



## Short Communication

## Highly pathogenic avian influenza A (H5N1) virus outbreak in Peru in 2022–2023



Nieves Sevilla<sup>a,1,\*</sup>, Wendy Lizarraga<sup>a,1</sup>, Victor Jimenez-Vasquez<sup>a</sup>, Veronica Hurtado<sup>a</sup>, Iris S. Molina<sup>a</sup>, Lilian Huarca<sup>a</sup>, Priscila Lope-Pari<sup>a</sup>, Ivan Vargas<sup>b</sup>, Gloria Arotinco<sup>a</sup>, Carlos Padilla-Rojas<sup>a</sup>

<sup>a</sup> Instituto Nacional de Salud, Lima 15066, Peru

<sup>b</sup> Centro Nacional de Epidemiología, Prevención y Control de Enfermedades, Lima 15072, Peru

## ARTICLE INFO

## Keywords:

Avian influenza A  
H5N1  
HPAI  
Clade 2.3.4.4b  
Sequencing  
2022–2023 outbreak

## ABSTRACT

**Background:** An epizootic of highly pathogenic avian influenza A (H5N1) has spread worldwide since 2022. Even though this virus has been extensively studied for many decades, little is known about its evolution in South America.

**Methods:** Here, we describe the sequencing and characterization of 13 H5N1 genomes collected from wild birds, poultry, and wild mammals in Peru during the genomic surveillance of this outbreak.

**Results:** The samples belonged to the highly pathogenic avian influenza (H5N1) 2.3.4.4b clade. Chilean and Peruvian samples clustered in the same group and therefore share a common ancestor. An analysis of the hemagglutinin and neuraminidase genes detected new mutations, some dependent upon the host type.

**Conclusions:** The genomic surveillance of highly pathogenic avian influenza is necessary to promote the One Health policy and to overcome the new problems entailed by climate change, which may alter the habitats of resident and migratory birds.

## 1. Introduction

The highly pathogenic avian influenza (HPAI) A (H5N1) virus causes bird flu, a highly contagious and fatal disease that affects both birds and mammals. Some isolated cases in humans have also been reported since 1997 when the first infection of the H5N1 subtype in humans was identified in Hong Kong [1–4]. Although it remains unclear whether HPAI will emerge as the next pandemic virus, its rapid spread will have a substantial impact on the economy, particularly on the global poultry industry [4] and consequently on food safety.

In late 2022, the Pan American Health Organization confirmed the presence of avian influenza A (H5N1) in domestic poultry and wild birds across several countries,

including Canada, Mexico, Colombia, Peru, and the United States. In Peru, the Servicio Nacional de Sanidad Animal (SENASA, Spanish acronym) is responsible for safeguarding the country against livestock-related diseases. On November 14, 2022, SENASA reported the first case of influenza A in free-ranging Peruvian pelicans (*Pelecanus thagus*) in the Piura region, where a mass mortality event involving shorebirds with systemic clinical signs (respiratory, digestive, and neurological) was recorded. Subsequently, SENASA issued a national sanitary alert [5] after the detection of HPAI A/H5N1 on November 23, 2022, to strengthen epidemiological surveillance. This was extended to December 31, 2023 [6,7].

**Abbreviations:** HPAI, highly pathogenic avian influenza; SENASA, Servicio Nacional de Sanidad Animal; HA, Hemagglutinin; NA, Neuraminidase.

\* Corresponding author.

E-mail address: [lsevilla@ins.gob.pe](mailto:lsevilla@ins.gob.pe) (N. Sevilla).

<sup>1</sup> These authors contributed equally to this study.

