

8. Meyer Sauter PM, Beeton ML, Pereyre S, Bébéar C, Gardette M, Hénin N, et al.; ESGMAC the ESGMAC MAPS study group. *Mycoplasma pneumoniae*: gone forever? *Lancet Microbe*. 2023;4:e763. [https://doi.org/10.1016/S2666-5247\(23\)00182-9](https://doi.org/10.1016/S2666-5247(23)00182-9)
9. Meyer Sauter PM, Beeton ML, Pereyre S, Bébéar C, Gardette M, Hénin N, et al. European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Mycoplasma and Chlamydia Infections (ESGMAC), and the ESGMAC *Mycoplasma pneumoniae* Surveillance (MAPS) Study Group. *Mycoplasma pneumoniae*: delayed re-emergence after COVID-19 pandemic restrictions. *Lancet Microbe*. 2024;5:e100-1. [https://doi.org/10.1016/S2666-5247\(23\)00344-0](https://doi.org/10.1016/S2666-5247(23)00344-0)
10. Opota O, Brouillet R, Greub G, Jaton K. Methods for real-time PCR-based diagnosis of *Chlamydia pneumoniae*, *Chlamydia psittaci*, and *Chlamydia abortus* infections in an opened molecular diagnostic platform. *Methods Mol Biol*. 2017;1616:171-81. https://doi.org/10.1007/978-1-4939-7037-7_11

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Highly Pathogenic Avian Influenza A(H5N1) Viruses from Multispecies Outbreak, Argentina, August 2023

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We report full-genome characterization of highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus from an outbreak among sea lions (August 2023) in Argentina and possible spillover to fur seals and terns. Mammalian adaptation mutations in virus isolated from marine mammals and a human in Chile were detected in mammalian and avian hosts.

In February 2023, the first case of highly pathogenic avian influenza (HPAI) A(H5N1) in Argentina was detected in a wild goose near the border with Bolivia and Chile (Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/30/4/23-1725-App1.pdf>) (1). In contrast with Peru and Chile, where extensive mortality of seabirds and marine mammals had been attributed to the virus in the preceding months (2,3), the initial spread of HPAI H5N1 in Argentina was largely limited to backyard and industrial poultry (94 outbreaks), causing the death or disposal of 2.2 million birds. Argentina declared itself free from the disease in poultry on August 8, 2023; before then, HPAI H5N1 detections in wildlife in Argentina had been scarce (7 events during February–April) and limited to aquatic birds (Anatidae, Laridae, and Rallidae) (1,4). However, soon thereafter, the national animal health services confirmed HPAI H5N1 in South American sea lions (*Otaria byronia*) from Río Grande, southernmost Argentina. Over subsequent weeks, the virus was detected in sea lions northward along the Argentina coast, and sporadic cases also occurred in South American fur seals (*Arctocephalus australis*). The most affected site was Punta Bermeja (Appendix Figure 1), the largest sea lion colony in Argentina, where an estimated 811 sea lions died over 2 months; minimal numbers (<5) of fur seals and terns were also affected (1,4).

In collaboration with provincial authorities and park rangers, we collected swab samples (oronasal, rectal, tracheal, lung, and brain) from 16 deceased sea lions, 1 fur seal, 1 great grebe (*Podiceps major*), and 1 South American tern (*Sterna hirundinacea*) discovered at Punta Bermeja on August 26, 2023. A sampled adult male sea lion was seen alive showing clinical signs consistent with HPAI infection (inability to stand or walk, muscular tremors and spasms, difficulty breathing, and abundant oral mucus). We tested the samples by real-time reverse transcription PCR targeting influenza A virus (5) and confirmed that all were positive. On the basis of viral RNA yields, we selected brain samples from 4 sea lions, 1 fur seal, and 1 tern for full-genome sequencing (Appendix Figure 2). We used maximum-likelihood tree phylogenetic analysis (6) and mutational analysis

