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Detection of avian influenza in the Cape Horn Biosphere Reserve: preliminary sampling at the Beagle Channel, Chile, 2024-2025

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ABSTRACT

Avian influenza viruses (AIVs) are important pathogens in wild aquatic birds worldwide, however, their presence in sub-Antarctic regions remains largely unknown. To address this gap, we report surveillance data from the Cape Horn Biosphere Reserve, in southernmost Chile, for the austral reproductive seasons of 2024–2025. In total, 352 fecal samples were collected from coastal sites, islets, and passerines in long-term monitoring programs. Only one environmental sample obtained at Punta Gusano in January 2024 tested positive by real-time RT-PCR (Ct 36.78), giving a detection rate of 0.625%, while all passerine and 2025 season samples were negative. This single weak positive result suggests occasional virus introduction, but there is no evidence of sustained circulation. The result agrees with previous reports showing active AIV dynamics in northern and central Chile, but very limited activity in Patagonia. The finding also matches the few confirmed highly pathogenic avian influenza H5N1 clade 2.3.4.4b cases recorded in southernmost Chile during the 2023 outbreak. Together, these observations suggest that southernmost Chile remains minimally affected by AIV. However, its proximity to Antarctica and location at a major migratory flyway intersection highlight the importance of continued surveillance in these remote ecosystems.

Key words: *Avian influenza, wild birds, viral surveillance, Beagle Channel, sub-Antarctic Magellanic ecoregion*

Detección de influenza aviar en la Reserva de la Biosfera Cabo de Hornos: muestreo preliminar en el canal Beagle, Chile, 2024-2025

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RESUMEN

Los virus de influenza aviar (VIA) son patógenos importantes en aves acuáticas silvestres a nivel mundial; sin embargo, su presencia en regiones subantárticas sigue siendo en gran medida desconocida. Para abordar esta brecha en el conocimiento, se presentan aquí datos de vigilancia obtenidos en la Reserva de la Biosfera Cabo de Hornos, en el extremo sur de Chile, durante las temporadas reproductivas australes de 2024-2025. En total, se recolectaron 352 muestras fecales de sitios costeros, islotes y aves paserinas en programas de monitoreo a largo plazo. Solo una muestra ambiental obtenida en Punta Gusano en enero de 2024 resultó positiva mediante RT-PCR en tiempo real (Ct 36,78), lo que corresponde a una tasa de detección del 0,625%, mientras que todas las muestras de paserinos y de la temporada 2025 fueron negativas. Este único resultado débilmente positivo sugiere introducciones virales ocasionales, pero no hay evidencia de circulación sostenida. El hallazgo coincide con informes previos que muestran una dinámica activa de VIA en el norte y centro de Chile, pero una actividad muy limitada en la Patagonia. Asimismo, concuerda con los pocos casos confirmados de influenza aviar altamente patogénica H5N1 del clado 2.3.4.4b registrados en el extremo sur de Chile durante el brote de 2023. En conjunto, estas observaciones sugieren que el extremo sur de Chile sigue estando mínimamente afectado por los VIA. No obstante, su proximidad a la Antártida y su ubicación en la intersección de importantes rutas migratorias resaltan la importancia de mantener la vigilancia en estos ecosistemas remotos.

Palabras clave: *Influenza aviar, aves silvestres, vigilancia viral, canal Beagle, ecorregión subantártica de Magallanes*

INTRODUCTION

Avian influenza viruses (AIVs) are globally distributed pathogens that circulate predominantly in wild aquatic birds, particularly in the orders *Anseriformes* and *Charadriiformes*, which serve as natural reservoirs for both low pathogenic (LPAI) and highly pathogenic (HPAI) lineages (Olsen et al., 2006; Webster et al., 1992). Regarding the antigenic diversity of the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), 17 distinct HA and nine NA subtypes have been identified in avian species (Karakus et al., 2024). Much of the foundational knowledge about AIV ecology stems from long-term surveillance programs in the Northern Hemisphere, where migratory patterns, seasonal peaks in virus prevalence, and reassortment dynamics are well described (Araujo et al., 2018; Fourment et al., 2017; Hanson et al., 2008; Herrick et al., 2013; Krauss et al., 2004; Lam et al., 2012; Verhagen et al., 2015). In contrast, the Southern Hemisphere, particularly South America and the Antarctic region, has received disproportionately less attention (Chatziprodromidou et al., 2018). This generates significant knowledge gaps about viral persistence, genetic diversity, and host-virus interactions.