<https://doi.org/10.1016/j.imj.2024.100108>

Received 31 October 2023; Received in revised form 6 December 2023; Accepted 3 April 2024

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To date, extensive wild bird mortality (reaching 563,233 recorded cases) has been confirmed along the entire coast of Peru. The most frequently recorded species are the Peruvian pelican, Peruvian booby, and Guanay cormorant. Furthermore, the mass mortality of marine mammals (11,008 recorded cases) has also been reported in Peru, with the South American sea lion the most frequently recorded species (<https://www.dge.gob.pe/influenza-aviar-ah5/#aves>). Thus, the H5N1 subtype has been detected in wild birds (*Pelecanus thagus*, *Calidris alba*, and *Leucocarbo bougainvillorum*) and marine mammals (*Otaria flavescens* and *Delphinus delphis*) [8,9]. On January 9, 2023, the first case of human infection with influenza A (H5) in Latin America was reported in a 9-year-old girl in Ecuador [10], followed by a case in Chile on March 29, 2023 [11]. Currently, there is no evidence of any cases of influenza A (H5) infection among humans in Peru.

Here, we describe an analysis of the 2022–2023 outbreak of influenza A H5N1 in Peru, focusing on the genomic surveillance of emerging mutations with next-generation sequencing.

## 2. Materials and methods

The Instituto Nacional de Salud (INS, Spanish acronym) initiated the genomic surveillance of HPAI A virus (H5N1) towards the end of 2022. The present study was undertaken within the framework of the Avian Influenza Health Emergency under Resolution No. 0180-2022-MIDAGRI-SENASA of SENASA. The samples and epidemiological information were collected by authorized personnel of SENASA and the Servicio Nacional Forestal y de Fauna Silvestre (SERFOR, Spanish acronym). The types of samples collected were lung tissue and tracheal, cloacal, and rectal swabs. A more detailed description is given in Table 1.

Influenza A (H5N1) was detected in the samples by the INS with a reverse transcription (RT)-quantitative PCR (qPCR) protocol. Sixty-nine samples were processed, 14 of which were positive and 55 negative for H5N1. One sample was not included in the present study due to a lack of sequencing information. From November 12, 2022 to July 24, 2023, these 13 positive samples from wild birds (9), poultry (2), and wild mammals (2), were collected by SERFOR and SENASA in the regions of Lima, Tacna, Lambayeque, and Piura, and the genomes were sequenced by INS. Data on the numbers of positive cases and deceased animals were obtained from the National Center for Epidemiology, Disease Prevention, and Control of Peru (CDC, Spanish acronym) up to August 28, 2023.

RNA was extracted from the 13 samples with the QIAamp Viral RNA Kit (Qiagen, Hilden, Germany). The influenza A virus gene segments were amplified simultaneously with a multisegment RT-PCR, as described by Zhou

**Table 1** Epidemiological data on avian-flu-positive samples (H5N1) sequenced at the Instituto Nacional de Salud (INS) in Peru.

Virus name	Accession number	Collection date	Region of Peru	Host (scientific name)	Host (common name)	Sample type	Ct value	Collected by
A/pelican/Peru/PIU-INS-001/2022	EPI_ISL_16249274	2022-11-12	Piura	<i>Pelecanus thagus</i>	Peruvian Pelican	Lung tissue	22	SENASA
A/chicken/Peru/LAM-INS-002/2022	EPI_ISL_16249681	2022-11-28	Lambayeque	<i>Gallus gallus domesticus</i>	Rooster	Tracheal swab	25	SENASA
A/chicken/Peru/LIM-INS-003/2022	EPI_ISL_16249730	2022-11-29	Lima	<i>Gallus gallus domesticus</i>	Rooster	Tracheal swab	24	SENASA
A/peruvian_booby/Peru/LIM-INS-004/2023	EPI_ISL_17777525	2023-04-03	Lima	<i>Sula variegata</i>	Peruvian Booby	Cloacal swab	19.58	SERFOR
A/peruvian_booby/Peru/LIM-INS-005/2023	EPI_ISL_17777526	2023-03-20	Lima	<i>Sula variegata</i>	Peruvian Booby	Tracheal swab	28.42	SERFOR
A/gull/Peru/LIM-INS-006/2023	EPI_ISL_17777527	2023-03-16	Lima	<i>Larosterna inca</i>	Inca Tern	Tracheal swab	25.02	SERFOR
A/peruvian_booby/Peru/CAL-INS-007/2023	EPI_ISL_17777528	2023-03-17	Lima	<i>Sula variegata</i>	Peruvian Booby	Tracheal and cloacal swab	17.49	SERFOR
A/peruvian_booby/Peru/CAL-INS-008/2023	EPI_ISL_17777529	2023-03-17	Lima	<i>Sula variegata</i>	Peruvian Booby	Tracheal and cloacal swab	16.55	SERFOR
A/guanay_cormorant/Peru/CAL-INS-009/2023	EPI_ISL_17777530	2023-03-17	Lima	<i>Leucocarbo bougainvillorum</i>	Guanay Cormorant	Tracheal and cloacal swab	16.06	SERFOR
A/sea_lion/Peru/TAC-INS-010/2023	EPI_ISL_17777531	N/A	Tacna	<i>Otaria flavescens</i>	South American sea lion	Tracheal swab	29.01	SENASA
A/sea_lion/Peru/TAC-INS-011/2023	EPI_ISL_17777532	N/A	Tacna	<i>Otaria flavescens</i>	South American sea lion	Rectal swab	32.12	SENASA
A/peruvian_booby/Peru/LIM-INS-012/2023	EPI_ISL_17777533	2023-04-12	Lima	<i>Sula variegata</i>	Peruvian Booby	Tracheal swab	26.72	SERFOR
A/duck/Peru/CAL-INS-013/2023	EPI_ISL_18217104	2023-07-24	Lima	<i>Spatula cyanoptera</i>	Cinnamon Teal	Cloacal swab	29.76	SERFOR