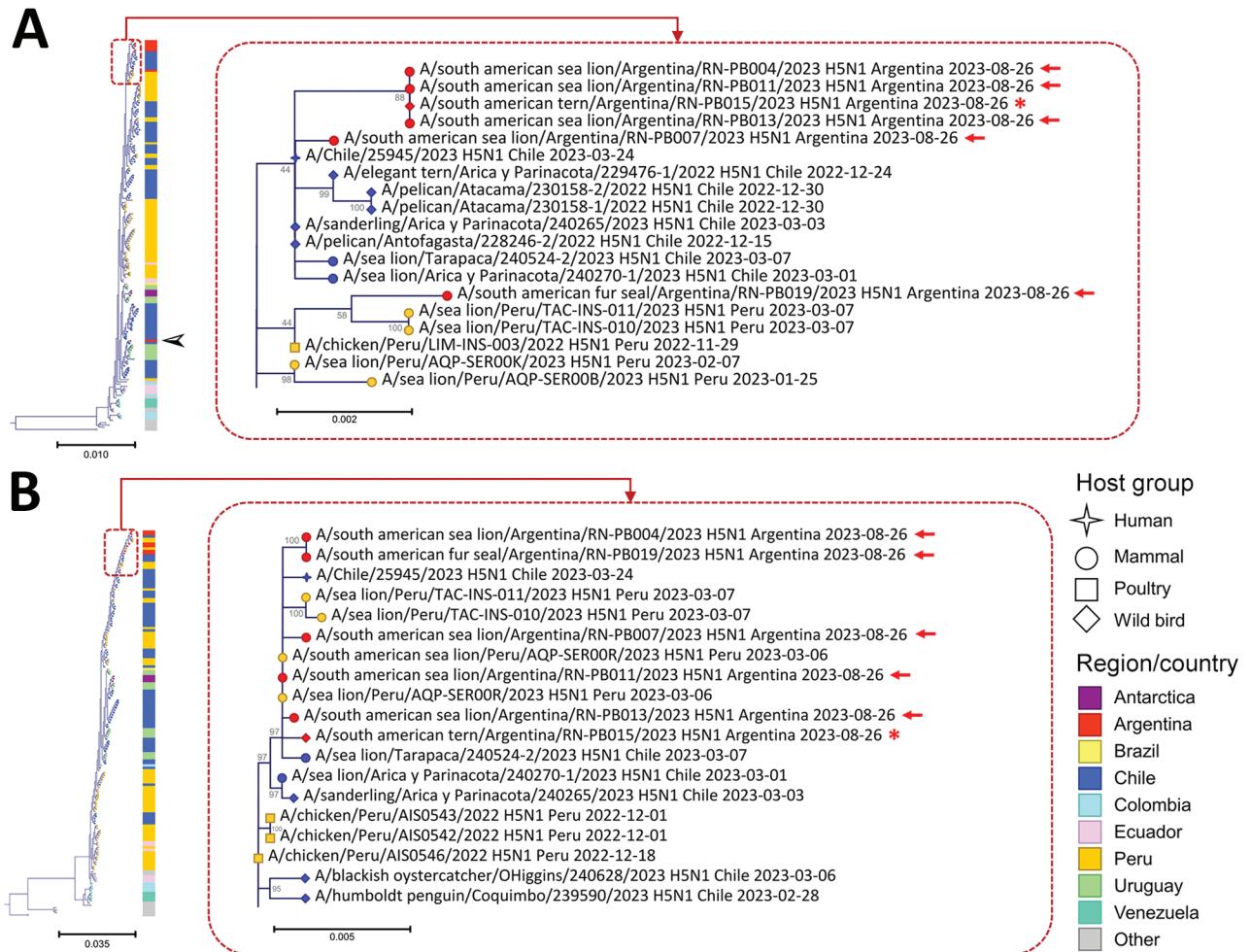


Figure. Maximum-likelihood trees for hemagglutinin (A) and polymerase basic 2 (B) gene segments evaluated in study of highly pathogenic avian influenza A(H5N1) in Argentina compared with reference strains from other countries in South America. Tree areas have been enlarged at right to show detail. Red arrows indicate virus from marine mammals in Argentina; red asterisk indicates virus from a tern in Argentina. Black arrowhead along full tree in panel A indicates the hemagglutinin sequence from the first detection of HPAI H5N1 in a wild goose in Argentina. Node shape represents host group, and node color (and bars adjacent to trees) represents the region/country. Branch lengths are drawn proportionally to the extent of changes. Values adjacent to nodes represent bootstrap support ≥ 40 . Scale bars indicate nucleotide substitutions per site.

to compare the sequences (GenBank accession nos. OR987081–128) with representative HPAI H5N1 strains from South America.

