Biological Risk Assessment of Highly Pathogenic Avian Influenza in the Southern Ocean

Document prepared by

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1. Executive Summary

Since October 2021, the world has been facing an unprecedented global panzootic caused by High Pathogenic Avian Influenza (HPAI) H5N1 clade 2.3.4.4b. During this time, millions of birds died throughout the Northern Hemisphere, the Americas and Southern Africa, with Australia, New Zealand and Antarctica the only areas free from HPAI H5N1 as of August 2023 (Klaassen & Wille, 2023). In July 2022, significant outbreaks occurred in wildlife of the Northern Hemisphere, particularly affecting seabirds (Falchieri et al. 2022), increasing concerns about the risk to Southern Ocean seabird populations. The arrival and rapid southerly spread of HPAI H5N1 in South America in 2022/23 has significantly increased concerns that the virus could spread to the Southern Ocean during the upcoming 2023/24 Austral summer. Due to this heightened concern, the Antarctic Wildlife Health Network (AWHN) investigated the potential risks for the 2023/24 Austral spring and summer and provided the Antarctic and sub-Antarctic community with recommendations and guidelines for the monitoring and mitigation of HPAI in the region.

In October 2022, HPAI arrived in Central and South America. Since its arrival in South America, the virus rapidly spread south along the Pacific coast of South America, particularly impacting Peru, and Chile (e.g., Jimenez-Bluhm et al. 2023; Leguia et al. 2023). The virus travelled over 6,000 km in just three months, eventually reaching the southernmost tip of Tierra del Fuego. Since its arrival in South America, over 500,000 seabirds have died due to HPAI H5N1, with pelicans, boobies, cormorants and penguins most heavily impacted. Its arrival into South America also saw the first significant outbreaks in marine mammals with approximately 20,000 South American sea lions (*Otaria flavescens*) dying in Peru and Chile alone. In August 2023, further sea lion mortalities were observed on the Atlantic coast of Argentina, with cases detected from Tierra del Fuego all the way north to the shores of Buenos Aires. This most recent northerly spread up the Atlantic coast has covered more than 2,500 km in only a couple of weeks. The arrival of HPAI H5N1 in Tierra del Fuego and the increasing number of cases in all major sea lion rookeries on the Argentinean coast significantly increases the risk of HPAI H5N1 arriving in the sub-Antarctic and Antarctic Peninsula during the 2023/24 Austral spring and summer.

Given the potential threat posed by HPAI H5N1 to the Southern Ocean this upcoming Austral spring and summer, the AWHN undertook a biological risk assessment of the region and its bird and marine mammal biodiversity. This risk assessment considers the most likely potential pathways for HPAI H5N1 into the region, the risk posed to distinct geographical areas, and identifies the species most susceptible to mass mortalities. In addition, the AWHN has prepared guidelines and recommendations for surveillance, monitoring, and responding to HPAI, research priorities and enhanced biosecurity measures for all operators in the Southern Ocean.

1.1. Glossary

Term	Definitions
AIV	Avian influenza virus
PPE	Personal Protective Equipment, equipment worn to minimize exposure to hazards that cause serious injury or illness
HPAI	High Pathogenicity Avian Influenza
LPAI	Low Pathogenicity Avian Influenza
Trained Personnel	Personnel who have undergone recommended training as outlined in Section 6.1 prior to participating in sample collection or molecular testing for HPAI.
AIV Clade	A group of avian influenza viruses that share a common ancestor and possess similar genetic characteristics, e.g., clade 2.3.4.4b HPAI H5N1.
NAP	National Antarctic Programmes
COMNAP	Council of Managers of National Antarctic Programs
SCAR	Scientific Committee for Antarctic Research
IAATO	International Association of Antarctica Tour Operators
Reservoir	Host species in which a pathogen endemically circulates and is considered to have coevolved with.
AWHN	Antarctic Wildlife Health Network
GVS	Group Vulnerability Score
Class 3 Pathogen	A pathogen or biological agent which can cause serious and potentially lethal disease via the inhalation route. Appropriate precautions should be taken when working with or handling these pathogens.
WAHIS	World Animal Health Information System from the World Organisation for Animal Health.

	This portal provides information on reported wildlife disease outbreaks.
Panzootic	A panzootic is a disease outbreak that affects animals over an extensive geographic area, or even worldwide. It is the animal equivalent of a pandemic in humans.
Seabirds	Birds adapted to life within the marine environment, comprising all species of the orders Sphenisciformes, Procellariiformes and Phaethontiformes and of the families Alcidae, Stercorariidae, Sulidae and Fregatidae, as well as some species of the families Pelecanidae, Phalacrocoracidae and Laridae.
Shorebirds	Birds of the suborders Charadrii or Limicoli (order Charadriiformes), which are commonly found near the edge of bodies of water (lakes, estuaries, coastline, etc.)
Waterfowl	Birds of the order Anseriformes, which spend the majority of their lives on or near water bodies.