Abbreviation: N/A, not available.

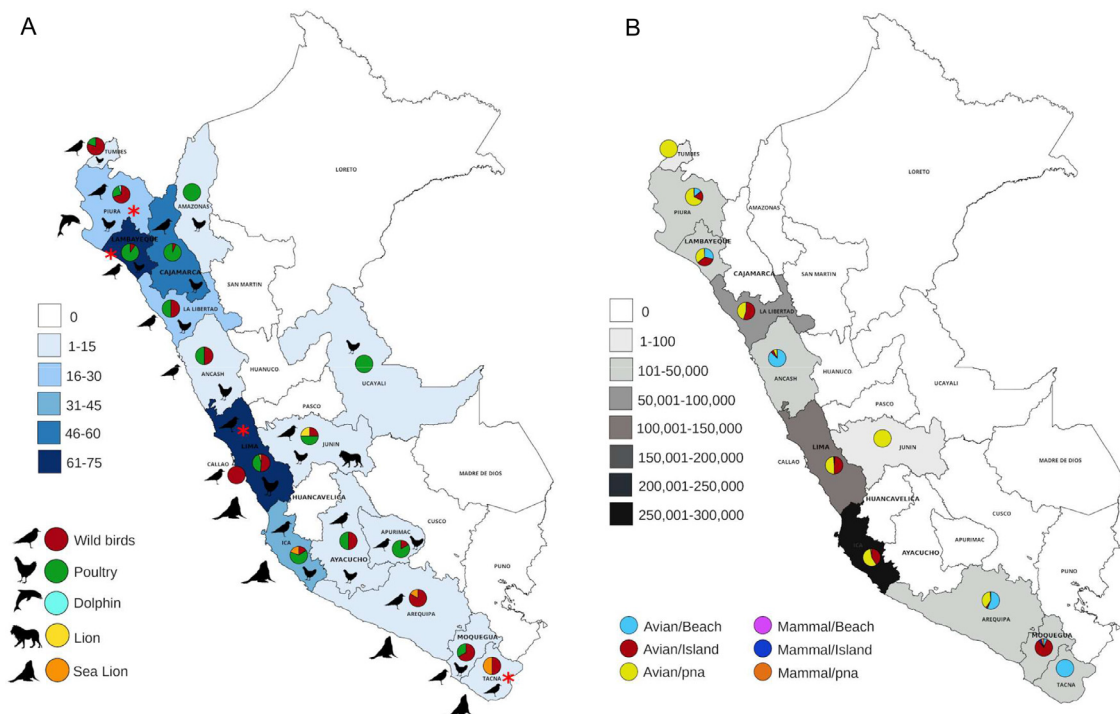
et al. [12]. Genomic libraries were then prepared with the Nextera® XT DNA Library Preparation Kit (Illumina Inc., San Diego, USA), according to the manufacturer’s instructions, for sequencing on the MiSeq platform (Illumina Inc.).

