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1 **Highly Pathogenic Avian Influenza A (H5N1) Suspected in penguins and shags on the**  
2 **Antarctic Peninsula and West Antarctic Coast**

3

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26

## 27 **Abstract**

28 Suspected cases of Highly Pathogenic Avian Influenza (H5N1) were detected in Adélie  
29 penguins and Antarctic shags at the southernmost latitude so far in Antarctica, at two  
30 breeding sites out of 13 visited, using highly specific PCR assay. These first records mark the  
31 progression of the H5N1 panzootic into Antarctica.

32

## 33 **Introduction**

34 The HPAI H5N1 clade 2.3.4.4b rapidly spread across continents within a two-year period  
35 starting from 2020, facilitated primarily by wild bird movements (Alkie et al., 2022). As the  
36 intensity of H5N1 clade 2.3.4.4b HPAI outbreaks continues to rise, the impact on wildlife has  
37 become significantly more severe (Charostad et al., 2023). The recent panzootic poses a  
38 significant conservation challenge, particularly on the health and survival of Antarctic  
39 wildlife. The challenge is exacerbated by the limited surveillance of Antarctic regions,  
40 complicating human monitoring and preventive measures. Moreover, colonial nesting

41 seabirds are particularly vulnerable to disease transmission (Boulinier, 2023). These birds,  
42 whose reproductive behaviors involve nesting closely together for prolonged periods, face  
43 heightened risks of disease spread (Dewar et al., 2023). These circumstances significantly  
44 increase the threat to their health and population stability.

45 Although other strains of H5 avian influenza virus have been reported in seabirds in  
46 Antarctica before (Hurt et al., 2014; Barriga et al., 2016; Ogrzewalska et al., 2022), the  
47 pathogenic H5N1 has never been reported up to 2023. In October 2023, a sudden and marked  
48 increase in strandings and mortality was noted among skuas, kelp gulls, elephant seals,  
49 gentoo penguins and fur seals in Falkland/Malvinas, Bird Island, South Georgia, and the  
50 South Shetland Islands (Bennison et al., 2023), raising significant concern. Suspected  
51 mortalities of skuas were observed in Heroína Island, and Esperanza bay in Antarctica. More  
52 recently, in February 2024, positive cases were detected among Skuas in the Western  
53 Antarctic Peninsula (<https://scar.org/library-data/avian-flu>).

54 We conducted epidemiological surveys of seabird nesting sites in the Antarctic Peninsula,  
55 Weddell Sea, and the Antarctic western coast (Bellinghausen, Amundsen, and Ross Seas) in  
56 December 2023 and January 2024, and report our findings here.

57

## 58 **Methods**

59 From December 2023 to January 2024, 13 seabird breeding sites were visited along the East  
60 and West coasts of the Antarctic Peninsula and the Antarctic western coast (Figure 1), on  
61 board Le Commandant Charcot PONANT vessel. A total of 115 birds from 4 species were  
62 captured, sampled, and released (see Table 1 for species and sample sizes). The procedure  
63 was conducted taking into account the recommendations provided by Biological Risk

64 Assessment of Highly Pathogenic Avian Influenza in the Southern Ocean of SCAR (Dewar et  
65 al., 2023). The cloacal sampling protocol was carried out using 45 cm swabs. Samples were  
66 preserved in denaturant Viral Transport Medium VTM and refrigerated at 4°C. They were  
67 processed within the weeks following sampling.

68 RNA was extracted using HiBind RNA Minicolumns (RNA extraction Omega) and amplified  
69 by RT-PCR against segment 4 of HPAI H5N1, with specific primers AH5-918F (5'-  
70 CCARTRGGKGCKATAAATC-3') and AH5-R1618 (5'-CCARTRGGKGCKATTAATC-  
71 3'), with an expected amplicon size of 782 bp. RT-PCR conditions were retrotranscription at  
72 45°C for 30', followed by denaturation of 95°C for 3', and 40 cycles of 95°C for 15'', 52°C  
73 for 30'', and 72°C for 60'', followed by final extension of 72°C for 10'. The procedure was  
74 repeated and confirmed three times for the suspected cases and randomly chosen negative  
75 cases, with a blank control included for each PCR run.

76 Importantly, the 16 Adélie penguins sampled in Beagle Island were also equipped with Argos  
77 satellite trackers within the framework of another study (Le Bohec, *unpublished data*), which  
78 allowed us to assess their fate in the period following sampling.

