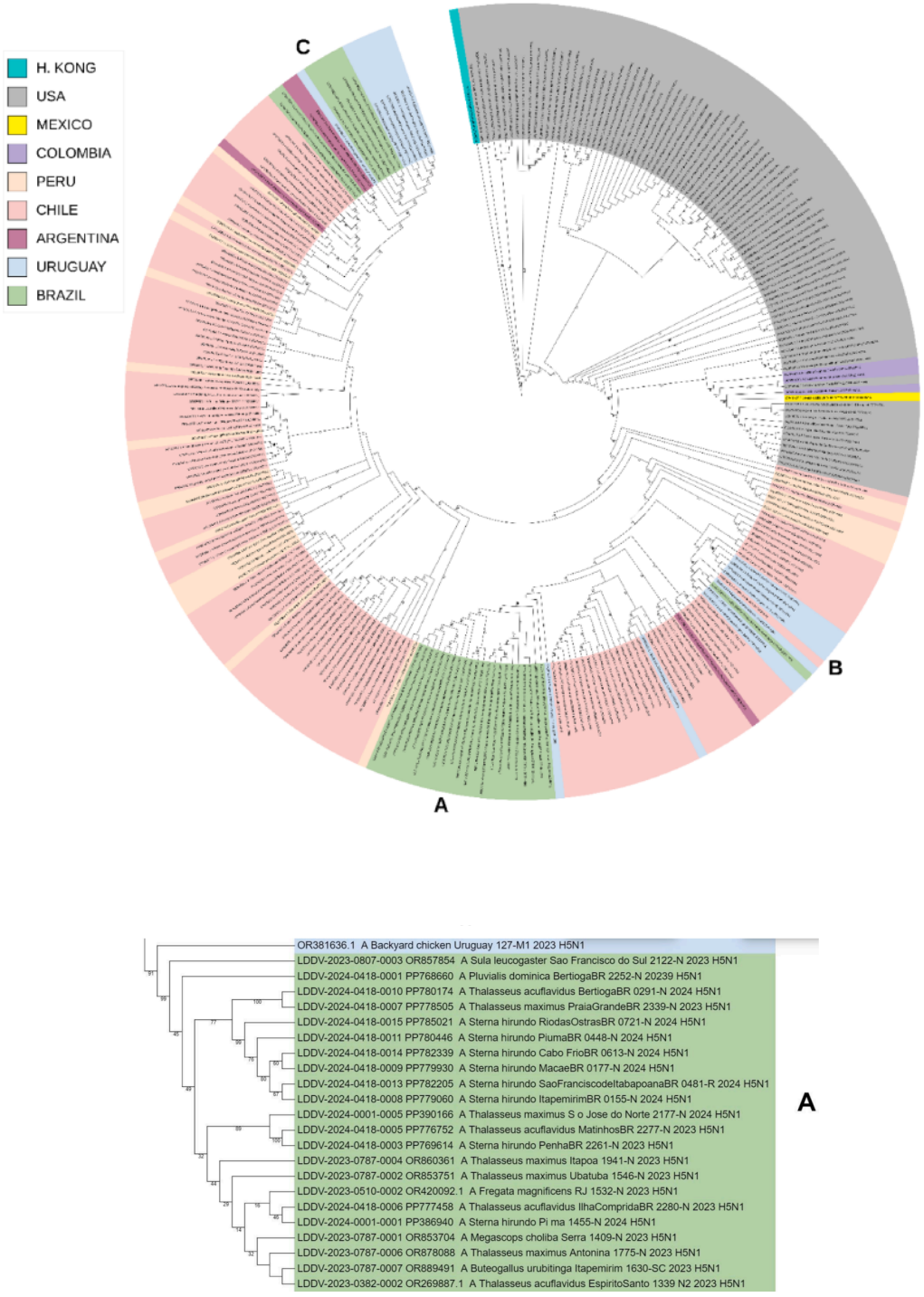
4.4 Probable origin of the viruses

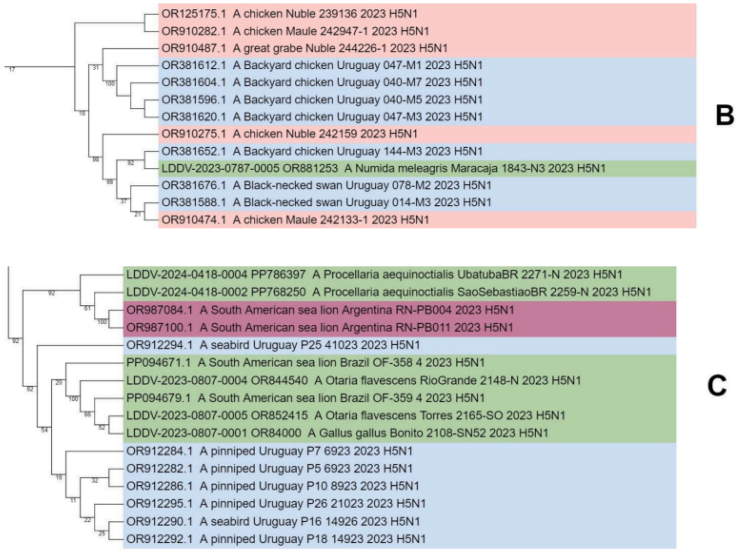
Phylogenetic and phylogeographic testing based on HPAI A/H5N1 viruses detected in Brazil indicate that the dynamic of viral introduction in the country was not uniform throughout the period under analysis, and there was evidence of a range of introduction routes between 2023 and 2026.

The viruses characterized during the 2023–2024 period belonged to clade 2.3.4.4b and were consistently classified as genotype B3.2, exhibiting high genetic similarity with viruses previously detected in Chile, Uruguay, and Argentina. Phylogeographic analysis suggests spread from the Northern Hemisphere towards South America, passing through Central

America and the Andes region, with subsequent dispersal to southern Brazil and other areas in the country. This pattern was observed above all along the Pacific migratory route, also taking into account connections between bird populations associated with the Pacific and Atlantic routes by intraregional movements.

Figure 7. Phylogenetic analysis of highly pathogenic avian influenza viruses detected in Brazil in 2023 and 2024.





Legend: The phylogeny of the H5 hemagglutinin sequences was reconstructed using the maximum likelihood method, employing IQ-TREE multicore v.1.6.12. The sequences were aligned using MAFFT in Geneious Prime® 2024.0.3, and the phylogenetic tree was generated within the same software and edited using the iTOL tool. The best-fitting evolutionary model was selected based on the Bayesian Information Criterion (BIC), and the robustness of the tree topology was assessed through 1,000 bootstrap replicates. The green color indicates the clustering of Brazilian sequences, including those from this study, identified by the acronym "LDDV". Viruses not belonging to the goose/Guangdong lineage, coming from Eurasia, were used as the outgroup.