To analyze the data, we used the software packages BWA (<https://bio-bwa.sourceforge.net/>) and Samtools (<https://www.htslib.org/>) to index and map the sequences against the reference genome A/gray gull/Chile/C61947/2022 (GenBank accession: OQ352553.1). Consensus genome sequences for each sample were obtained with the iVar package [13]. The sequences are available in the GISAID (Global Initiative on Sharing All Influenza Data) database (Table 1). To identify new mutations and establish the phylogenetic relationships among the samples from South American regions, phylogenetic trees were constructed from the influenza A (H5N1) hemagglutinin (HA) and neuraminidase (NA) genes. The genomes were compared with the BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and similar sequences were downloaded and aligned with MAFFT [14]. A local database containing sequences from GISAID (<https://gisaid.org/>) was also prepared and used in the phylogenetic analysis. The phylogenetic trees were constructed with the Nextstrain

tool [15] using the iqtree method [16] and the GTR substitution model, and were rooted with the A/goose/Guangdong/1/1996 strain sequence. The phylogenetic trees were visualized with the Figtree program (<http://tree.bio.ed.ac.uk/software/figtree/>). A complete description of the mainly nonsynonymous mutations of interest by segment is compiled in Table 2.

### 3. Results and discussion

A global perspective on the avian influenza A (H5N1) outbreak of 2022–2023 in Peru (Fig. 1) shows that most cases of infection were widely distributed on the Peruvian coast, and mainly affected domestic poultry and wild birds, or sea lions in the mid-south coast of Peru (in the regions of Lima, Ica, Arequipa, and Tacna). Isolated cases were also identified in a dolphin in Piura and a zoo lion in Junin. The number of dead animals was high all along the Peruvian coast, totaling more than 30 thousand deaths in many regions, including Arequipa, Ica, Lima, Ancash, and La Libertad. The greatest number of dead animals were birds, collected on beaches and islands and in protected natural areas. Although a large number of animal deaths were detected along the Peruvian coast, only a few geographically representative cases could be



**Fig. 1.** Graphical representation of the avian flu (H5N1) outbreak in Peru in 2022–2023 compiled by the National Center for Epidemiology, Disease Prevention, and Control of Peru until August 28, 2023. (A) Number of H5N1-subtype-positive cases is shown by region as a blue gradient, where the different types and frequencies of hosts (poultry and wild birds, dolphin, lion, sea lion) are represented in a circular graph at the edge of each region. There was a wide distribution of positive cases in wild birds and poultry across almost all Peruvian regions, and in sea lions in southern Peru. Specific positive cases were observed in other mammals, such as a dolphin and lion, in the regions of Piura and Junin, respectively. Each type of host is represented in black figures. Asterisks (\*) on the map indicate the places of collection of the sequenced sample. (B) Global view of dead animals in Peru as a gray gradient. The frequencies of different types of animals (avian/mammalian) and environments at the collecting sites (beach, island, pna) are depicted in a circular graph. pna: protected natural area. Data were compiled by Agrorural, SERFOR, SENASA, and SERNANP.





**Fig. 2.** Phylogenetic tree of H5N1 subtype influenza virus hemagglutinin (A) and neuraminidase (B) genes collected during the 2022–2023 outbreak in Peru. Data were downloaded from GISAID up to September 4, 2023. The final figure was obtained with Figtree. Samples sequenced by the INS in Peru are highlighted in bold and colored red. The reference sequence used to root the phylogenetic tree was A/goose/Guangdong/1/1996 (available in the NCBI database under the accession numbers NC\_007362.1 and NC\_007361.1); the reference is not shown in the figure. The clade containing the Peruvian samples is shaded in orange. Samples from other countries are colored as follows: Ecuador (blue), Colombia (purple), Venezuela (turquoise), and Mexico (green). Samples from Chile, the USA, and other countries are not highlighted. Pictorial representations of each host is shown in the figure. New mutations are highlighted on the appropriate branch.

