Revista Argentina de Microbiología 57 (2025) 327–335

asociación argentina de microbiología

REVISTA ARGENTINA DE MICROBIOLOGÍA

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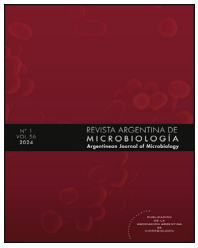

ORIGINAL ARTICLE

# Detection and characterization of highly pathogenic avian influenza A (H5N1) clade 2.3.4.4b virus circulating in Argentina in 2023

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Received 26 January 2024; accepted 28 August 2024
Available online 12 November 2024

**KEYWORDS**
Avian influenza;
H5N1;
South America;
Epidemiological surveillance;
Outbreak

**Abstract** In 2021, avian influenza A (H5N1) clade 2.3.4.4b virus spread to North America and then to Central and South America in October 2022, extending from Colombia to Chile in three months. During 2023, several countries, mostly in the Americas, reported outbreaks in poultry, wild birds and mammals, as well as the emergence of two cases in humans (one in Ecuador in January and one in Chile in March). As of September 20th, 2023, 17 countries in the Americas Region have recorded cases of A (H5N1) in birds and mammals. On February 14th, 2023, Argentina confirmed the first case of avian influenza in wild birds, which was later detected in backyard and commercial poultry, and in the South-American sea lion (*Otaria flavescens*) in Tierra del Fuego, in the south of the country. So far, 21 suspected cases have been recorded in humans; however, all of them tested negative for Influenza A virus. Hemagglutinin sequence

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https://doi.org/10.1016/j.ram.2024.08.002

data of animal viruses analyzed in this report showed that Argentinian viruses clustered together with those isolated in other countries of the region. Epidemiological data suggested the possibility of multiple simultaneous entries of the avian virus, highlighting the role of migratory avian populations in the introduction and dissemination of the disease in Argentina. Continued comprehensive surveillance of these viruses in animals and people worldwide, along with ongoing preparedness efforts, are critical to determine the public health risk.


**PALABRAS CLAVE**
Influenza aviar;
H5N1;
América del Sur;
Vigilancia epidemiológica;
Brote

**Detección y caracterización del virus de la influenza aviar A(H5N1) del clado 2.3.4.4b circulante en Argentina en 2023**

**Resumen** En 2021, el virus de influenza aviar A(H5N1) del clado 2.3.4.4b se propagó hacia América del Norte, para luego pasar a América Central y alcanzar América del Sur en octubre de 2022, esparciéndose desde Colombia hasta Chile en tres meses. Durante 2023, varios países americanos reportaron brotes en aves de corral, aves silvestres y mamíferos. En ese año se informaron dos casos en humanos, uno en Ecuador y otro en Chile. Al 20 de septiembre de 2023, 17 países de la región de las Américas habían registrado casos de influenza A(H5N1) en aves y mamíferos. Argentina confirmó el primer caso de gripe aviar en aves silvestres el 14 de febrero de 2023, luego de lo cual también se detectaron casos en aves de traspatio, aves de corral y lobos marinos sudamericanos (*Otaria flavescens*) en Tierra del Fuego, hacia el sur del país. Hasta el momento se han registrado 21 casos sospechosos en humanos; en todos ellos, el virus de la influenza A resultó no detectable. Los datos de las secuencias de hemaglutinina de los virus animales analizados mostraron que los virus argentinos se agruparon junto con los aislados en otros países de la región. Los datos epidemiológicos sugieren la posibilidad de múltiples entradas simultáneas del virus aviar y destacan el papel de las aves migratorias en la introducción y diseminación de la enfermedad en el país. La vigilancia integral continua de estos virus en animales y personas en todo el mundo es fundamental para determinar el riesgo que estos entrañan para la salud pública, junto con los esfuerzos de preparación en curso.


## Introduction

The subtype H5 influenza virus is the most widely detected avian influenza virus and has caused numerous disease outbreaks in domestic poultry and wild birds around the world[10]. The currently circulating highly pathogenic avian influenza virus (HPAI) in America belongs to subtype H5N1 clade 2.3.4.4b. In 2020, the first outbreaks emerged in Asia, Africa and Europe and since then, through migratory wild birds, the virus reached North America in 2021 and spread all over the continent[3–5,15,20,23]. In October 2022, the virus reached South America and was officially reported in Colombia; later on it was also reported in Peru, Ecuador, and Venezuela[2].

This virus is of major concern because of its high transmission capacity and adaptability to different hosts, including mammals[1,17]. In this sense, not only backyard and commercial poultry are in danger, but the biodiversity of the wild bird population and other species is also threatened[18].