The highlighted clusters A, B, and C share a most recent common ancestor with viral lineages identified in Chile, Uruguay, and Argentina between 2022 and 2024, with bootstrap support values between 82% and 92%, indicating closer phylogenetic proximity to sequences from South America.

The high phylogenetic proximity between Brazilian sequences and those from other South American countries reinforces the hypothesis that the initial detections in Brazil were part of the regional dispersion process of H5N1 already occurring on the continent, primarily mediated by movements of wild birds.

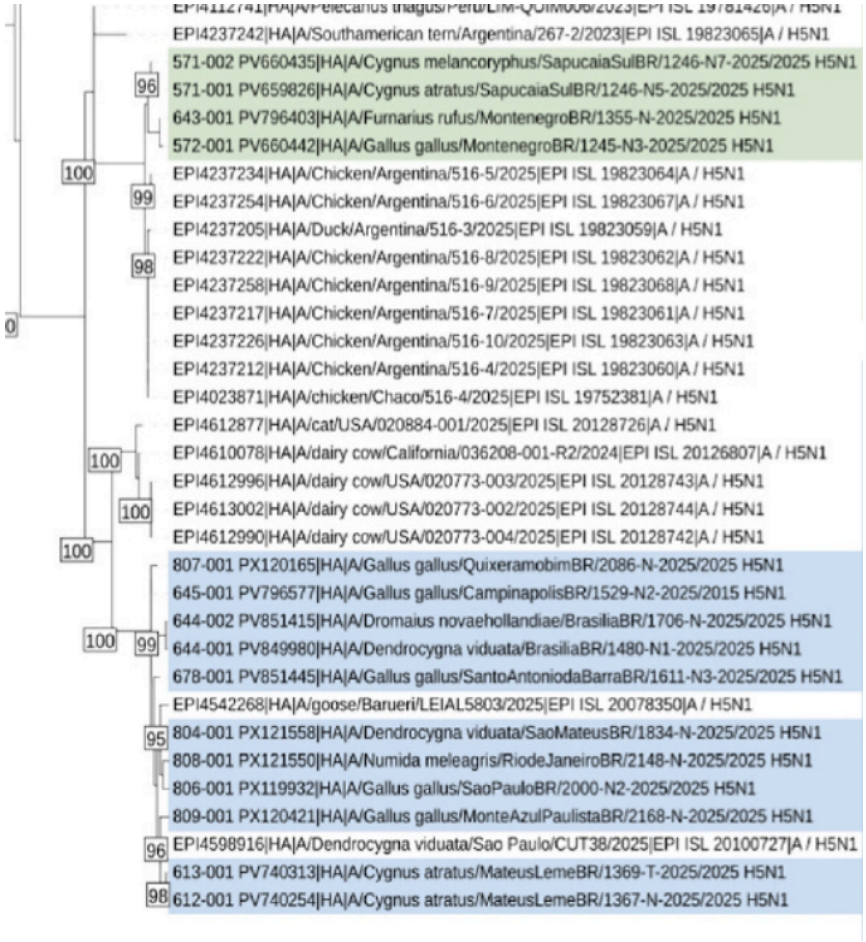
However, from 2025 onward, a significant change in this pattern is observed. Unlike the relative genetic homogeneity seen in 2023–2024, the Brazilian sequences analyzed in 2025 were distributed into two distinct phylogenetic clusters, indicating multiple viral incursions.

The first cluster included viruses detected in Rio Grande do Sul, both in the commercial poultry establishment in Montenegro and in wild birds from Sapucaia do Sul. These viruses presented raised identity with viruses detected in Argentina and Bolivia in 2025, supporting the hypothesis of regional epidemiological connectivity in the Southern Cone, possibly mediated by migratory birds.

The second cluster brought together viruses detected in the Northeast, Midwest, and Southeast regions of Brazil, which showed greater phylogenetic proximity to viruses from North America. As previously described in RIVETTI JUNIOR et al., 2025, this cluster is

compatible with an independent introduction, unrelated to the events identified in the south of the country, reinforcing the existence of more than one pathway for incursion and dispersion for H5N1 in Brazil.

Figure 8. Phylogenetic analysis of highly pathogenic avian influenza viruses detected in Brazil in 2025



Legend: The phylogenetic tree was constructed using the maximum likelihood method, utilizing IQ-TREE and the K3Pu + F + G4 nucleotide substitution model, and edited in iTOL. The Brazilian sequences from 2025 and their respective locations (B) are highlighted in green for the clade phylogenetically related to sequences from Argentina, and in blue for the clade related to sequences from dairy cattle in the United States.

Additionally, the analysis of the eight genomic segments revealed, in 2025, events of genomic reassortment, with segments related to lineages from South America, North America, and Eurasia. In Rio Grande do Sul, a pattern similar to that described in Argentina in 2025 was identified, while another set of viruses exhibited segments that were genetically related to North American and Colombian viruses. These findings demonstrate that the dynamics observed from 2025 involve not only different introductions but also a greater complexity in the genomic composition of circulating viruses in the Americas.

The test results for samples from 2026 underscored the continuity of genetic diversity among A(H5N1) viruses detected in Brazil and indicated distinct phylogenetic relationships

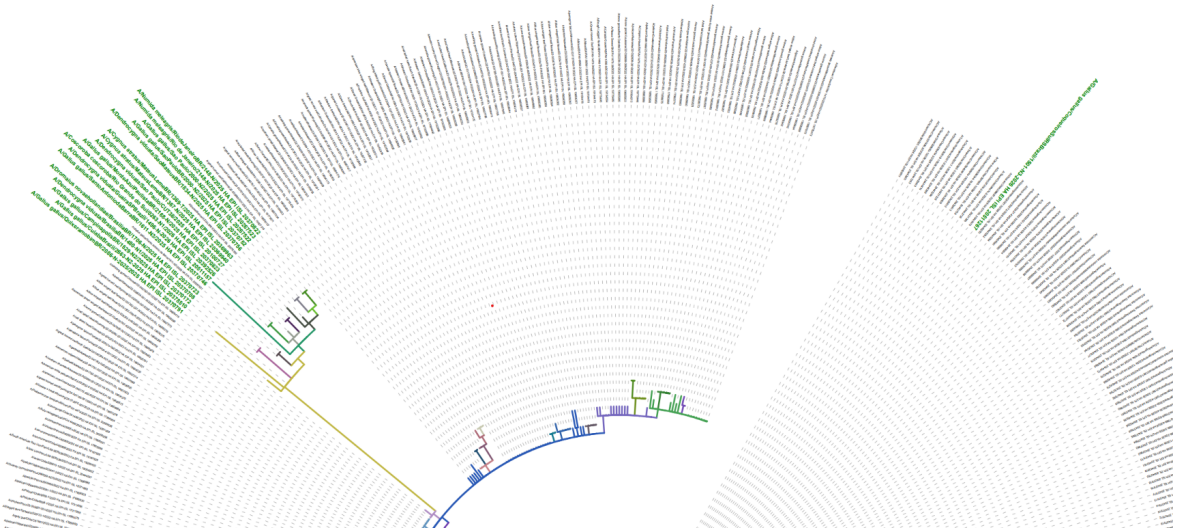
among the events analyzed, compatible with different connections to viruses circulating in the Americas.