**Table 2**

Description of nonsynonymous mutations of interest, by segment, present in Peruvian genomes of influenza H5N1 sequenced at the Instituto Nacional de Salud (INS) in Peru.

Gene ID	Description	Mutations of interest
PB2	Polymerase	D701N <sup>a*</sup> ;Q591K <sup>*</sup> ;L607X;V613X;E627X
PB1	Polymerase	P598L
PA	Polymerase	R57Q
HA	Hemagglutinin	S149A <sup>a*</sup> ;D110S;L131Q;S139P;D142E;A143T;S145L;H154Q;S157P;S171D;D199N;Q208K;K201R;V226A;K234Q;S239R;Q338L;I352K
NP	Nucleocapsid protein	None
NA	Neuraminidase	S339P;L269M
MP	Matrix protein	K101R
NS	Nonstructural protein	A42S;Y103F;I205S;Y221K;A223E;K224R;R225T;V226I;Q14M;S48A;N67E;L100M

*Note:* Data were compiled with the research tool FluSurver against the reference genome A/goose/Guangdong/1/1996(H5N1). The mutations shown occurred at a site of interaction that could be involved in drug binding, drug resistance, and/or the alteration of host cells. The mutations reported were present in a minimum of three of the 13 genomes sequenced.

<sup>a</sup> Most significant mutation.

\* Only present in sea lions.

analyzed and attributed definitively to avian influenza (Fig. 1A).

Sequence analyses of the influenza A HA and NA genes indicated that the Peruvian samples were infected with HPAI H5N1, clade 2.3.4.4b, and shared a common ancestor with the virus infecting samples from Chile (Fig. 2). They were characterized by two synonymous mutations in the HA gene (606G>A and 1107G>A) in the presence of L131Q, a mutation related to antigenic variability [17], and two nonsynonymous mutations in the NA gene (805T>A, L269M; 1015T>C, S339P). Analysis of the HPAI H5N1 strain detected in *Otaria flavescens* samples revealed both I352K and 1368C>T mutations in the HA gene. Other nonsynonymous mutations of importance were also detected within other segments of the virus (Table 2). In the *Otaria flavescens* samples, the PB2 segment contained the D701N and Q591K mutations, which are associated with mammalian adaptation of the virus and its enhanced pathogenicity in mammalian hosts [18,19]. The PB1 segment also contained a P598L mutation in birds and mammals, previously linked to enhanced polymerase activity [20]. The results of this analysis highlight the introduction of some novel mutations into the virus in both birds and mammals, and further research is required to understand their biological implications and to prevent any consequences for global health or the poultry industry.

The well-known migratory pathways over the Pacific coast of the Americas could explain why the HPAI strains found in samples from Ecuador, Colombia, and Mexico are closely related to the Peruvian-Chilean clade. These findings imply that the potential ancestral host of these HPAI H5N1 viruses traveled from North America to Ecuador, Peru, and Chile via the American coastline. This observation is consistent with reports of H5N1 subtype infections in the USA since the beginning of 2022 and with the prevalence of HPAI in migratory birds, such as *Spatula cyanoptera*, in Peru. A distinct flyway on the Atlantic coast of the Americas could be used by birds in the Venezuela clade. More research is required to understand

the biological significance of the new mutations identified here and to determine whether they are associated with the rapid transmission and high fatality rate of the recent outbreak.

#### 4. Conclusions

The influenza A HPAI (H5N1) outbreak in 2022–2023 had a great impact on both birds and mammals in Peru. According to our study, the H5N1 found in Peru clustered with that in Chilean samples, is a member of the 2.3.4.4b clade, and may have originated in North America. The rapid dispersion and magnitude of this outbreak should motivate us to take public health precautions in the event of a possible avian flu epidemic in the future.

#### Funding

This research received no specific grant from a funding agency in the public, commercial, or not-for-profit sector.