HPAI viruses can spread rapidly among poultry through direct contact with infected waterfowl or other poultry, or through contact with contaminated fomites, surfaces or water[9,7].

On February 14th 2023, the Official Laboratory of the National Service for Agrifood Health and Quality of Argentina (SENASA) confirmed the first case of HPAI A (H5N1) infection in an Andean guayata (*Chloephaga melanoptera*) at Parque Nacional Laguna de los Pozuelos Monument and Biosphere Reserve located in the Province of Jujuy. This was the first detection of avian influenza A (H5N1) in Argentina since widespread active monitoring of both domestic and wild birds was implemented in 1997. From February to July 27th, 2023, a total of 104 outbreaks had been detected, affecting only birds.

Starting on August 10th, several outbreaks of HPAI in marine mammals were also detected along the Argentinian Atlantic coast, beginning in the south and extending even to Uruguay. Since the first Influenza A (H5N1) case in Argentina, intensified epidemiological surveillance in exposed humans (in contact with sick or dead birds in the context of avian influenza outbreaks) was encouraged by the Ministry of Health to early detect any potential transmission from animals to humans.

This manuscript describes the epidemiological and partial genomic characterization of highly pathogenic avian influenza (HPAI) H5N1 viruses circulating in Argentina between February and August 2023. The aim of this research is to present the phylogenetic study based on the hemagglutinin (HA) gene of 53 Argentinian viruses obtained by whole genome sequencing from viruses detected in poultry, wild birds and mammals, and evaluate their relationship with the published strains circulating in neighboring countries in order to contribute to the epidemiological study of the region. Furthermore, it is important to highlight that epidemiological surveillance was conducted by both the Official Animal Health Organization and the Human Health Organization under the ''One Health'' concept.

## Materials and methods

### Animal surveillance: sample collection and viral detection

Tracheal and/or cloacal swabs collected from birds and mammals during the active and passive surveillance performed by SENASA were sent to the Official Laboratory of SENASA for influenza A testing and subtyping by RT-qPCR according to the recommendations of the WOAH (World Organization for Animal Health) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2022 following the standard operating protocols for NVSL-USDA[19].

During 2022, active surveillance included sampling activities aimed at demonstrating the absence of HPAI in commercial and backyard flocks and wild birds. Active surveillance in commercial flocks targeted breeders and layers, and both commercial and backyard surveillance were risk-based. Surveillance in wild birds was opportunistic and conducted in collaboration with researchers and authorized duck hunters (Suppl. Table 1). During 2023, active surveillance was suspended due to the HPAI outbreak, implementing passive surveillance on notifications of any suspicion or event for the earliest possible detection of HPAI. When an HPAI outbreak was confirmed in backyard poultry, bird movements were suspended, all birds were immediately culled, and an epidemiological survey was conducted to identify the possible source of the outbreak. The survey included gathering information on biosecurity, bird movements, visitor registers, proximity to other poultry farms, proximity to water bodies, and the presence of wild birds. When the outbreak involved only wild birds, only the epidemiological survey was conducted. In outbreaks involving commercial poultry, in addition to the previously mentioned measures, the movements of fertile eggs were traced, and hatcheries were visited and eggs were destroyed and disposed of. The origin and storage of feed were also investigated. All commercial farms within the perifocal and surveillance zones (3 km and between 3 and 10 km around the outbreak, respectively) were visited, and sampled for molecular diagnosis.

### Human surveillance: Sample collection and viral detection

Respiratory samples (nasopharyngeal aspirates, nasopharyngeal swabs, nasal swabs, pharyngeal swabs) were collected from patients who met the definition of suspected avian influenza cases established by the Ministry of Health (https://bancos.salud.gob.ar/sites/default/files/2023-02/comunicacion_influenza_aviar-20230210.pdf). As this was an observational study undertaken as part of the routine virological surveillance (Anonymously, without identification of patients), written informed consent and explicit ethical approval were not required. This procedure is indicated in the Terms of Reference for WHO-NICs (World Health Organization-National Influenza Centres), which describe the bases of WHO Surveillance in more than 130 countries[21]. These samples were sent to the National Reference Laboratory (NRL) INEI-ANLIS ''Dr. Carlos G. Malbrán''. RNA was extracted using the QIAamp® Viral RNA Mini Kit (Qiagen). For the simultaneous detection of Influenza and SARS-CoV-2, the CDC (Centers for Disease Control and Prevention) reference technique was used, following PAHO (Pan American Health Organization)/WHO guidelines[24]. Human samples negative for influenza and SARS-CoV-2 were also screened for other respiratory viruses using the Viasure Respiratory Panel IV Real Time PCR Detection Kit, which included respiratory syncytial virus (RSV), human adenovirus (ADV), parainfluenza viruses 1–4 (PARA I–IV), human metapneumovirus (HMPV), human rhinovirus (HRV), human coronavirus (HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1).