For example, the virus detected in Coqueiros do Sul/RS in a backyard chicken (*Gallus gallus*) was phylogenetically closer to viruses previously identified in chickens in Chile and in a goose in Argentina. This cluster suggests a relationship with circulating viruses in the Southern Cone and is compatible with the regional connectivity observed among the HPAI viruses identified in southern South American countries.

On the other hand, the virus detected in Guaíra/SP in a whistling duck (*Dendrocygna viduata*) showed greater phylogenetic proximity to the virus identified in Santa Vitória do Palmar/RS in a Coscoroba swan. The latter, in turn, exhibited greater similarity to a virus identified in a Cackling goose in Colorado, United States (FIGURE 8). This pattern suggests a phylogenetic connection other than that observed for the sample from Coqueiros do Sul and points to the possible involvement of viruses related to lineages circulating in North America.

The analysis of the eight genomic segments complements these findings, showing genomic constellations compatible with genetic reassortment in the samples from Guaíra/SP and Coqueiros do Sul/RS. In both, the HA, NA, PA, and MP segments showed greater proximity to the Eurasian lineage, while PB2, PB1, NP, and NS were related to lineages of American wild birds. Despite this similar composition, their different phylogenetic relationships reinforce the need to evaluate individual segments and not solely the overall genotypic classification of the viruses.

Figure 9. Phylogenetic analysis of highly pathogenic avian influenza viruses detected in Brazil in a portion of samples from 2025 to 2026



Legend: The circular phylogenetic tree of Brazilian sequences is highlighted in green at the tips of the branches, with internal colors indicating different clades and evolutionary lineages. This shows two distinct patterns through the separation of clades.

Collectively, the findings from 2026 suggest that, although the number of sequences analyzed cannot yet provide certainty, there may be at least two phylogenetic patterns among the analyzed viruses: one related to viruses previously detected in the Southern Cone, represented by the sample from Coqueiros do Sul/RS, and another relating to the sequence from Santa Vitória do Palmar/RS, and more distantly, to viruses from North America, represented by the sample from Guaíra/SP. These results are compatible with the continuity of different viral incursion and dispersion pathways on the continent, potentially associated with connectivity among populations of wild birds. It is to be emphasized that the data available to date are not conclusive regarding the routes and origins of the viruses; the results presented should be interpreted as an overview based on the information currently available.

4.5 Genotypes that have been identified

The analysis of sequences obtained over the period of time under study shows changes in the genomic profile of the avian influenza viruses detected in Brazil. In the initial phase of detection, genotype B3.2 predominated and was identified in several species and geographical regions in 2023 as well as in samples harvested in the first half of 2024. This pattern demonstrates the broad distribution of this genotype during the initial phase of detection of the virus in Brazil. However, since 2024, no new identifications of genotype B3.2 were recorded among the presented sequences, suggesting a change in the profile of viruses detected in the country.

Since 2025 new genotypes have been identified, including K.8 and N.1 (ANNEX). The K.8 genotype was identified in a black swan sample taken in Rio Grande do Sul, while N.1 was detected in samples from different species taken in the Federal District, Minas Gerais, and Mato Grosso. These findings demonstrate the occurrence of viruses genetically distinct from those predominant in the initial phase of detections.

In addition to the three genotypes already identified (B3.2, K.8, and N.1), there remain samples with complete genomes without any genotypic classification reported in the database, including records from 2025 and 2026. Thus, the interpretation of the genomic diversity observed during this period may still be expanded as these sequences become classified and incorporated into phylogenetic analyses.

Together, the results demonstrate a temporal change in the genetic composition of viruses detected in Brazil, characterized by the initial predominance of genotype B3.2 in the early years of the animal health emergency. Subsequently, there was greater diversity in genotypes, with the identification of K.8 and N.1, as well as viruses not yet classified as to their genotype. This scenario highlights a more diversified genomic dynamics over time, potentially related to new incursion events and the circulation of different viral variants in South America, reinforcing the importance of continuous genomic surveillance to monitor these changes.

4.6 Genetic mutations and adaptation markers

Mutations PB1-F2 N66S and PA N383D have been present in all the sequences analyzed over the entire historical monitoring period in the country (total of 48 genomes).

In the genetic profile analysis of the virus in 2026, additional changes were identified in three proteins: in HA (which affects binding to host cells), in NS1 (linked to immune response), and in M1 (associated with the virus's pathogenicity). On the other hand, the virus still does not have the classic adaptations to mammals (such as PB2 Q591K and D701N), which characterized the group of cases in 2023.

Table 4. Frequency and temporal distribution of mutations and molecular markers of interest identified in highly pathogenic avian influenza A(H5N1) viruses in Brazil, 2023–2026