#### Author contributions

**Nieves Sevilla:** Conceptualization, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing. **Wendy Lizarraga:** Conceptualization, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing. **Victor Jimenez-Vasquez:** Data curation, Investigation, Software, Writing—review & editing. **Veronica Hurtado:** Investigation, Methodology, Supervision, Writing—review & editing. **Iris S. Molina:** Investigation, Methodology, Supervision, Writing—review & editing. **Lilian Huarca:** Methodology, Investigation, Data curation, Writing—review & editing. **Priscila Lope-Pari:** Investigation, Resources, Supervision, Writing—review & editing. **Ivan Vargas:** Data curation, Investigation, Writing—review & editing. **Gloria Arotinco:** Investigation, Resources, Supervision, Writing—review & editing. **Car-**

**los Padilla Rojas:** Investigation, Methodology, Supervision, Writing—review & editing.

## Acknowledgments

We express our gratitude to the Servicio Nacional Forestal y de Fauna Silvestre (SERFOR) and the Servicio Nacional de Sanidad Animal (SENASA) of Peru for collecting and sending samples to be sequenced by the INS. We acknowledge the researchers who share H5N1 genomic sequences in public repository databases, such as GISAID, which greatly facilitates research into influenza A H5N1.

## Declaration of competing interest

The authors declare that they have no conflicts of interest. The views expressed in this publication do not necessarily reflect the views of the National Center for Epidemiology, Prevention, and Control of Diseases.

## Data available statement

The data that support the findings of this study are openly available in the GISAID database at <https://gisaid.org/>, reference numbers: EPI\_ISL\_16249274, EPI\_ISL\_16249681, EPI\_ISL\_16249730, EPI\_ISL\_17777525, EPI\_ISL\_17777526, EPI\_ISL\_17777527, EPI\_ISL\_17777528, EPI\_ISL\_17777529, EPI\_ISL\_17777530, EPI\_ISL\_17777531, EPI\_ISL\_17777532, EPI\_ISL\_17777533, EPI\_ISL\_18217104.

## Ethics statement

Not applicable.

## Informed consent

Not applicable.