2. Background

2.1. Epidemiology of avian influenza viruses and emergence of 2.3.4.4b HPAI H5N1

Avian influenza virus (AIV) comprises the avian strains of Influenza A virus (species *Alphainfluenzavirus influenzae*, family *Orthomyxoviridae*) which are grouped into low pathogenicity avian influenza (LPAI), which usually causes no clinical signs of disease in wild birds, and high pathogenicity avian influenza (HPAI), which tends to cause disease with high morbidity and mortality in poultry and wild birds (Olsen et al. 2006). The AIV strains maintained in wild birds are LPAI, whereas the emergence of HPAI strains has invariably been linked to poultry (Olsen et al. 2006; Lee et al. 2021). There is a substantial diversity of LPAI viruses, comprising 16 haemagglutinin (HA) subtypes and nine neuramindase (NA) subtypes, generally reported in HA-NA subtype combinations. The main host reservoirs for LPAI viruses are members of the Anseriformes (ducks, geese, swans) and Charadriiformes (shorebirds, gulls, terns). Dabbling ducks of the genus *Anas* are reservoirs for the largest diversity (HA-NA subtypes) of LPAI viruses with very high prevalence (~20% of the population infected at any given time during the autumn) in northern hemisphere populations (Olsen et al. 2006; Latorre-Margalef et al. 2014).

Of particular concern are AIV strains of subtypes H5 and H7, which can become highly pathogenic in poultry, with the potential to spillover into wild populations (Horimoto & Kawaokade 2005; Monne et al. 2014; de Bruin et al. 2022). Infection of birds with HPAI viruses can result in up to 100% mortality. While most HPAI viral lineages were eradicated by eliminating infections in poultry, the Gs/GD lineage (for Goose/Guangdong, the species and location in which this lineage was first identified) has been enduring and diversifying (Caliendo et al. 2022a; Sonnberg et al. 2013; Xie et al. preprint). The viruses of the Gs/GD lineage are thought to have emerged in Asia in 1996, before spreading throughout Asia, Africa, and Europe to become established as endemic in Asian and African poultry (Lycett et al. 2019; Wille & Barr 2022). Outbreaks of this lineage in wild birds have occurred sporadically, with notable outbreaks occurring in 2005, 2014, 2016 and the current panzootic that commenced in 2021 (Lycett et al. 2019; Xie et al. preprint; Wille & Barr 2022). Prior to 2016, there was limited evidence that wild birds were a reservoir for these viruses, rather, continual spill over from poultry was considered the cause of the outbreaks in wild birds (Xie et al. preprint). This changed in 2016 (Poen et al. 2018) and a recent study determined that the virus now preferentially replicates in waterfowl (James et al. 2023).

In 2021, through key genetic changes in the virus, emergence of clade 2.3.4.4b HPAI H5N1 was noted, and the clade has since replaced most genetic clades of HPAI H5Nx circulating globally (Wille & Barr 2022). Further, there has been a dramatic increase in the range of HPAI H5N1, facilitated by wild bird migration with two independent introductions into North America and at least three independent introductions into South America (Ruiz-Saenz et al. 2023), an increase in the number and magnitude of wild bird and poultry outbreaks, a substantial expansion in host range (that is, widespread outbreaks in seabirds for the first time) (Klaassen & Wille 2023), and infections observed throughout the year rather than being limited to the northern hemisphere Autumn and Winter (EFSA et al. 2022; Gass, et al. 2022).

2.2 Avian influenza in Seabirds

2.2.1 Role of seabirds, particularly Antarctic seabirds, in the ecology of avian influenza virus

2.2.1.1 LPAI in seabirds as context for HPAI in Antarctica

While the main reservoirs of AIV are members of the Anseriformes (ducks, geese, swans) and Charadriiformes (shorebirds, gulls, terns) (Olsen et al. 2006), a variety of seabird species have also been linked to the ecology of these viruses (Lang et al. 2016). An outbreak of HPAI H5N3 (unrelated to current HPAI H5 strains) in common terns (*Sterna hirundo*) in South Africa in 1961 was the first time a seabird mortality event was attributed to HPAI (Becker 1966). Further, the isolation of H6N5 from wedge-tailed shearwaters (*Ardenna pacifica*) in Australia was the first detection of AIV in healthy (disease-free) seabirds (Downie & Laver 1973). Decades of work since have determined that seabirds including guillemots, murres, shearwaters, penguins, gulls, and terns can be reservoirs for LPAI (Lang et al. 2016).