Gene / Segment	Protein	Mutation / Marker	2023 (n=21)	2024 (n=7)	2025 (n=16)	2026 (n=4)	Brief Report on Effect / Biological Impact
PB2	PB2	Q591K	5 (23.8%)	0 (0%)	0 (0%)	0 (0%)	Efficient viral replication in mammalian cells.
PB2	PB2	D701N	5 (23.8%)	0 (0%)	0 (0%)	0 (0%)	Increases nuclear transport and transmission between mammals (key zoonotic marker).
PB2	PB2	K389R / V598T	0 (0%)	0 (0%)	0 (0%)	4 (100%)	Increases polymerase activity and replication in mammalian/mouse cells.
PB2	PB2	E627K	0 (0%)	0 (0%)	0 (0%)	0 (0%)	Classic marker of mammal adaptation (absent in all Brazilian samples; PB2 627E conserved).
PB1	PB1-F2	N66S	21 (100%)	7 (100%)	16 (100%)	4 (100%)	Associated with increased virulence and inhibition of the innate immune response in mammals.
PB1	PB1	D3V / D622G	0 (0%)	0 (0%)	0 (0%)	4 (100%)	Increases polymerase activity and replication efficiency in mammalian/mouse cells.
PA	PA	N383D (383D)	21 (100%)	7 (100%)	16 (100%)	4 (100%)	Conserved in avian lineages; maintains high viral polymerase efficiency in birds and mammals.
PA	PA	M86I	5 (23.8%)	0 (0%)	0 (0%)	0 (0%)	Adaptation marker in mammals associated with increased virulence (present in the 2023 cluster).
PA	PA	S37A / N409S	0 (0%)	0 (0%)	0 (0%)	4 (100%)	Increases RNA polymerase activity and replicative kinetics.
HA	HA1 / HA2	S107R + T108I	0 (0%)	0 (0%)	0 (0%)	4 (100%)	Elevates fusion pH and increases virulence in animal models.
HA	HA1 / HA2	S133A / S154N / T156A	0 (0%)	0 (0%)	0 (0%)	4 (100%)	Increases binding affinity to human/mammalian-type receptors (alpha2,6-sialyl).
HA	HA1 / HA2	K218Q + S223R	0 (0%)	0 (0%)	0 (0%)	4 (100%)	Modulation of dual binding affinity (alpha2,3 and alpha2,6).
HA	HA1 / HA2	K64E (HA2)	0 (0%)	0 (0%)	0 (0%)	4 (100%)	Stability of hemagglutinin and modulation of membrane fusion threshold.
NP	NP	A184K / K198R	0 (0%)	0 (0%)	0 (0%)	4 (100%)	Modulation of interferon response and activity of the ribonucleoprotein complex (RNP).
NA	NA	Point polymorphisms	—	—	—	Present	Secondary structural variations without an impact on catalytic activity.
MP	M1	N30D / I43M / T215A	0 (0%)	0 (0%)	0 (0%)	4 (100%)	Molecular markers associated with increased pathogenicity and virulence in birds and mice.
NS	NS1	D26K	5 (23.8%)	0 (0%)	0 (0%)	0 (0%)	Adapted to mammals and immune evasion (2023 cluster).
NS	NS1	P42S / I106M / C138F / V149A	0 (0%)	0 (0%)	0 (0%)	4 (100%)	Suppression of innate immune response by attenuation of interferon (IFN) and facilitation of cellular replication.

Table 5. Molecular Resistance Markers to Antivirals Identified in HPAI A(H5N1) Viruses Analyzed in Brazil, 2023–2026

Antiviral Class	Evaluated Drug	Key Markers Investigated	2023 (n=21)	2024 (n=7)	2025 (n=16)	2026 (n=4)	Consolidated Sensitivity Profile
Neuraminidase inhibitors (NAIs)	Oseltamivir	NA H275Y, N295S, I223V	0% mutated	0% mutated	0% mutated	0% mutated (Sensitive)	100% Sensitive (no resistance detected in any year).
Neuraminidase inhibitors (NAIs)	Oseltamivir	V203M	0% mutated	0% mutated	0% mutated	1 (25%)	Reduced inhibition for Oseltamivir
Neuraminidase inhibitors (NAIs)	Zanamivir	NA E119V, T438I, R292K	0% mutated	0% mutated	0% mutated	0% mutated (Sensitive)	100% Sensitive.
Neuraminidase inhibitors (NAIs)	Peramivir	NA H275Y, T438I	0% mutated	0% mutated	0% mutated	0% mutated (Sensitive)	100% Sensitive.
Endonuclease Inhibitor (PA)	Baloxavir Marboxyl	PA I38T/F/M, E23G/K, E199G	0% mutated	0% mutated	0% mutated	0% mutated (Sensitive)	Sensitive (no mutations in PA polymerase active site).
Adamantanes	Amantadine / Rimantadine	M2 S31N, V27A, L26F	0% mutated	0% mutated	0% mutated	0% mutated (V27I/S31S)	Sensitivity (no classical M2 channel blocking mutations detected).

Beyond the differences in genotype and the origin of genomic segments, the testing of the A/H5N1 virus sequences detected in Brazil identified replacements of amino acids in viral proteins of interest for evaluating adaptation to different hosts, transmissibility, and zoonotic potential.

In samples analyzed between 2023 and 2024, mutations were identified in the virus's surface protein (hemagglutinin - HA), with changes in its two main regions (HA1 and HA2) and a total of 12 significant single nucleotide polymorphisms (SNPs).

On the other hand, PB2-D701N and PB2-Q591K substitutions were identified in the Brazilian samples, both of which are described in the literature as markers associated with an increased efficiency of replication and/or adaptation of influenza A virus in mammalian hosts. These alterations occurred in five of the samples evaluated and were also present in phylogenetically related viruses detected in Argentina, Uruguay, and Chile.

The presence of these markers warrants attention from a public health perspective but should not be interpreted in isolation as evidence of the virus's adaptation to mammals or increased transmission among these hosts.

Substitutions related to resistance to antiviral neuraminidase inhibitors were also identified. No NA-N295S, NA-H275Y, or NA-T438I substitutions, previously associated with reduced susceptibility or resistance to oseltamivir, zanamivir, and/or peramivir, were detected in Brazilian samples. Likewise, the M1 and M2 proteins did not present markers associated with adaptation to mammals in the analyzed set.

Thus, the available results demonstrate that genomic monitoring should consider point mutations, molecular markers of adaptation, and antiviral resistance, as well as events of genetic reassortment. While substitutions of interest, particularly in PB2, have been identified, the data do not in themselves alone demonstrate a sustained adaptation of H5N1 to mammals in Brazil, reinforcing the need for continuous monitoring of these markers through genomic surveillance.

CHAPTER 5

The integration of epidemiological and genomic data

5.1. Introduction

In Brazil, the integration of genomic surveillance into epidemiological investigations has allowed the interpretation of events observed during the emergence of HPAI to be broadened, providing additional evidence on possible independent incursions, dispersion events, changes in the genetic composition of viruses, and potential ecological interfaces involved in viral circulation.

However, it is important to recognize that the interpretation of these data is inherently limited because of the dynamic nature of HPAI epidemiology. Viral circulation among multiple hosts, continuous occurrences of new introductions, the genetic evolution of viruses, and sampling gaps all mean that the available evidence allows for increasingly robust epidemiological hypotheses rather than conclusions that can always be definitive. In this context, the continuous generation of epidemiological, laboratory, and genomic data, combined with long-term monitoring, is fundamental to progressively improving knowledge of the disease dynamics.

The following sections discuss the main contributions of integrating epidemiological and genomic data to interpret the emergence of HPAI in Brazil. The results presented herein should be understood as supplementary evidence that strengthens epidemiological hypotheses and reduces uncertainties, contributing to the ongoing improvement of surveillance and risk assessment without intending to establish definitive conclusions about all the mechanisms involved in the introduction and spread of the virus.

5.2 The structure of the integration of the data

Each HPAI outbreak generates different sets of information which, when analyzed together, allow for a more comprehensive interpretation of the event.