### Genomic characterization of the hemagglutinin gene

Fifty-three influenza A (H5N1) viruses detected by RT-qPCR (1 from wild bird, Andean guayata, 50 from domestic birds and 2 from sea lions) were selected for next-generation sequencing (NGS). RNA was extracted directly from the samples using the QIAamp® Viral RNA Mini Kit (Qiagen), following the manufacturer's instructions.

RNA was reverse transcribed, and the entire influenza genome was amplified in a single multi-segment real-time polymerase chain reaction (M-RTPCR)[27] using the Uni/Inf primer set. The amplification was performed in 25 μl reactions containing 8 μl nuclease-free water, 12.5 μl 2× RT-PCR buffer, 1 μl pool InfA (Uni12/Inf-1, Uni12/Inf-3. Uni13/Inf-1. 10 μM from each of them), 0.3 μl Uni12/Inf3 (10 μmol/l), 0.5 μl SuperScript III One-Step RT-PCR with Platinum Taq High Fidelity (Invitrogen), and 3 μl extracted RNA. Following PCR, the amplicons were purified with Exo SAP IT.

Library preparation was performed from PCR products using an adapted protocol with the COVIDseq kit (Illumina, USA), and sequencing was performed on the MiSeq or NovaSeq 6000 platform (Illumina, USA) by paired-end sequencing (2 × 150 nucleotides).

Contiguous nucleotide sequence (contigs) assembly was conducted using the FLU module of the Iterative Refinement Meta-Assembler (IRMA v.1.0.3), employing IRMA default settings.

### Evolutionary analysis by the maximum likelihood method (timetree)

Consensus sequences of HA were blasted in order to identify subtypes from GISAID (Global Initiative on Sharing

all Influenza Data)[16]. Sequences were downloaded and aligned using BioEdit version 7.2.5, by the MUSCLE (Multiple Sequence Comparison by Log-Expectation) command. Cleavage site of H5 HPAI virus was also analyzed by aminoacidic translation and alignment by Bioedit. Phylogenetic trees were created using MEGA (Molecular Evolutionary Genetics Analysis Software) Version 11 by the maximum likelihood method based on the Kimura 2-parameter model according to the best model suggested by the software. The timetree was generated using the RelTime method[25]. Divergence times for all branching points in the topology were calculated using the maximum likelihood method and the Hasegawa–Kishino–Yano model, according to the best model suggested by the software[11]. The estimated log likelihood value of the topology shown is −4264.57. A discrete gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3062)). The tree is drawn to scale, with branch lengths measured in the relative number of substitutions per site. This analysis involved 115 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 1659 positions in the final dataset. Evolutionary analyses were conducted in MEGA11[26].

## Results and discussion

Since the first case of H5 HPAI virus detected on February 14th, 2023, at epidemiological week 6 (EW6), 526 notifications were addressed. Samples were sent to the SENASA Official Laboratory to diagnose avian influenza by the RT-qPCR technique. Between February and August 2023, 113 (23.6%) positive notifications were reported (69.9% backyard poultry, 15.9% commercial poultry and 14.2% wild animals). Of these, 53 were selected from different regions of Argentina in order to perform whole genome sequence: 36 backyard poultry (which accounts for 45.6% of the total positive notifications in the backyard category), 14 commercial poultry (77.8% of the total positive notifications in this category) and 3 wild animals (18.8% of the total positive wild notifications) including the first wild bird case and the first two sea lion (*Otaria flavescens*) cases. The eight segments of the genome of each virus were uploaded to the GISAID database (Table 1).

The analysis of the hemagglutinin (HA) and neuraminidase (NA) segments demonstrated that all viruses belonged to subtype H5N1. Furthermore, based on the cleavage site analysis of the HA gene confirmed high pathogenicity by the presence of a polybasic motif (Suppl. Fig. 1) according to the Offlu Influenza Cleavage Site Guide[22]. All viruses had the aminoacid motif PLREKRRKR/GLF, except for one from an *Anatidae* spp. which had the mutation of an S in position 339 (PLSEKRRKR/GLF).

In order to perform the phylogenetic analysis of the HA gene, selected data were downloaded from GISAID and aligned together with the Argentinian sequences. The phylogenetic tree confirmed that all samples belonged to the 2.3.4.4b clade (Suppl. Fig. 2).