Recent evidence supports the role of migratory seabirds as likely vectors for the introduction of HPAI H5N1 clade 2.3.4.4b into the Antarctic region, marking the first historic detection of HPAI in the region (Banyard et al., 2024). On the other hand, a study by Pardo-Roa et al., 2025 provided evidence of possible transmission among marine mammals in Chile. In 2023, the National Fisheries Service (SERNAPESCA, 2025) detected HPAI in two South American sea lions (*Otaria flavescens*) and one Southern river otter (*Lontra provocax*) in the Magallanes region. Additionally, in 2024, the Scientific Committee on Antarctic Research (SCAR) reported positive cases in a Southern elephant seal (*Mirounga leonina*) in several areas of the Antarctic region, a migratory species that also occurs in Chile. These findings raise concerns for public health and highlight new hosts capable of infecting wild birds (Pardo-Roa et al., 2025). Phylogenetic analyses have linked viruses detected in South Georgia to strains circulating in South America, suggesting movement along avian migratory pathways that connect the Southern Cone to sub-Antarctic and Antarctic ecosystems (Banyard et al., 2024; Verhagen et al., 2015). However, comprehensive data remains limited, making it difficult to assess the epidemiological significance of these remote yet biologically rich systems.

Patagonia and the sub-Antarctic Magellanic ecoregion are intersected by major migratory flyways (Piersma, 2007; Rozzi & Jimenez, 2014). However, long-term ecological research as well as long-term ornithological monitoring and studies are limited in this region (Rozzi et al., 2012). In particular, surveys of avian viruses are minimal and limited to sporadic sampling efforts.

Along the whole latitudinal range of Chile, surveillance between 2012 and 2015 revealed 16 isolates from over 4,000 samples, most of which were from central zones rather than extreme southern latitudes (Jiménez-Bluhm et al., 2018). Other surveillance studies in Chile have described the presence of avian influenza viruses in wild birds from northern and central regions of the country, indicating a high level of viral circulation in these areas (Mathieu et al., 2015; Ruiz et al., 2023, 2021). By contrast, a study conducted in Argentina between 2006 and 2007 included the single documented AIV sampling effort in the Beagle Channel, with negative results (Pereda et al., 2008). Nevertheless, antibody studies in Antarctic penguins and seabirds in Bahia Lomas, Tierra del Fuego, suggest exposure to other avian viruses such as *Avian Orthoavulavirus 1* (Newcastle disease virus), supporting the need to examine viral circulation in these supposedly pristine environments (Ariyama et al., 2021).

More recent efforts have expanded the known viral diversity and raised concern over increasing incursions of HPAI into Antarctic and sub-Antarctic systems. For example, H11N2 viruses were detected in Adélie and chinstrap penguins in the South Shetland Islands, suggesting active local AIV circulation (Ogrzewalska et al., 2022). At the same time, an H6N8 strain isolated from a brown skua in King George Island demonstrated genetic connectivity with viruses from both Chile and North America (de Seixas et al., 2022). The emergence of HPAI H5N1 clade 2.3.4.4b in South America in late 2022 and its subsequent southward spread has been particularly alarming. The first documented outbreak in the sub-Antarctic area occurred in South Georgia (Bird Island) in late 2023, where H5N1 was detected in brown skuas, kelp gulls, and multiple marine mammal species (Bennison et al., 2024). Genetic analyses of these viruses revealed close relationships with strains circulating in Chile, Argentina, and Uruguay (Banyard et al., 2024), underscoring the role of southern South America, and the Patagonia in particular, as both a viral source and a potential gateway to Antarctic ecosystems. Moreover, the Chilean Agricultural and Livestock Service (SAG) documented over 94,000 wild bird deaths between December 2022 and 2023. Although not all individuals were tested, HPAI H5N1 was the most likely cause, raising significant concerns for wildlife conservation. In the Magallanes region, only 282 deaths were reported; however, this figure is likely underestimated due to the challenging surveillance conditions in the area, where wild bird data from this region, as reported by SAG (2023), were largely obtained through passive monitoring during the outbreak.