From an epidemiological viewpoint, the elements taken into consideration include: geographic location; date of occurrence; the species affected; the chronology of the event; epidemiological links; the movements of animals, products, people, and vehicles; the risk factors identified; and environmental characteristics.

From a laboratory and genomic perspective, the elements taken into consideration include: confirmation of diagnosis; the genomic sequence; classification of the subtype and

clade; the viral genotype; the phylogenetic position; the genetic relation to viruses identified previously; and the presence of genetically relevant changes of epidemiological interest.

The integrated interpretation should also incorporate such ecological and spatial information as: migratory routes; the distribution of wild species; aquatic environments; the density of poultry; the location of susceptible establishments; and connectivity between poultry-producing units.

The relative contribution of each source of information varies in accordance with the epidemiological question being investigated. While some questions may be clarified predominantly by epidemiological or genomic data, others depend on the joint analysis of multiple pieces of evidence in order to increase the confidence of the interpretation.

Chart 2 synthesizes the contributions of epidemiology, genomics and the integration of this evidence in order to answer the major queries concerning the origin, spread and evolution of HPAI outbreaks.

Chart 2 – Contribution of epidemiological and genomic data to the interpretation of HPAI outbreaks

Epidemiological question	Epidemiology	Genomics	Integration of evidence
Where did the outbreak occur?	High	Low	High
When did it occur?	High	Low	High
What animal species were involved?	High	Low	High
What is the probable origin of the virus?	Moderate	High	**Very high**
Is it an independent incursion?	Moderate	High	**Very high**
Are the two outbreaks related?	Moderate	High	**Very high**
Has there been lateral dissemination?	Moderate	Moderate	**Greater confidence**
Has there been local persistence of the virus?	Moderate	High	**Greater confidence**
Has the viral genotype changed?	Low	High	High
Has there been genetic reassortment?	Low	High	High
What are the most plausible dissemination routes?	High	Moderate	**Very high**

5.3 Origin and incursion events

The first recorded outbreaks in Brazil, in 2023 and 2024, were strongly associated temporally, spatially, and ecologically with wild birds, particularly migratory species in coastal environments, consistent with the dispersal pattern of H5N1 clade 2.3.4.4b observed in the Americas. Genomic testing corroborated this epidemiological pattern, indicating a high genetic

homogeneity among the viruses characterized during this period, with the predominance of a single genotype among samples from distinct outbreaks that were analyzed.

The viruses identified in 2025 began to show greater genomic diversity. The tests showed Brazilian viruses as being divided into two main clades: one genetically related to the viruses detected in Argentina in 2025, consistent with the regional spread of new viruses with genetic reassortment in South America, and the other as part of a group of viruses from North America.

Additionally, the identification of viruses that are genetically related to lineages previously detected in other regions, combined with the absence of epidemiological links between certain outbreaks, is compatible with the **occurrence of independent events of incursion into Brazil**, possibly related to the circulation and movement of infected wild birds.

5.4 Dispersion of the virus and ecological interfaces

After multiple initial incursions whereby the virus enters Brazil, an understanding of the dynamics of its dispersion begins to involve not only the movements of migratory birds and the location of the outbreaks but also the ecological connectivity between different bird populations and the environments they share.

In this context, the information presented in the Report on Areas of Concentration of Migratory Birds in Brazil (*Relatório de Áreas de Concentração de Aves Migratórias no Brasil*) - 4th edition, prepared by the National Center for Research and Conservation of Wild Birds (CEMAVE), is an important resource for interpreting the spatial dynamics of HPAI in Brazil. The report identifies the major migratory routes and maps out sites along the coast and at inland locations in Brazil that are regularly used by migratory birds for landing, resting, feeding, and breeding. These environments form a network of ecologically connected areas, where they may be a spatial and temporal overlap between migratory and resident birds.

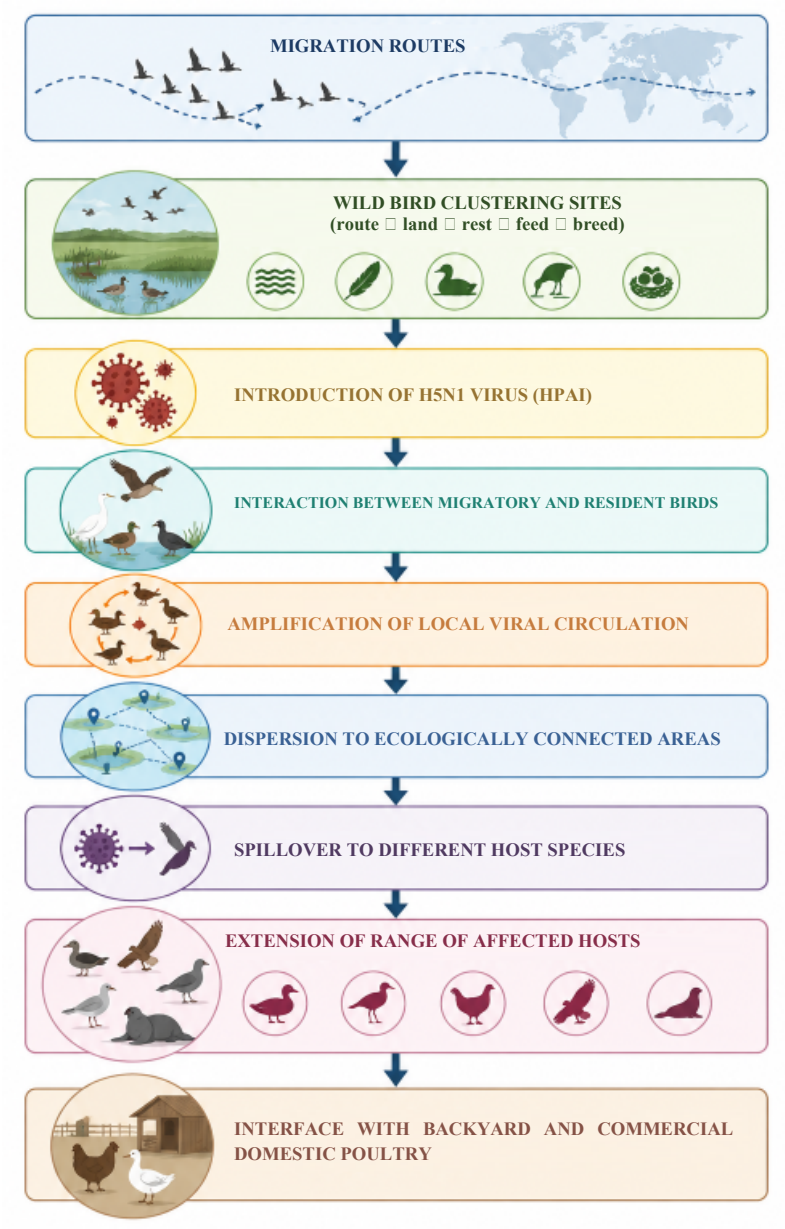
The international literature shows that areas where birds congregate can constitute ecologically significant areas for avian influenza viruses. The concentration of different species and the sharing of aquatic and terrestrial environments increase the opportunities for exposure and transmission among hosts. Viral dispersion may therefore involve not only the long-distance movements associated with migratory routes but also regional and local movements and successive events involving transmission between different bird populations that use ecologically connected environments.