Figure 1 shows that Argentinian viruses clustered together with others detected in neighboring countries. It should be noted that viruses isolated from sea lions (GISAID id: 18698754, 18698755) are grouped with those from Chile and Peru, including the human case reported by Chile in 2023 (GISAID id: 17468386), suggesting an introduction from the Pacific Way. The clusters obtained are consistent with the evolution of the outbreaks observed throughout the Region of the Americas.

The first case detected in wild birds (*C. melanoptera*) in Argentina, occurred in the province of Jujuy (GISAID id: 18698459), located 540 km from the Bolivian outbreaks and 470 km from the Chilean outbreaks detected at the same time during EW6. There was a peak in EW7 with the emergence of clinical signs in several outbreaks, documented in the provinces of Santa Fe, Buenos Aires, San Luis, Santiago del Estero, Córdoba and Neuquén, most of them situated less than 300 km from those registered in the previous week, with the exception of those that occurred in the southern region of Buenos Aires and Neuquén, which extended beyond this radius. The latter were closer to cases detected in Chile, approximately 300 km away (Fig. 2). Given the distribution of the outbreaks that began in the same epidemiological week, with respect to the initial ones, it is reasonable to hypothesize that the virus entered our country simultaneously through several locations.

In the Department of Confluencia in Neuquén (Fig. 3), three outbreaks were detected in the course of 53 days, with only 11 days between the second and third case (GISAID id: 18698502, 18698517, 18698520). Although the farthest outbreaks were 4.5 km away, the sequences obtained share more similarities with those from Peru and Chile than with each other (Fig. 1).

In contrast, it was observed that the two outbreaks detected in Pilar, Buenos Aires, (GISAID id: 18698519, 18698521) and the three outbreaks detected in Diamante, Entre Ríos (GISAID id: 18698524, 18698525, 18698526) are closely related as determined by the phylogenetic tree (Fig. 1), despite a distance of 310 km between both groups (Fig. 3). The outbreaks were detected 25 days apart during the month of May, the first week in Buenos Aires and the last two weeks in Entre Ríos. The maximum distance between the three outbreaks in Pilar was 1500 m, while the outbreaks in Diamante were 500 m apart from each other. It is worth noting that the five outbreaks involving this genetic group from Pilar and Entre Ríos occurred in commercial farms. The window of detections (between 10 and 25 days) between these two regions may reflect overlapping infection dynamics. The incursion of the virus might be attributed to a biosecurity failure, suggesting a common source of infection related to wild birds. However, horizontally transmission between farms cannot be excluded, due to their proximity within the same area.

Two outbreaks in Chaco (GISAID id: 18698518, 18698523) were detected 4 km apart with a difference of 10 days. Both viruses were closely related as revealed by the phylogenetic tree shown in Figure 1, indicating a common source, likely associated with wild birds. Notably, they are phylogenetically close to a sequence obtained from an outbreak that had been detected a week earlier in Chubut (GISAID id: 18698514) 2000 km away (Fig. 3), which indicates the widespread dispersion probably associated with wild birds.

With regard to the hypothetical origins of the outbreaks, in 77% of them, veterinarians investigating the cases