## 79 **Results**

80 Of the 115 individuals sampled, 9 tested positive, including 8 Adélie penguins and 1  
81 Antarctic shag, corresponding to a positivity rate of ~12% for all the Adélie penguins  
82 sampled. On Beagle Island (Danger Islands, at the northern tip of the Antarctic Peninsula),  
83 nearly half the Adélie penguins sampled were positive for RT-PCR amplification. All  
84 suspected positive cases came from the Antarctic Peninsula, and no suspected cases were  
85 detected in the 64 samples collected in the western part of the Bellingshausen Sea, in the  
86 Amundsen Sea, or in the Ross Sea. None of the birds sampled, including the suspect cases,  
87 showed any symptoms. Crucially, the 7 Adélie penguins that tested positive in Beagle Island

88 and also carried an Argos tracker were still alive and engaged in breeding-like foraging  
 89 behavior as of early March 2024, over 2 months after the positive test, thus establishing the  
 90 low-pathogenicity of H5N1 infection in these 7 cases. Molecular analysis confirmed the  
 91 presence of PCR amplicons of the expected size for H5N1 in positive cases and their  
 92 replicates, while replicates of negative cases consistently yielded negative results.

93

94 **Table 1.** Detailed breakdown of the total number and suspected samples per species, status,  
 95 period, and location sampled during the summer 2023/2024.\* stands for suspected cases.

Species	Period	Locality	Sample size	N° of suspected cases	Status	Geographical coordinates (Lat, Long)
<i>Pygoscelis adeliae</i>	December 2023	Beagle Island	16	7 (43.75%)*	Adult	-63.41°, -54.66°
<i>Aptenodytes forsteri</i>	January 2024	Snow Hill	10	0	Chick	-64.57°, -57.36°
<i>Aptenodytes forsteri</i>	January 2024	Jason Peninsula	10	0	Adult	-66.16°, -60.36°
<i>Pygoscelis adeliae</i>	January 2024	Red Rock Ridge	9	1 (11.11%)*	Adult	-68.29°, -67.18°
<i>Leucocarbo bransfieldensis</i>	January 2024	Red Rock Ridge	4	1 (25.00%)*	Adult	-68.29°, -67.18°
<i>Stercorarius mackormmicki</i>	January 2024	Red Rock Ridge	1	0	Chick	-68.29°, -67.18°
<i>Stercorarius mackormmicki</i>	January	Stonington	1	0	Chick	-68.18°, -

	2024	Island				66.99°
<i>Aptenodytes forsteri</i>	January 2024	Carroll Inlet	1	0	Adult	-73.18°, -78.43°
<i>Pygoscelis adeliae</i>	January 2024	Sims Island	7	0	Adult	-73.27°, -78.54°
<i>Stercorarius mackormmicki</i>	January 2024	Sims Island	6	0	Chick	-73.27°, -78.54°
<i>Aptenodytes forsteri</i>	January 2024	Siple Island	7	0	Adult	-72.77°, -127.15°
<i>Stercorarius mackormmicki</i>	January 2024	Cape Burks	1	0	Chick	-74.75°, -136.87°
<i>Pygoscelis adeliae</i>	January 2024	Cape Burks	7	0	Adult	-74.75°, -136.87°
<i>Stercorarius mackormmicki</i>	January 2024	Stephen Island	1	0	Chick	-75.65°, -146.92°
<i>Aptenodytes forsteri</i>	January 2024	Cape Colbeck	3	0	Adult	-77.07°, -155.70°
<i>Pygoscelis adeliae</i>	January 2024	Cape Royds	13	0	Adult	-77.55°, -166.16°
<i>Pygoscelis adeliae</i>	January 2024	Cape Hallett	15	0	Adult	-72.34°, -170.21°
<i>Stercorarius mackormmicki</i>	January 2024	Cape Hallett	3	0	Chick	-72.34°, -170.21°

96

97 **Discussion**

98 The first-time detection of suspected cases of H5N1 influenza in penguins and cormorants in  
99 the Antarctic continent marks a significant expansion of the panzootic into this isolated  
100 continent and puts highly vulnerable bird populations at risk. Individual cases were already  
101 reported for migratory Antarctica skuas in the peninsula region in the previous months  
102 (<https://scar.org/library-data/avian-flu>), confirming the fear that this widely-dispersing bird  
103 may be the first vector or the virus to the continent. Our findings now establish the  
104 transmission of the virus to two Antarctic resident species. Although this study covered a  
105 large part of the West Antarctic, at this stage positive cases seem restricted to the Antarctic  
106 peninsula (Beagle Island and Red Rock Ridge in our sampling). However, it is now clear that  
107 the HPAI virus has penetrated the Antarctic continent and has a high prevalence in two  
108 highly abundant resident species. There is thus significant apprehension regarding the further  
109 spread of the virus, due to the high density and mobility of these birds and their gregarious  
110 reproductive behavior - two traits that enhance intra and inter-colony virus spread, and that  
111 are thought to have largely worsened the HPAI panzootic in Northern Hemisphere seabirds  
112 (Paradell et al. 2023)