Given the evident risk posed by emerging epizootics and the critical lack of data in the southernmost regions of South America, this study aims to describe new AIV surveillance efforts in the Beagle Channel, specifically around Navarino Island, to determine the presence of AIVs in wild birds in this area, in the context of the ongoing H5N1 panzootic. Since the 2008 work by Pereda et al., no published reports have examined this area for avian influenza, despite its ecological relevance and proximity to Antarctica. Our study presents preliminary results of an ongoing surveillance of AIVs in wild birds in the Beagle Channel, which responds directly to the call for improved surveillance in remote, southern high-latitude regions. By focusing on a previously neglected region at the interface of the Southern Cone and Antarctica, we aim to contribute to the global understanding of avian influenza ecology and inform early warning systems within a One Health framework.

MATERIALS AND METHODS

Sampling and Study Area

A total of 330 fresh environmental feces were collected along the shores of lakes and the sea at multiple sites along the northern coast of Navarino Island, including islets in the Beagle Strait in an opportunistic manner (Figure 1). Navarino Island is located at the southern end of the sub-Antarctic Magellanic ecoregion (Rozzi et al., 2006), and sampling efforts were concentrated in areas close to the city of Puerto Williams (54°55'S 67°37'W). The highest sampling effort was focused on the nesting colony of shorebirds at the Punta Gusano Peninsula, north of Puerto Williams, which included species such as kelp gulls (*Larus dominicanus*) and imperial cormorants (*Leucocarbo atriceps*) (Figure 2).

In January 2024, a set of 138 environmental samples were collected (Figure 3). Additionally, 21 fresh fecal samples originated from 5 species: (*Zonotrichia capensis* (7), *Phrygilus patagonicus* (5), *Aphrastura spinicauda* (4), *Troglogytes musculus* (3) and *Elaenia albiceps* (2)) were also obtained. They were collected from February 1st to 6th 2024 from birds captured during the long-term bird monitoring program in Omora Park (54°56'S, 67°39'W) (Rozzi & Jimenez, 2014). A second set of 192 environmental samples was collected in January 2025.

Briefly, fresh fecal samples were collected from the ground at various sample sites or from the holding bags in which the birds were kept after being captured in the mist nets, using sterile flocked swabs (Copan, Italy) and placed into tubes containing 1 mL of Universal Transport Medium (Copan, Italy). Samples were kept at 4 °C for no more than 5 days or stored at -80°C until analysis (World Health Organization, 2009) at the Laboratory of Infectious Veterinary Diseases, School of Veterinary Medicine, Pontificia Universidad Católica de Chile.

Sample analysis

Sample extraction was performed under BSL-2 conditions in a Class II, Type A2 biosafety cabinet. RNA was extracted using the MagMAX-96 AI/ND Viral RNA Isolation Kit (Ambion, USA), following the manufacturer's instructions. Reverse transcription real-time PCR was conducted to detect the influenza matrix gene, as previously described (World Health Organization, 2009). A cutoff value of Ct 38 was used to determine positivity (Shu et al., 2011), and amplification was performed on an Mx3000P real-time PCR thermocycler (Agilent Technologies, USA). Samples were analyzed individually and processed alongside two positive controls of A/California/04/2009 (H1N1) (GenBank accession number ACP41105.1) and two negative controls consisting of distilled water and master mix.

RESULTS

Of the samples analyzed, only one tested positive during the 2024 sampling season. It was an environmental sample obtained at Punta Gusano, a narrow peninsula north of Puerto Williams. Overall, we detected a low positivity of 0.625% (95% CI: 0.02%–3.44%). Due to the high Ct value (36.78), no further processing of the sample was possible.

None of the samples taken from trapped passerines tested positive. Additionally, none of the samples of the 2025 season yielded positive results.