**Table 1** Virus selected for genomic characterization.

| Isolate name | GISAID accession number | Sample collection date | Category | Geographic location |
|---|---|---|---|---|
| A/Goose/Argentina/389-1/2023 | 18698459 | 11/2/2023 | Wild | JUJUY/RINCONADA |
| A/Chicken/Argentina/464-4/2023 | 18698460 | 16/2/2023 | Backyard | CÓRDOBA/UNIÓN |
| A/Chicken/Argentina/477-5/2023 | 18698462 | 18/2/2023 | Backyard | CÓRDOBA/RÍO SEGUNDO |
| A/Chicken/Argentina/481-2/2023 | 18698464 | 18/2/2023 | Backyard | CÓRDOBA/MARCOS JUÁREZ |
| A/Chicken/Argentina/485-6/2023 | 18698466 | 19/2/2023 | Backyard | CÓRDOBA/GRAL. ROCA |
| A/Chicken/Argentina/491-2/2023 | 18698469 | 20/2/2023 | Backyard | BUENOS AIRES/PUAN |
| A/Chicken/Argentina/501-1/2023 | 18698471 | 20/2/2023 | Backyard | CÓRDOBA/RÍO PRIMERO |
| A/Chicken/Argentina/506-2/2023 | 18698474 | 20/2/2023 | Backyard | CÓRDOBA/TULUMBA |
| A/Chicken/Argentina/509-2/2023 | 18698476 | 21/2/2023 | Backyard | SANTA FE/SAN JERÓNIMO |
| A/Chicken/Argentina/556-6/2023 | 18698478 | 23/2/2023 | Backyard | BUENOS AIRES/SAN CAYETANO |
| A/Chicken/Argentina/559-8/2023 | 18698480 | 22/2/2023 | Backyard | SAN LUIS/LA CAPITAL |
| A/Chicken/Argentina/578-2/2023 | 18698482 | 23/2/2023 | Backyard | RÍO NEGRO/AVELLANEDA |
| A/Avian/Argentina/579-5/2023 | 18698485 | 24/2/2023 | Backyard | BUENOS AIRES/AZUL |
| A/Avian/Argentina/586-4/2023 | 18698487 | 24/2/2023 | Backyard | CÓRDOBA/UNIÓN |
| A/Chicken/Argentina/588-4/2023 | 18698490 | 24/2/2023 | Backyard | CÓRDOBA/JUÁREZ CELMAN |
| A/Chicken/Argentina/606-1/2023 | 18698492 | 27/2/2023 | Backyard | BUENOS AIRES/RAUCH |
| A/Chicken/Argentina/736-1/2023 | 18698494 | 3/3/2023 | Commercial | BUENOS AIRES/GRAL.PUEYRREDON |
| A/Chicken/Argentina/747-1/2023 | 18698730 | 4/3/2023 | Commercial | BUENOS AIRES/GRAL.PUEYRREDON |
| A/Turkey/Argentina/753-1/2023 | 18698497 | 4/3/2023 | Backyard | SANTA FE/LAS COLONIAS |
| A/Chicken/Argentina/858-1/2023 | 18698499 | 8/3/2023 | Backyard | CÓRDOBA/SANTA MARÍA |
| A/Chicken/Argentina/895-1/2023 | 18698731 | 9/3/2023 | Backyard | SAN LUIS/JUNÍN |
| A/Chicken/Argentina/919-3/2023 | 18698502 | 13/3/2023 | Backyard | NEUQUÉN/CONFLUENCIA |
| A/Chicken/Argentina/1035-1/2023 | 18698504 | 16/3/2023 | Backyard | LA PAMPA/TOAY |
| A/Chicken/Argentina/1147-2/2023 | 18698505 | 21/3/2023 | Backyard | NEUQUÉN/ZAPALA |
| A/Chicken/Argentina/1200-1/2023 | 18698506 | 27/3/2023 | Backyard | SANTA FE/GRAL.LOPEZ |
| A/Chicken/Argentina/1340-2/2023 | 18698507 | 31/3/2023 | Commercial | BUENOS AIRES/GRAL.ALVEAR |