Phylogenetic trees (Figure; Appendix Figure 2) showed that the viruses we identified belong to HPAI H5N1 clade 2.3.4.4b and are closely related to H5N1 viruses that circulated in South America during 2022–2023. Our finding supports the hypothesis that, after introduction from North America into Peru in November 2022, HPAI H5N1 viruses continued spreading across the continent and into Argentina. Of note, the viruses from Punta Bermeja did not cluster with the hemagglutinin and neuraminidase sequences available from HPAI H5N1 first detected in a wild goose in Argentina. Instead,

all gene segments from the viruses were closely related to virus sequences from sea lions in Chile and Peru (2; C. Pardo Roa, unpub. data, <https://www.biorxiv.org/content/10.1101/2023.06.30.547205v1>); 6 gene segments (all except polymerase basic protein 1 and nucleocapsid protein) also clustered with the virus isolated from a human in Chile (7). That finding suggests that viruses from Punta Bermeja may have been derived from a separate HPAI H5N1 introduction into Argentina. Because of the lack of genomic data for HPAI H5N1 viruses circulating in Argentina during February–July 2023, the finer scale pathways (local geographic routes and host species involved) of how these viruses arrived at Punta Bermeja remain unclear. Even so, the viruses

that we report did not cluster with those from birds in Uruguay, Brazil, or Bird Island (Antarctica), possibly suggesting separate pathways of virus spread.

On the basis of previous comparisons with HPAI H5N1 isolates from other countries in South America, we identified 9 mutations already present in viruses infecting sea lions in Peru and Chile but not in the goose/Guangdong reference strain or in viruses from birds and mammals from North America in 2022 (Table, <https://wwwnc.cdc.gov/eid/article/30/4/23-1725-t1>). Specifically, we found Q591K and D701N mutations in polymerase basic 2 associated with increased pathogenicity to mammals (8). The virus we detected in the South American tern also has those mutations, but they were absent from previously reported HPAI H5N1 viruses from avian hosts in South America (except for A/sanderling/Arica y Parinacota/240265/2023, which has the D701N mutation). That finding further supports the hypothesis that HPAI H5N1 viruses from sea lions from Peru and Chile acquired mammalian adaptation mutations that improved their ability to infect pinnipeds while possibly retaining the ability to infect avian hosts. Given the rapid and widespread dissemination of the viruses among pinnipeds in South America and the substantial associated mortalities (3,9), it seems likely that pinniped-to-pinniped transmission played a role in the spread of the mammal-adapted HPAI H5N1 viruses in the region. It is alarming that the HPAI H5N1 viruses infecting pinnipeds and seabirds in Argentina share the same mammalian adaptation mutations as the virus from the affected human in Chile, which highlights the potential threat posed by these viruses to public health.

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bioinformatic analyses; A.R., R.E.T.V., and M.M.U. wrote the manuscript. All authors have read, revised, and approved the manuscript.

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References

1. Servicio Nacional de Sanidad y Calidad Agroalimentaria. State of the epidemiological situation in Argentina [in Spanish] [cited 2023 Dec 12]. <https://www.argentina.gob.ar/senasa/estado-de-la-situacion-epidemiologica-en-la-argentina>
2. Leguia M, Garcia-Glaessner A, Muñoz-Saavedra B, Juárez D, Barrera P, Calvo-Mac C, et al. Highly pathogenic avian influenza A (H5N1) in marine mammals and seabirds in Peru. *Nat Commun.* 2023;14:5489. <https://doi.org/10.1038/s41467-023-41182-0>
3. Ulloa M, Fernández A, Ariyama N, Colom-Rivero A, Rivera C, Nuñez P, et al. Mass mortality event in South American sea lions (*Otaria flavescens*) correlated to highly pathogenic avian influenza (HPAI) H5N1 outbreak in Chile. *Vet Q.* 2023;43:1–10. <https://doi.org/10.1080/01652176.2023.2265173>
4. World Organisation for Animal Health. WAHIS: World Animal Health Information System [cited 2023 Dec 12]. <https://wahis.woah.org>
5. Pan American Health Organization. CDC protocol for real-time RT-PCR for the new subtype of influenza A(H1N1) virus [in Spanish] [cited 2023 Dec 12]. <https://www.paho.org/es/node/36933>
6. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol.* 2015;32:268–74. <https://doi.org/10.1093/molbev/msu300>
7. Castillo A, Fasce R, Parra B, Andrade W, Covarrubias P, Hueche A, et al. The first case of human infection with H5N1 avian Influenza A virus in Chile. *J Travel Med.* 2023; 30(5):taad083. <https://doi.org/10.1093/jtm/taad083>
8. Lee CY, An SH, Choi JG, Lee YJ, Kim JH, Kwon HJ. Rank orders of mammalian pathogenicity-related PB2 mutations of avian influenza A viruses. *Sci Rep.* 2020;10:5359. <https://doi.org/10.1038/s41598-020-62036-5>
9. Campagna C, Uhart M, Falabella V, Campagna J, Zavattieri V, Vanstreels RET, et al. Catastrophic mortality of southern elephant seals caused by H5N1 avian influenza. *Mar Mamm Sci.* 2024;40:322–5. <https://doi.org/10.1111/mms.13101>

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