This mechanism is consistent with the epidemiological patterns observed in Brazil. Taken all together, the findings suggest that after independent viral incursion events, the regional dispersion of HPAI may have been influenced by the interaction between migratory and resident birds and by the ecological connectivity between the areas utilized by these populations. The spatial and temporal overlap of different species at sites where birds feed,

rest, breed, and cluster areas may favor inter-species transmission and the exposure of species that have different levels of susceptibility, thus contributing to the dynamics of maintaining and dispersing the virus. However, outbreaks in these species, which are not natural reservoirs of the virus, do not in themselves alone provide evidence of maintenance, viral persistence, or sustained transmission in these populations.

This dynamic may also boost interfaces between wild and domestic birds. The risk is particularly significant in backyard flocks, small-scale systems, and other establishments with lower biosafety levels, where access of domestic birds to the external environment, proximity to sites where wild birds cluster, and water, food, or other environmental resources are shared, may increase opportunities for exposure and facilitate the transmission of the virus from wild birds to domestic birds.

Figure 9: Dynamics of the introduction and spread of the avian influenza virus



5.5 Relationship between genomic diversity and epidemiological behavior

Another important benefit of integrated genomic surveillance is the ability to monitor changes in the genetic composition of viruses over time.

Influenza viruses have a segmented genome, allowing for reassortments when different viruses simultaneously infect the same host. Thus, viruses belonging to the same clade can exhibit different combinations of genomic segments and constitute distinct genotypes.

The fact that different genotypes and genetic reassortment events were identified over the course of the animal health emergency in Brazil demonstrates that H5N1 clade 2.3.4.4b

viruses have evolved genetically throughout their circulation in the Americas. To date, however, there has been no evidence that the genotypical changes identified in Brazil have been accompanied by consistent changes in the epidemiological or phenotypical behavior of viruses, such as changes in the spectrum of affected species, in their clinical presentation, pathogenicity, or capacity for transmission among domestic birds.

The international literature indicates that the rise in the number of species affected as observed during the current panzootic is primarily related to high environmental infection pressure, the expansion of viral circulation among wild birds, and increased ecological interfaces between different hosts, rather than the emergence of a specific genotype. Similarly, mutations associated with adaptation to mammals have been described, above all following spillover events to species of mammals, albeit not constituting evidence of widespread phenotypical changes in viruses circulating among birds. Thus, the epidemiological interpretation of genomic changes should take jointly into consideration the phylogenetic, epidemiological, ecological, and (when available) phenotypical studies, and avoid inferring biological changes solely based on the identification of new genotypes.

5.6 Reducing uncertainties

Epidemiological investigations of HPAI outbreaks often involve multiple plausible hypotheses of incursion or spread. In certain situations, information regarding movement, geographic proximity, or exposure to wild birds may not be sufficient to distinguish between competing hypotheses.

The incorporation of genomic data adds a new layer of evidence to the investigation. When genomic results align with the chronology, with location, and with epidemiological links, confidence in the hypothesis that is formulated increases. When they are discordant, this may indicate a need to review the investigation or consider alternative hypotheses.

However, reducing uncertainty is not the same as eliminating it. The absence of sequences from certain hosts or periods, differences in the intensity of surveillance, and samples of more limited representativeness may all hinder a complete reconstruction of transmission chains.

CHAPTER 6

Applications and prospects for the integration of epidemiological and genomic surveillance to strengthen official veterinary services

6.1. Introduction

Rather than merely enhancing understanding of the events that occurred, the integrated analysis of epidemiological and genomic information offers new possibilities for application in official surveillance, particularly in epidemiological investigation, in identifying potential pathways for the introduction and dispersion of viruses, in assessing risk, and in prioritizing populations and areas for surveillance.

Linking this data to laboratory, spatial, and ecological information enables a more comprehensive understanding of the dynamics of HPAI to be constructed, and the results produced by surveillance to be turned into information that is applicable for planning and decision-making by the official veterinary services (OVSs). At the same time, it enables the identification of knowledge gaps and new opportunities for improving prevention, early detection, and response strategies.

The present chapter discusses the observed and potential contributions resulting from the integration of epidemiological and genomic data for the understanding of HPAI dynamics in Brazil, as well as how they can be applied in order to improve the strategies executed by the OVS.

6.2 The integration of evidence and enhancement of surveillance

The knowledge produced by the epidemiological and genomic surveillance of HPAI has increasingly been incorporated into the OVS's activities, and has enabled adjustments to be made to strategies, populations expanded and interfaces to be taken into account in surveillance, and allowed higher risk situations to be identified and prioritized. Connecting these findings to information on the distribution of wild and domestic birds, migratory routes, animal movements, and environmental factors has transformed the data obtained from different sources into understanding that can be applied to prevention, investigation, surveillance, and response actions.

This approach aligns with the concept of epidemiological intelligence, which involves the systematic gathering, integration, analysis, and interpretation of information from a range of sources in order to identify and assess health risks and inform the OVS's decision making process. In the context of the emergence of HPAI in Brazil, applying the approach has resulted in the practical adaptation of surveillance strategies as new epidemiological, genomic, and ecological evidence has emerged.

One such application is the enhancement of risk-based surveillance. Incorporating information on sites where wild birds gather, migration routes, the spatial distribution of outbreaks, and the results obtained from epidemiological investigations has improved risk assessments, identified regions at a higher likelihood of viral introduction, and refined the prioritization of municipalities and areas for intensifying surveillance and biosafety actions.

The changing nature of the emergency has also underscored the ongoing need to adapt surveillance to changes in the epidemiological profile of the disease. Initially, the passive surveillance of wild birds focused primarily on species of the orders Anseriformes and Charadriiformes, internationally acknowledged to be important reservoirs for avian influenza viruses. However, over the course of the emergency, the detection of infection in species of different taxonomic orders, including resident wild birds, showed that the spectrum of hosts is widening. Some of these species may potentially act as bridge hosts, facilitating the interface between infected migratory birds and other populations of wild, peri-urban, and domestic birds. In the light of this scenario, passive surveillance was expanded to take wild birds of different species into account as target populations, provided that the criteria for establishing a suspect case laid down by the Official Veterinary Services (OVS) have been met, thereby increasing the system's capacity to detect changes in the epidemiological profile of HPAI.