| A/Turkey/Argentina/1348-3/2023 | 18698508 | 31/3/2023 | Backyard | CORRIENTES/SAN COSME |
| A/Chicken/Argentina/1375-6/2023 | 18698509 | 3/4/2023 | Commercial | CHUBUT/GAIMAN |
| A/Chicken/Argentina/1416-3/2023 | 18698510 | 3/4/2023 | Backyard | RÍO NEGRO/PICHI MAHUIDA |
| A/Chicken/Argentina/1495-4/2023 | 18698719 | 10/4/2023 | Backyard | FORMOSA/PIRANÉ |
| A/Chicken/Argentina/1530-3/2023 | 18698511 | 12/4/2023 | Backyard | CHUBUT/BIEDMA |
| A/Chicken/Argentina/1708-1/2023 | 18698512 | 19/4/2023 | Backyard | MENDOZA/SAN CARLOS |
| A/Turkey/Argentina/1710-1/2023 | 18698513 | 17/4/2023 | Backyard | SANTA CRUZ/GÜER AIKE |
| A/Turkey/Argentina/1711-2/2023 | 18698514 | 19/4/2023 | Backyard | CHUBUT/FUTALEUFÚ |
| A/Duck/Argentina/1712-5/2023 | 18698515 | 19/4/2023 | Backyard | CORRIENTES/CONCEPCIÓN |
| A/Avian/Argentina/1762-2/2023 | 18698516 | 21/4/2023 | Backyard | BUENOS AIRES/PERGAMINO |
| A/Avian/Argentina/1790-5/2023 | 18698517 | 24/4/2023 | Commercial | NEUQUÉN/CONFLUENCIA |
| A/Chicken/Argentina/1976-2/2023 | 18698518 | 1/5/2023 | Backyard | CHACO/SAN FERNANDO |
| A/Chicken/Argentina/1984-4/2023 | 18698519 | 4/5/2023 | Commercial | BUENOS AIRES/PILAR |
| A/Chicken/Argentina/2016-2/2023 | 18698732 | 3/5/2023 | Backyard | FORMOSA/PILCOMAYO |
| A/Chicken/Argentina/2034-5/2023 | 18698520 | 5/5/2023 | Commercial | NEUQUÉN/CONFLUENCIA |
| A/Chicken/Argentina/2049-3/2023 | 18698521 | 8/5/2023 | Commercial | BUENOS AIRES/PILAR |
| A/Chicken/Argentina/2064-3/2023 | 18698522 | 8/5/2023 | Commercial | CHUBUT/RAWSON |
| A/Duck/Argentina/2197-1/2023 | 18698523 | 11/5/2023 | Backyard | CHACO/LIBERTAD |
| A/Chicken/Argentina/2305-1/2023 | 18698524 | 18/5/2023 | Commercial | ENTRE RÍOS/DIAMANTE |
| A/Chicken/Argentina/2305-6/2023 | 18698525 | 18/5/2023 | Commercial | ENTRE RÍOS/DIAMANTE |
| A/Chicken/Argentina/2483-3/2023 | 18698526 | 29/5/2023 | Commercial | ENTRE RÍOS/DIAMANTE |
| A/Chicken/Argentina/2796-2/2023 | 18698527 | 13/6/2023 | Commercial | BUENOS AIRES/LA PLATA |
| A/Chicken/Argentina/3346-1/2023 | 18698528 | 6/7/2023 | Backyard | FORMOSA/PATIÑO |
| A/Chicken/Argentina/3657-2/2023 | 18698529 | 26/7/2023 | Backyard | SALTA/METÁN |
| A/Chicken/Argentina/3695-3/2023 | 18698530 | 27/7/2023 | Commercial | CÓRDOBA/PUNILLA |
| A/SeaLion/Argentina/3849-4/2023 | 18698754 | 8/8/2023 | Wild | TIERRA DEL FUEGO/RÍO GRANDE |
| A/SeaLion/Argentina/3893-1/2023 | 18698755 | 11/8/2023 | Wild | RíO NEGRO/ADOLFO ALSINA |