DISCUSSION

Our findings, which include only a single positive sample out of 160 collected in January 2024 and none of the 192 samples collected in January 2025 across multiple sites along the Beagle channel and Omora Park, are consistent with previous reports from southernmost South America. Previous surveillance studies have shown a rich circulation of AIVs in parts of South America, mainly in Argentina, northern and central Chile, as well as in Brazil, where multiple subtypes have been detected in wild birds (Alvarez et al., 2010; Araujo et al., 2018; de Araujo et al., 2014; Jiménez-Bluhm et al., 2018; Mathieu et al., 2015; Pereda et al., 2008; Rimondi et al., 2018; Ruiz et al., 2021; Xu et al., 2012). These findings indicate active viral dynamics in some regions of the continent. However, Patagonia has remained notably silent, with no sustained evidence of AIV circulation reported other than during the HPAI H5N1 2.3.4.4b outbreak in 2023. The low detection rate in our study is also in accordance with the limited number of confirmed HPAI cases recorded during the peak of the H5N1 panzootic in southernmost Chile and Argentina, where only two confirmed cases were documented in the surrounding area to Navarino Island (SAG, 2023). This geographic discrepancy highlights a gap in our understanding of how environmental and ecological factors, like monthly minimum temperature and vegetation coverage (Ruiz et al., 2021) may limit or influence the maintenance and transmission of viruses in southern high-latitude regions. Due to the high Ct value of the sample, we were unable to determine the subtype of the positive case. In this regard, it is relevant to note that LPAI H5N5, H6N8, and H11N2 have been detected in the Antarctic regions (de Seixas et al., 2022; Hurt et al., 2016, 2014). Thus, additional studies in this region are needed to clarify the subtypes present, although sampling efforts in this study were comparable to those conducted in other regions of Chile, that is, a high number of environmental samples per site (Jiménez-Bluhm et al., 2018; Ruiz et al., 2023, 2021). It is important to note that fieldwork in this area is logistically challenging due to harsh weather conditions, which restrict sampling to the summer months.

Although the positive sample could not be linked to a specific host species, the fecal morphology was consistent with that of gulls or cormorants. Moreover, the sampling site at Punta Gusano was dominated by breeding kelp gulls and imperial cormorants. LPAI has been detected in kelp gulls in other regions of Chile (Mathieu et al., 2015; Ruiz et al., 2023), and during the HPAI H5N1 outbreak, several individuals of this species tested positive; however, only one case was reported in the Magallanes region (Puerto Natales)(SAG, 2023). In contrast, there are no reports of LPAI in imperial cormorants in Chile, and only a single individual tested positive during the H5N1 outbreak in the Los Lagos region (SAG, 2023). Additionally, Argentina also reported HPAI H5N1–positive cases in this species (WOAH, 2025). These species were the most prevalent species observed during fieldwork, although other bird species were also present in the area. Thus, it is plausible that the positive sample originated from one of these abundant breeders, but this cannot be confirmed with certainty if it originated from a migratory or resident species. Determining the host species in future studies will be essential to provide further insight into the role that different species may play in the dispersal of AIVs.

Together, these results support the notion that, despite growing concerns about virus spread via migratory birds into Antarctic and sub-Antarctic ecosystems (Araujo et al., 2018; Banyard et al., 2024), the area surrounding the Beagle Channel has so far remained only minimally affected by AIV circulation. Nonetheless, continued surveillance in this region remains essential for the early detection of future introductions, particularly as environmental change and shifts in wild bird migration patterns driven by climate change may alter the current scenario.