The observed spectrum of hosts broadened over the course of the animal health emergency beyond avian species. The occurrence of H5N1 virus infections in different domestic and wild mammalian species in other countries showed that spillover events were possible and that there was a need to pay more attention to non-avian species that might be exposed. Given this evolution in the epidemiological landscape, Brazil's surveillance strategy has been progressively adapted in order to take these interfaces into consideration, particularly in situations involving outbreaks of HPAI in domestic birds. In these events, a risk assessment for domestic mammals (cattle and pigs) present in the affected epidemiological unit has been included, particularly when there was a possibility of direct or indirect contact with infected birds or potentially contaminated materials. As identified risks arise, investigations may include sample-taking in order to help detect possible transmission events between species and to monitor changes in the spectrum of hosts of the virus.

Genomic surveillance added another dimension to this interpretation, now being incorporated systematically into investigations and evaluations of the epidemiological landscape. Linking it to epidemiological data has helped evaluate the connections between outbreaks, investigate any potential pathways for the introduction and dispersion of the virus, and identify changes in the profile of viruses detected in the country.

The integration of epidemiological, genomic, and ecological information has thus enabled new findings to be incorporated into official surveillance, progressively adjusting its strategies to fit the changes observed in the epidemiological profile of HPAI, contributing to the definition of priorities and enhancing risk-based surveillance.

6.3 Prospects

The rapid evolution of HPAI and the growing diversity and volume of information produced by surveillance both underscore the need for a continuous evolution in the capacity of the OVS to integrate, process, and analyze data from various sources. In this context, a strategic prospect for Brazil is to advance in its incorporation of epidemiological intelligence systems focused on the integration and analysis of data that can systematically and in timely fashion compile epidemiological, laboratory, genomic, ecological, and environmental information, as well as data from the poultry-raising industry.

The process should encompass both the central level, strengthening Brazil's capacity in analysis, risk assessment, and the definition of strategies, and the state level services (SVSs) which generate a significant amount of the data and perform surveillance activities in the field. Strengthening this capacity at different levels of the OVS could enhance system interoperability, data standardization and quality, the automation of analyses, and the generation of timely strategic information.

Consolidating this structure will allow a shift from systems that focus above all on recording and monitoring occurrences to models able continuously to integrate and analyze a range of sources of information, bolstering the early identification of changes in epidemiological profiles, prioritizing areas and populations at higher risk, and directing surveillance activities in a timely manner.

Another important advance has been the integration of databases currently produced by different institutions. An integrated approach is needed for analyzing information from the official epidemiological surveillance, active surveillance of asymptomatic wild birds conducted by environmental and research institutions, wildlife monitoring, and environmental data, so as to significantly enhance the capacity to understand the ecological determinants of viral circulation. This approach would allow more precise identification of priority areas for surveillance; the probable pathways to dispersion; interfaces between migratory birds, resident birds, and domestic birds; enabling the identification of environmental factors associated with the maintenance and dissemination of the virus; and it would function as an alert system for outbreaks in the poultry-raising industry.

The integration of this evidence will also contribute to the development of models able to identify hotspots, to estimate areas at greater risk of viral introduction and dispersion, and continuously support the updating of risk-based surveillance strategies for Brazil's poultry-raising industry.

The expansion of genomic surveillance could also enable a more systematic monitoring of viral diversity, mutations, genetic reassortment events, and molecular markers of interest. When associated with epidemiological evidence and phenotypical data, when available, this information can aid in evaluating changes in host adaptation, transmissibility, and other significant characteristics. In scenarios where vaccination strategies are being considered, the genetic and antigenic characterization of circulating viruses may also bolster the evaluation and selection of potential vaccine strains.

Finally, the consolidation of the One Health approach is one of the main prospects for strengthening HPAI surveillance. The integration of animal health, human health, the environment, and wildlife conservation will allow for a more comprehensive understanding of the ecological interfaces involved in the circulation of influenza viruses; enable the early identification of transmission events between species; and enhance the capacity to respond to cross-border diseases.

CHAPTER 7

Final remarks

The experience accumulated since the introduction of HPAI in Brazil in 2023 has demonstrated the robustness and adaptability of Brazil's national surveillance system, supported by the integrated actions of MAPA, the SVSs, the official laboratory network, the poultry-raising industry, and the several actors involved in surveillance and the response to zoosanitary emergencies. The structure in place has enabled the detection and investigation of outbreaks among different species and epidemiological contexts, expanded the diagnostic capability, and led to the timely implementation of prevention, control, and response measures.

At the same time, the results presented in the present report show that the epidemiology of HPAI remains dynamic and subject to continuous change. The expanding geographical distribution and widening spectrum of hosts, along with the identification of different genotypes, genetic reassortment events, and possible independent incursions, show clearly that the health risk is not limited to the periods or areas where the first detections were observed. In this scenario, the reduction over time in the number of outbreaks must not be interpreted as a reduction in the need for surveillance, but instead reinforces the importance of maintaining a continuous, sensitive, and risk-based system that is capable of recognizing changes in epidemiological patterns early, and directing actions toward higher-risk avian populations, areas, and interfaces.

The incorporation of genomic surveillance has represented a significant advance in this process. By integrating genomic surveillance with epidemiological, laboratory, spatial, and ecological information, the capacity to interpret events; to formulate and assess hypotheses regarding potential pathways for the incursion and spread of the virus; to track the evolution of the virus; and to give direction to improvements in surveillance strategies, have all been enhanced. However, the results show that genomic data must remain integrated with epidemiological investigation in the field, serving as supplementary information sources for constructing the conclusions of the OVS.

The Brazilian experience also shows that the robustness of a surveillance system does not depend solely on its ability to respond to outbreaks but also on its capacity to learn and adapt continuously to changes in the epidemiological landscape. The increase in the number of species targeted by surveillance, the incorporation of new epidemiological interfaces, the strengthening of laboratory capacity, and the increasing use of genomic information are all examples of this adaptation over the course of the emergency.

For the coming years, it is strategic to consolidate these advances and strengthen the capacity for epidemiological intelligence at both central and state levels, promoting greater integration and interoperability between epidemiological, laboratory, genomic, ecological, environmental, and production-system data. More robust analytical systems will be able to

allow the large volume of information produced by surveillance to be converted into actionable knowledge for risk assessment, early warning, and prioritization by the OVS. This need has already been pointed out in the report's description of the outlook.