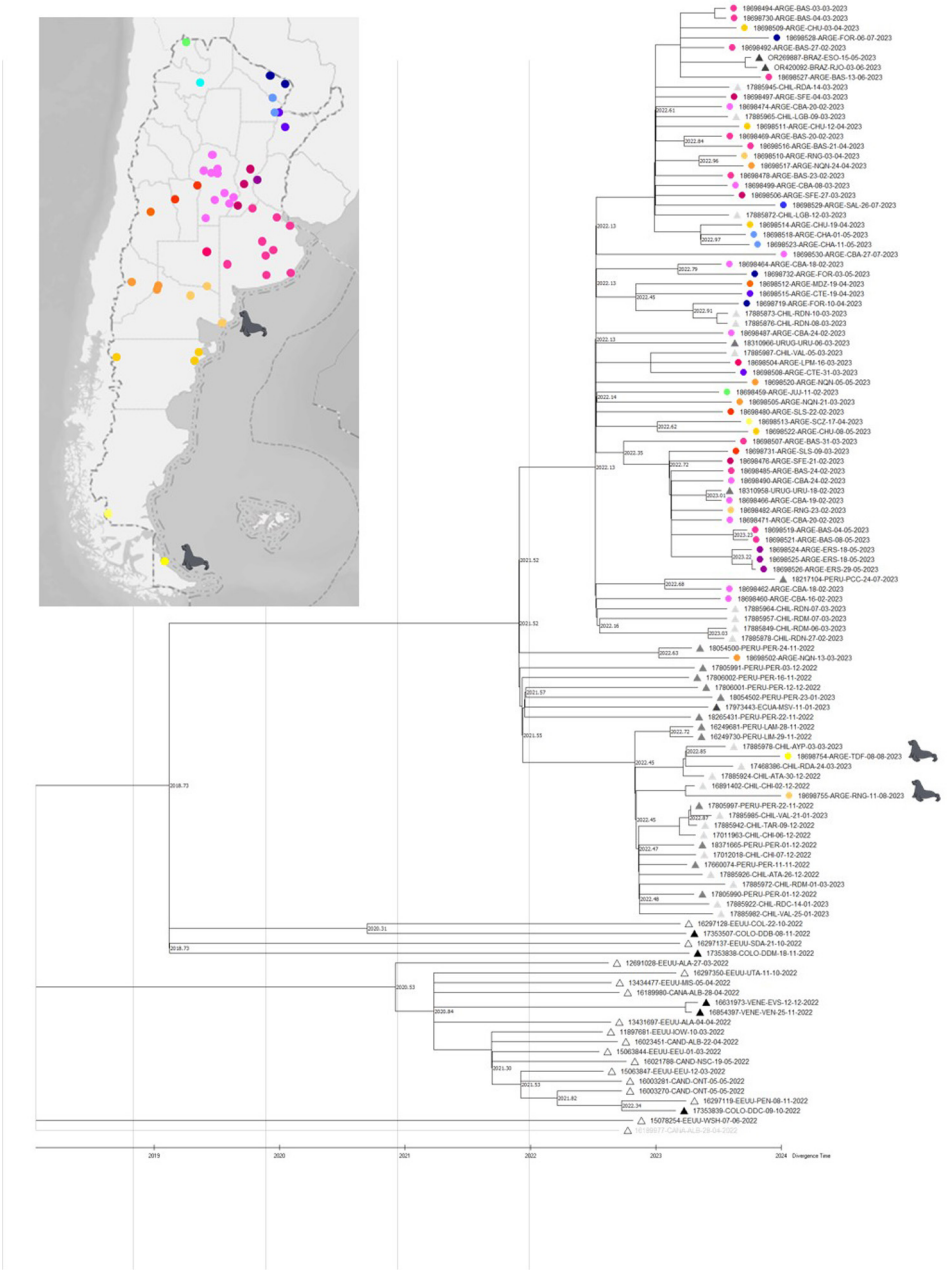

**Figure 1** Phylogenetic timetree of the HA gene. ● Sequences from Argentina. ▲ Sequences from other countries. Different colors indicate different Argentinian provinces.

reported contact with wild birds as a possible source of infection. Furthermore, 96% of the outbreaks were within 10 km of artificial or natural bodies of water, and in 23% wild birds were seen close to domestic birds.

During the time of the outbreaks, Argentina was suffering from a prolonged drought, which may relate to increased contact between wild and domestic birds. In the commercial outbreaks, the most likely point of entry for the avian influenza virus into the premises were biosecurity failures. Since Argentina only trades poultry products from countries free of HPAI, the introduction by poultry trade was dismissed.

The observed biosecurity failures included failure to change clothing and lack of footwear disinfection before entering the poultry houses, poor maintenance of footbaths, absence of wheel dips and the presence of wild bird feces in the vicinity of the poultry houses.

Based on the results of the epidemiological surveys conducted during the outbreaks, it was determined that horizontal transmission between farms was observed in very few cases. There was only one instance where it was confirmed that birds were traded between two backyard flocks. Regarding the outbreaks detected in Las Lajas, Neuquén, which were all within the perifocal zone (3 km from the out-