113 The absence of clinical signs in potentially HPAIV H5N1-infected birds, coupled with the  
114 lack of excess mortality at the sampled sites despite a positivity rate of roughly 50% among  
115 Adelie penguins in Beagle Island, raises the possibility that some seabird species may be  
116 more susceptible to H5N1 than others, as previously reported by Chilean authorities in  
117 Humboldt and Magellanic penguins (SERNAPESCA, [https://www.sernapesca.cl/influenza-](https://www.sernapesca.cl/influenza-aviar/)  
118 [aviar/](https://www.sernapesca.cl/influenza-aviar/)). These asymptomatic cases may seem reassuring for the species in question, but they  
119 have strong implications for Antarctic wildlife in general, potentially leading to unnoticed  
120 and widespread virus transmission, as asymptomatic carriers could serve as "trojan horses,"  
121 facilitating the introduction and spread of HPAIV to previously unaffected populations,  
122 eventually contaminating more susceptible species (such as pinnipeds, who died in large



123 numbers of HPAI in South America and South Georgia). These new findings emphasize the  
124 pressing need to monitor and address emerging diseases in vulnerable Antarctic seabirds.  
125 Implementing rigorous biosecurity measures during Antarctic activities is essential to  
126 minimize the potential spread of avian influenza.

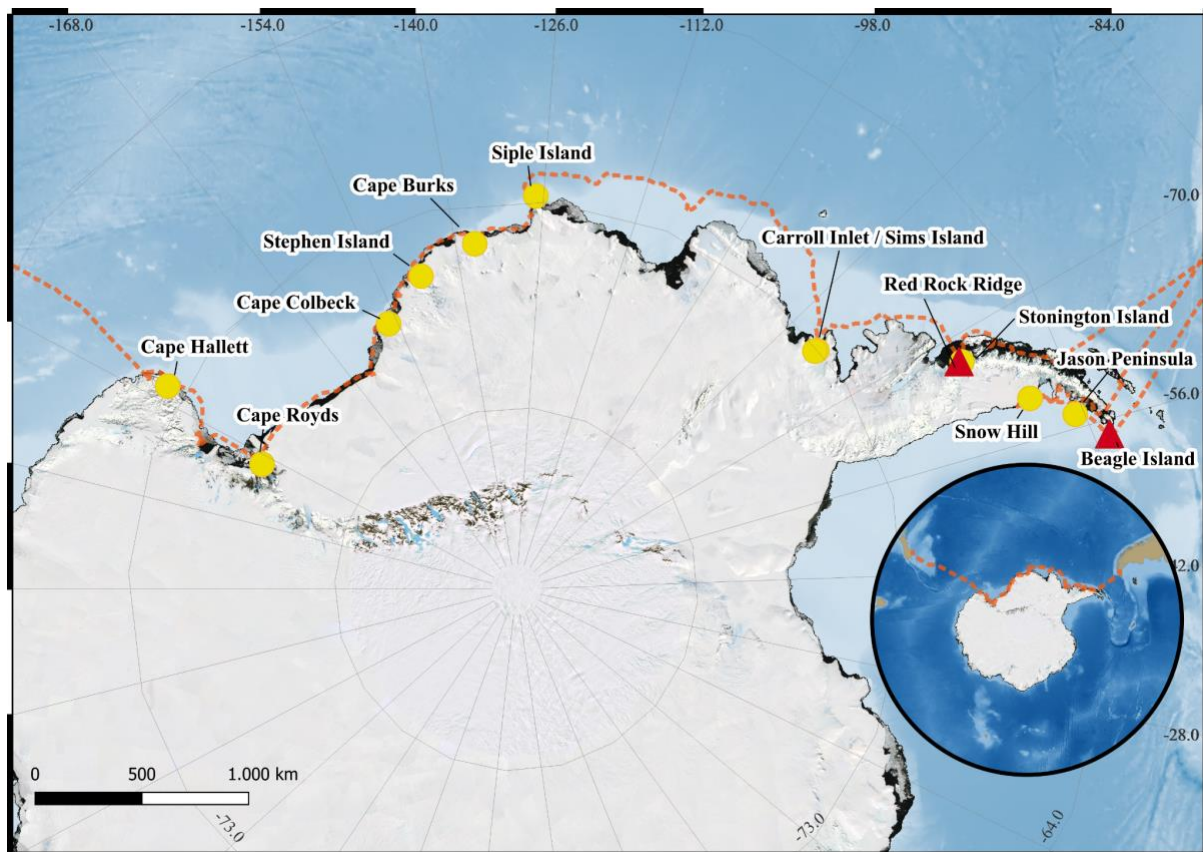
127

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140 and 151-2024).

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143

144 **Figure 1.** Sampling sites. Positive cases are denoted in red triangles and negative cases in  
145 yellow circles. The orange dashed line shows the ship track followed.

146

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