## References

- [1] C. Korteweg, J. Gu, Pathology, molecular biology, and pathogenesis of avian influenza A (H5N1) infection in humans, *Am. J. Pathol.* 172 (5) (2008) 1155–1170, doi:10.2353/ajpath.2008.070791.
- [2] M.D. Van Kerkhove, E. Mumford, A.W. Mounts, et al., Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review, *PLoS One* 6 (1) (2011) e14582, doi:10.1371/journal.pone.0014582.
- [3] I. Oliver, J. Roberts, C.S. Brown, et al., A case of avian influenza A(H5N1) in England, January 2022, *Euro Surveill* 27 (5) (2022) 2200061, doi:10.2807/1560-7917.ES.2022.27.5.2200061.
- [4] J. Peiris, W.C. Yu, C.W. Leung, et al., Re-emergence of fatal human influenza A subtype H5N1 disease, *Lancet* 363 (9409) (2004) 617–619, doi:10.1016/S0140-6736(04)15595-5.
- [5] Servicio Nacional de Sanidad Agraria, Declaran alerta sanitaria en todo el territorio nacional, ante la presencia de influenza aviar altamente patógena sub-tipificada como Influenza A H5N1 en pelicano, 2022. Available at: <https://www.gob.pe/institucion/senasa/normas-legales/3680104-0173-2022-midagri-senasa>. Accessed November 23, 2022.
- [6] Servicio Nacional de Sanidad Agraria, Declaran la emergencia sanitaria en todo el territorio nacional ante la presencia de influenza aviar de alta patogenicidad en aves domésticas (aves de traspatio), por el periodo de noventa (90) días calendario, 2022. Available at: <https://www.gob.pe/institucion/senasa/normas-legales/3693849-0180-2022-midagri-senasa>. Accessed November 29, 2022.
- [7] Servicio Nacional de Sanidad Agraria, Amplían emergencia sanitaria en todo el territorio nacional ante la presencia de influenza aviar de alta patogenicidad, modifican la R.J. N° 0180-2022-MIDAGRI- SENASA y dictan diversas disposiciones, 2023. Available at: <https://www.gob.pe/institucion/senasa/normas-legales/3943176-0028-2023-midagri-senasa>. Accessed February 24, 2023.
- [8] M. Leguia, A. Garcia-Glaessner, B. Muñoz-Saavedra, et al., Highly pathogenic avian influenza A (H5N1) in marine mammals and seabirds in Peru, *Nat. Commun.* 14 (2023) 5489, doi:10.1038/s41467-023-41182-0.
- [9] M. Fernández-Díaz, D. Villanueva-Pérez, L. Tataje-Lavanda, et al., Detection and genomic characterization of an avian influenza virus subtype H5N1 (clade 2.3.4.4b) strain isolated from a pelican in Peru, *Microbiol. Resour. Announc.* 12 (6) (2023) e0019923, doi:10.1128/mra.00199-23.
- [10] A. Bruno, A. Alfaro-Núñez, D. de Mora, et al., First case of human infection with highly pathogenic H5 avian influenza A virus in South America: a new zoonotic pandemic threat for 2023? *J. Travel Med.* 30 (5) (2023) 1–3, doi:10.1093/jtm/taad032.
- [11] Organización Panamericana de la Salud, Nota informativa: Infección humana causada por el virus de influenza aviar en Chile, 2023. Available at: <https://www.paho.org/es/documentos/nota-informativa-nota-informativa-infeccion-humana-causada-por-virus-influenza-aviar-ah5>. Accessed March 31, 2023.
- [12] B. Zhou, M.E. Donnelly, D.T. Scholes, et al., Single-reaction genomic amplification accelerates sequencing and vaccine production for classical and Swine origin human influenza A viruses, *J. Virol.* 83 (19) (2009) 10309–10313, doi:10.1128/JVI.01109-09.
- [13] M. Hunt, A. Gall, S.H. Ong, et al., IVA: accurate *de novo* assembly of RNA virus genomes, *Bioinform. Oxf. Engl.* 31 (14) (2015) 2374–2376, doi:10.1093/bioinformatics/btv120.
- [14] K. Katoh, J. Rozewicki, K.D. Yamada, MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization, *Brief. Bioinform.* 20 (4) (2019) 1160–1166, doi:10.1093/bib/bbx108.
- [15] J. Hadfield, C. Megill, S.M. Bell, et al., Nextstrain: real-time tracking of pathogen evolution, *Bioinformatics* 34 (23) (2018) 4121–4123, doi:10.1093/bioinformatics/bty407.
- [16] L.T. Nguyen, H.A. Schmidt, A. von Haeseler, et al., IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies, *Mol. Biol. Evol.* 32 (1) (2015) 268–274, doi:10.1093/molbev/msu300.
- [17] N. Ariyama, C. Pardo-Roa, G. Muñoz, et al., Highly pathogenic avian influenza a(H5N1) clade 2.3.4.4b virus in wild birds, Chile, *Emerg. Infect. Dis.* 29 (9) (2023) 1842–1845, doi:10.3201/eid2909.230067.
- [18] C. Wang, H.H. Lee, Z.F. Yang, et al., PB2-Q591K mutation determines the pathogenicity of avian H9N2 influenza viruses for mammalian species, *PLoS One* 11 (9) (2016) e0162163, doi:10.1371/journal.pone.0162163.
- [19] V. Czudai-Matwich, A. Otte, M. Matrosovich, et al., PB2 mutations D701N and S714R promote adaptation of an influenza H5N1 virus to a mammalian host, *J. Virol.* 88 (16) (2014) 8735–8742, doi:10.1128/JVI.00422-14.
- [20] K. Pabbaraju, R. Tellier, S. Wong, et al., Full-genome analysis of avian influenza a(H5N1) virus from a human, North America, 2013, *Emerg. Infect. Dis.* 20 (5) (2014) 887–891, doi:10.3201/eid2005.140164.