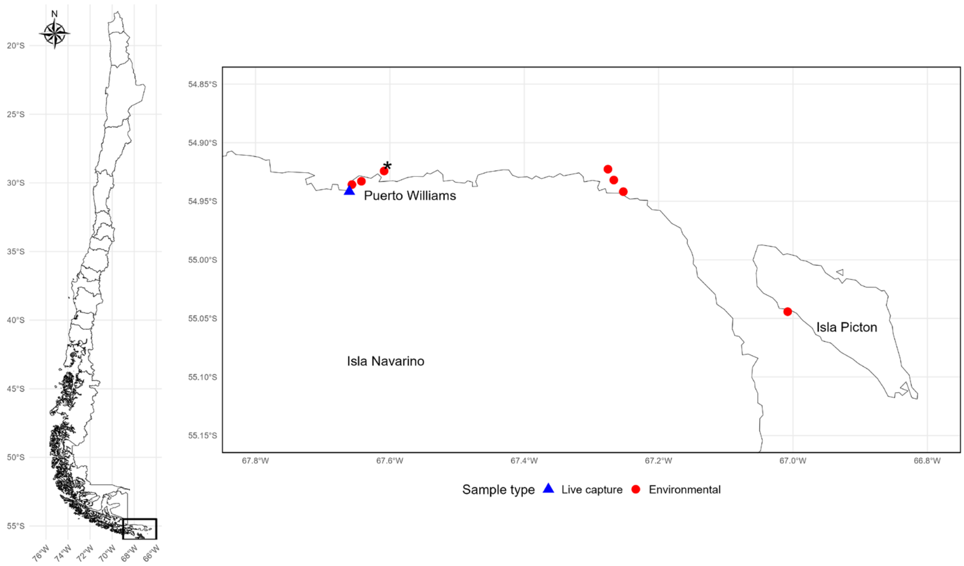

Figure 1. Sample sites on the Beagle Channel and its surroundings in 2024 and 2025. Red dots indicate environmental sample sites, and blue triangles indicate the live capture site. The dots that appear in the water correspond to points located on islets. The sampling site marked with an asterisk corresponds to the location of the positive sample.

Figure 2. Photographs of the primary sampling sites on the Beagle Channel. (A) View of the Punta Gusano peninsula with several individuals of kelp gulls (*Larus dominicanus*), including both adults and juveniles. (B) One of the sampled islets in the Beagle Channel, home to a breeding colony of imperial cormorants (*Leucocarbo atriceps*).

A)  B) 

Figure 3. Fieldwork collecting environmental samples of shorebirds on the southern coast of the Beagle Channel (A), and passerines at the Omora Ethnobotanical Park (B).

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ETHICS STATEMENT

Sampling was conducted under approval of the Animal Use and Welfare Committee of the Pontificia Universidad Católica de Chile (Ethics ID 230613022, June 5, 2024). Scientific bird capture permit issued by the Agricultural and Livestock Service (SAG): EXEMPT RESOLUTION No. 2220/2023.

COMPETITIVE INTEREST

The authors declare no competing interests.

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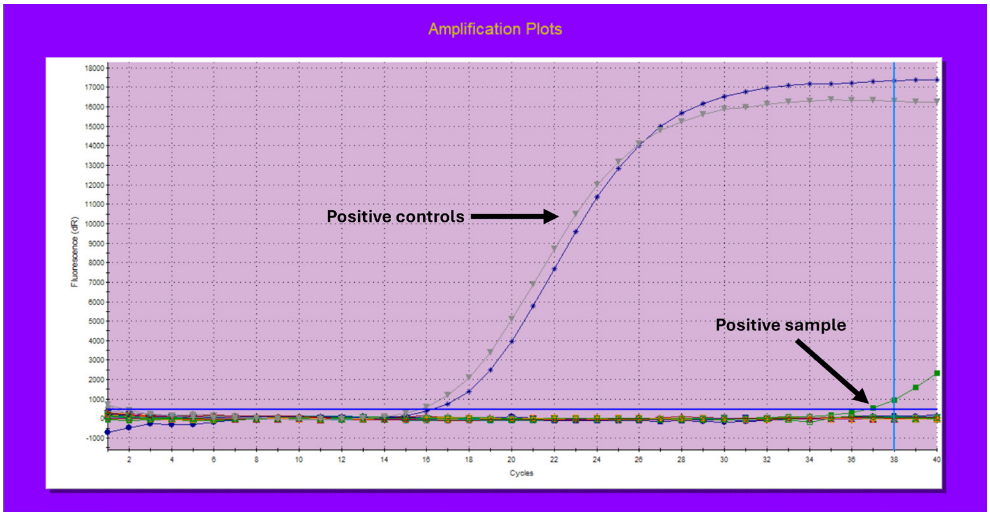
SUPPLEMENTAL MATERIAL

Supplementary Figure 1. Amplification curves of the reverse transcription real-time PCR of the positive sample. The positive sample had a Ct value of 36.78 and positive controls had values of 16.35 and 15.73. Negative controls showed no amplification.



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