The consolidated results contained in this report therefore show that Brazil has a comprehensive and technically robust surveillance structure capable of detecting, investigating, characterizing, and responding to HPAI events. Maintaining this capacity, however, requires continued investment in surveillance, in diagnostic capability, data qualification, inter-institutional integration, and the incorporation of new analytical tools. At the same time, given the dynamic nature of HPAI, the diversity of involved hosts, and the multiplicity of interfaces capable of favoring the introduction and spread of the virus, the **implementation of biosecurity measures in poultry establishments** assumes increasing importance as a fundamental component of prevention. The continuous strengthening of these measures, combined with risk-based surveillance and early detection, constitutes one of the main strategies to reduce the probability of the introduction of the virus into commercial domestic bird populations.

Rather than merely responding to the emergency that began in 2023, the challenge is now to consolidate a permanent, integrated, and risk-oriented surveillance system capable of tracking the evolution of HPAI in Brazil and the world, responding promptly to significant changes in the epidemiological landscape, and supporting the protection of Brazil's poultry-raising industry and public health.

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ANNEXES

Table 1. Sequenced samples and identification of genotypes resulting from the official surveillance of the Highly Pathogen Avian Influenza virus in Brazil

ID	State	Municipality	Species	Date of detection	Genotype	Sequencing coverage
1	ES	Cariacica	Cabot's tern	15/05/2023	—	Partial
2	ES	Cariacica	Cabot's tern	15/05/2023	B3.2	Complete genome
3	ES	Cariacica	Brown booby	15/05/2023	—	Partial
4	ES	Serra	Tropical screech owl	26/05/2023	—	Partial
5	ES	Serra	Tropical screech owl	26/05/2023	B3.2	Complete genome
6	RJ	Niterói	Magnificent Frigatebird	03/06/2023	B3.2	Complete genome
7	SP	Ubatuba	Royal tern	05/06/2023	—	Partial
8	SP	Ubatuba	Royal tern	05/06/2023	B3.2	Complete genome
9	BA	Caravelas	Royal tern	06/06/2023	—	Partial
13	SC	Itapoá	Royal tern	25/07/2023	B3.2	Complete genome
14	SC	—	Guinea fowl	15/07/2023	B3.2	Complete genome
15	PR	Antonina	Royal tern	23/06/2023	B3.2	Complete genome
16	ES	Itapemirim	Great black hawk	07/06/2023	B3.2	Complete genome
17	MS	Bonito	Hen	22/09/2023	B3.2	Complete genome
19	SC	São Francisco do Sul	Brown booby	27/09/2023	B3.2	Complete genome
20	RS	Rio Grande	South American sea lion	03/10/2023	B3.2	Complete genome
21	RS	Torres	South American sea lion	11/10/2023	B3.2	Complete genome
23	ES	Piúma	Common tern	27/05/2023	—	Partial
24	SC	Penha	Southern lapwing	29/08/2023	—	Partial
25	SP	Bertioga	Manx shearwater	18/09/2023	—	Partial
27	RS	São José do Norte	Royal tern	13/10/2023	B3.2	Complete genome
28	RS	—	South American fur seal	06/10/2023	—	Partial
30	SP	Bertioga	American Golden Plover	08/11/2023	—	Partial
31	SP	Bertioga	American Golden Plover	08/11/2023	B3.2	Complete genome
32	SP	São Sebastião	White-chinned petrel	09/11/2023	B3.2	Complete genome
33	SC	Penha	Common tern	09/11/2023	B3.2	Complete genome
34	SP	Ubatuba	White-chinned petrel	10/11/2023	B3.2	Complete genome
35	PR	Matinhos	Cabot's tern	14/11/2023	B3.2	Complete genome
36	SP	Ilha Comprida	Cabot's tern	16/11/2023	B3.2	Complete genome
37	SP	Praia Grande	Royal tern	13/12/2023	B3.2	Complete genome
38	ES	Itapemirim	Common tern	02/02/2024	B3.2	Complete genome
39	RJ	Macaé	Common tern	09/02/2024	B3.2	Complete genome
40	SP	Bertioga	Cabot's tern	22/02/2024	B3.2	Complete genome
41	ES	Piúma	Common tern	02/03/2024	B3.2	Complete genome
42	RJ	São João da Barra	Common tern	02/03/2024	—	Partial
43	RJ	São Francisco de Itabapoana	Common tern	02/03/2024	B3.2	Complete genome
44	RJ	Cabo Frio	Common tern	09/03/2024	B3.2	Complete genome
45	RJ	Rio das Ostras	Common tern	15/03/2024	B3.2	Complete genome
48	ES	Vitória	Cabot's tern	26/04/2024	B3.2	Complete genome
51	RJ	Macaé	Cabot's tern	12/04/2024	B3.2	Complete genome

52	RJ	São Francisco de Itabapoana	Common tern	05/04/2024	B3.2	Complete genome
56	RS	Sapucaia do Sul	Black swan	15/05/2025	K.8	Complete genome
57	RS	Sapucaia do Sul	Black-necked swan	15/05/2025	—	Complete genome
58	RS	Montenegro	Hen	15/05/2025	—	Complete genome
59	MG	Mateus Leme	Black swan	25/05/2025	—	Complete genome
60	MG	Mateus Leme	Black swan	25/05/2025	—	Complete genome
61	RS	Montenegro	Rufous hornero	28/05/2025	—	Complete genome
62	MT	Campinápolis	Hen	07/06/2025	—	Complete genome
63	DF	Brasilia	White-faced whistling duck	03/06/2025	N.1	Complete genome
64	DF	Brasilia	Emu	14/06/2025	—	Complete genome
65	GO	Santo Antônio da Barra	Hen	13/06/2025	—	Complete genome
67	ES	São Mateus	White-faced whistling duck	27/06/2025	—	Complete genome
69	SP	São Paulo	Hen	11/07/2025	—	Complete genome
70	CE	Quixeramobim	Hen	17/07/2025	—	Complete genome
71	RJ	Rio de Janeiro	Guinea fowl	22/07/2025	—	Complete genome
72	SP	Monte Azul Paulista	Hen	23/07/2025	—	Complete genome
74	MG	Mateus Leme	Domestic grey goose	25/05/2025	N.1	Complete genome
75	MT	Cuiabá	Indian peafowl	23/12/2025	—	Partial
76	MT	Acorizal	Hen	16/01/2026	—	Partial (HA, NP, NA, MP and NS)
77	MT	Cuiabá	Hen	23/12/2025	N.1	Complete genome
78	RS	Santa Vitória do Palmar	Coscoroba swan	03/03/2026	—	Complete genome
79	SP	Guaíra	White-faced whistling duck	15/07/2026	—	Complete genome
80	RS	Coqueiros do Sul	Hen	17/07/2026	—	Complete genome



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