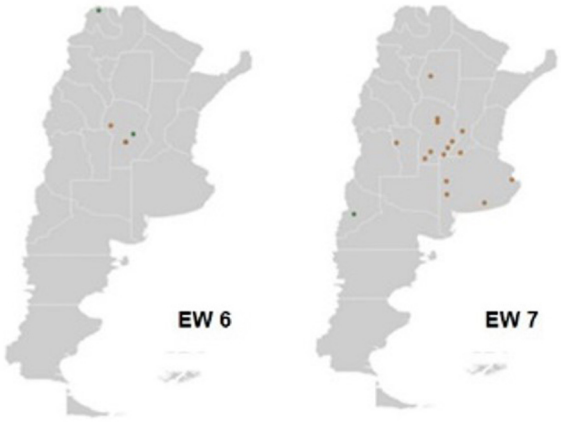


**Figure 2** Spatial distribution of HPAI outbreaks in Argentina in epidemiological weeks six and seven.

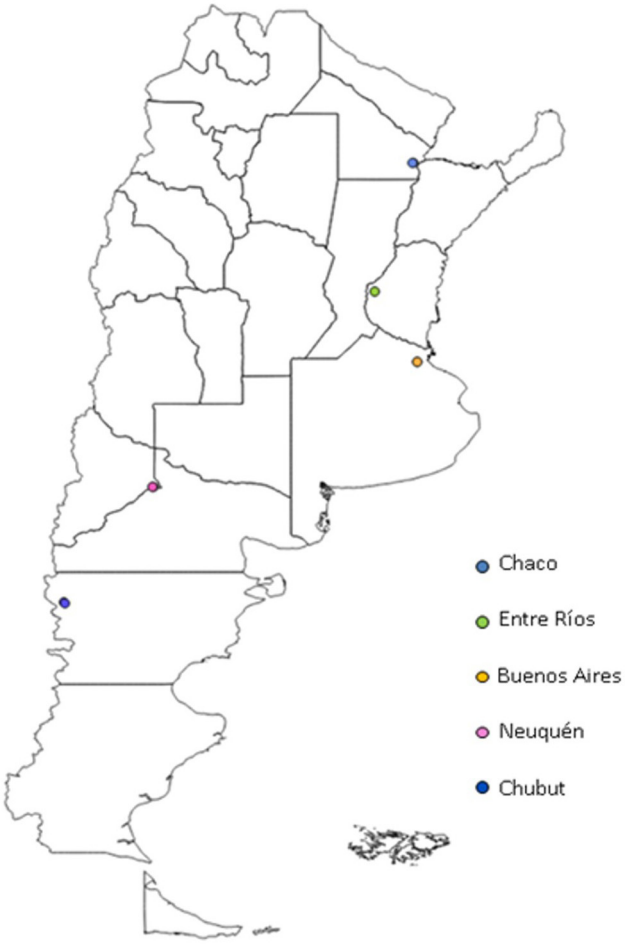


**Figure 3** Location of phylogenetically related outbreaks.

break), it is believed that the source of infection was due to direct contact between the birds in the involved backyard flocks. Among the outbreaks observed in the surveillance zone (between 3 and 10 km from the outbreak), there are two backyard flocks in Chubut whose owners were family members, and who frequently visited each other's properties. These were the only cases where a clear horizontal relationship between outbreaks was identified. It is possible that the outbreaks in Diamante, Entre Ríos, and Pilar, Buenos Aires, also had a common source of infection or that the virus was transmitted horizontally between the farms due to their proximity, although this could not be confirmed. In the remaining cases, according to the epidemiological information, wild birds served as the primary source of infection, similar to what happened in Europe[8]. The introduction of the pathogen was attributed to biosecurity lapses. This phenomenon may relate to the dispersed distribution of poultry farms across the territory, which differs from the more concentrated distribution observed in other countries or regions, such as in Europe, where horizontal transmission is more related to the high density[6]. These findings highlight the critical role of biosecurity measures in mitigating the risk of horizontal transmission, and emphasize the need for tailored strategies considering the spatial characteristics of poultry farm distribution in Argentina.

Between EW8 and EW22 2023, the NRL INEI – ANLIS ''Dr. Carlos G. Malbrán'' received 17 of 21 respiratory samples collected from suspected Human cases; the remaining cases were studied by other National Influenza Centers. The median age of these patients was 43 years and they lived in different locations, mostly in the southern and central provinces of Argentina. No avian influenza virus was detected in any of the samples studied. The multiplex PCR for other respiratory viruses detected the following viruses: 1 influenza A (H1N1) pdm09, 1 PARA II, 1 ADV, 2 HRV.

Only seven human infections caused by avian influenza A (H5N1) have been reported since its introduction in Americas. One in the United States of America in April 2022, one in Ecuador in January 2023, one in Chile in March 2023 and four more in the United States due to exposure to dairy cows between January and March 2024[14,12,13]. Fortunately, no human cases have been detected in Argentina to date.

## Conclusions

The molecular analysis of the HA and NA genes of sequenced Argentinian viruses showed that they all belonged to the H5N1 subtype, clade 2.3.4.4b of high pathogenicity. The phylogenetic study based on the HA gene, together with the epidemiological analysis, suggested multiple simultaneous entries through migratory birds, and a separate pathway of introduction of the virus in marine mammals along the Pacific coast.

The reported cases investigated by veterinarians indicated contact with wild birds as the main source of infection, attributing most of them to biosecurity lapses and in very few cases to horizontal transmission between farms. In this context, it is important to continue with active and passive surveillance in order to control a possible new outbreak and analyze the circulating strains in the region. Continued efforts in enhancing biosecurity protocols are essential for preventing and controlling future outbreaks, particularly in regions frequented by migratory bird populations.

The outbreak of Influenza A (H5N1) is of public concern since it represents an important threat, not only to animal but also to human health due to potential zoonotic transmission. In this sense, SENASA and the Anlis Malbrán Institute, in collaboration with the Ministry of Health, have been work-

ing in coordination to control the emergency under the ''One Health'' approach.

## Funding statement

All the investigations and actions of the case were covered with SENASA's and Anlis-Malbrán Institute's own funds.

## Conflict of interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

We would like to express our gratitude to the staff of SENASA and the Malbrán Institute, involved in the entire process, especially Dr. Cristina Lema from the Neurovirosis Laboratory and to members of the Ministry of Health and SENASA for promoting joint work between both.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ram.2024.08.002.

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