In the seabirds of Antarctica, LPAI strains have been detected on the Antarctic Peninsula and South Shetland Islands in adélie (*Pygoscelis adeliae*), gentoo (*P. papua*), and chinstrap (*P. antarcticus*) penguins (Sanfilippo 2010; Hurt et al. 2014, 2016; Barriga et al. 2016), southern giant petrel (M*acronectes giganteus*) (de Souza Petersen et al. 2017), brown skua (*Stercorarius antarcticus*) (de Seixas et al. 2022), and snowy sheathbill (*Chionis albus*) (Hurt et al. 2016). Antibodies against AIV have also been detected in a range of seabird species and localities on the Antarctic continent and sub-Antarctic islands (as reviewed by Lang et al. 2016; Smeele et al. 2018).

While most detections were not part of long-term studies, repeat detections of some subtypes (for example, H11N2; Hurt et al. 2014) and time-structured analysis of genome data (for example, LPAI H5N5; Barriga 2016; Ogrzewalska et al. 2022) indicate that some AIV lineages persist and are isolated to Antarctic birds. Other studies have demonstrated genetic connectivity of AIV in Antarctic seabirds with lineages from South, and potentially North America, particularly in highly mobile species such as brown skuas and southern giant petrels (de Seixas et al. 2022; de Souza Petersen et al. 2017). Overall, seabirds, including a diversity of Antarctic seabirds, are susceptible and likely play a role as reservoirs and vectors for AIVs.

2.2.2 Strains of the HPAI H5Nx Clade 2.3.4.4b

Since 2021, seabirds have been impacted by HPAI outbreaks globally, with 95 species affected to date. Seabird groups currently unaffected are those with no (or limited) exposure; that is, they occur in regions with no HPAI activity, such as Antarctica, or are highly pelagic, such as the Procellariiformes (Klaassen & Wille 2023). In general, HPAI has now been confirmed in approximately 20% of the Anseriformes, Charadriiformes, Gaviiformes, Pelecaniformes and Suliformes. Herein, the major events and outbreaks of HPAI in seabirds globally will be briefly outlined.

2.2.2.1. Asia

The H5N1 Gs/GD lineage has been endemic in Asian poultry since 1996 (Lycett et al. 2019; Wille & Barr 2022), with sporadic outbreaks in wild birds, with notable outbreaks since that time including the current panzootic that commenced in 2021 (Lycett et al. 2019; Xie et al. preprint; Wille & Barr 2022). Recent outbreaks in July 2023 were recorded in wildlife (unspecified) in the Xizang Province of the People's Republic of China with approximately 5,182 cases, and more recently in domestic cats in Republic of Korea (WOAH 2023). There were no reports of ongoing or new events of HPAI in poultry in Asia between 23/06/2023-13/07/2023, although there was a recurrence of H5N6 in Nueva Ecija, Philippines (WOAH 2023). There were also no reports of HPAI for Asia in non-poultry birds for the same time period (WOAH 2023).

2.2.2.2 Africa

Since the incursion of 2.3.4.4 H5Nx into Africa in 2017-18, seabirds have been heavily impacted by outbreaks. The first indication of an unusual spread of this virus towards southern Africa was the detection of HPAI in terns in Uganda, East Africa, in 2017, resulting in the death of approximately 1,200 white-winged black terns (*Chlidonias leucopterus*) in a population comprising only 2,000 individuals (Ndumu et al. 2018). The virus soon arrived in southern Africa causing

repeated and devastating outbreaks in seabirds of South Africa and Namibia. Between December 2017 and May 2018, an estimated 7,415 birds of 15 species, including greater crested terns (*Thalasseus bergii*, forming the majority), African penguins (*Spheniscus demersus*), cape gannets (*Morus capensis*), common terns, sandwich terns (*Thalasseus sandvicensis*), hartlaub's gulls (*Larus hartlaubii*), cape cormorants (*Phalacrocorax capensis*), crowned cormorants (*Microcarbo coronatus*) and African oystercatchers (*Haematopus moquini*) died due to clade 2.3.4.4 H5N8 in South Africa (Peyrot et al. 2022; Roberts et al. 2023). Between December 2018 and February 2019, more than 350 African penguins (~5% of the colony) died on Halifax Island in Namibia (Molini et al. 2020). These viruses were genetically similar to a virus found in a hartlaub's gull in South Africa, raising questions about the role of these gulls in viral movement (Peyrot et al. 2022; Roberts et al. 2023). Outbreaks have continued in Southern Africa with the arrival of clade 2.3.4.4b H5N1 in 2021-2022, with 24,463 cape cormorants (of 57,000 breeding pairs) and 230 African penguins dead in South Africa, and 6,500 cape cormorants dead in Namibia (McCain 2021; Abolnik et al. 2023; Molini et al. 2023; Roberts et al. 2023). Occasional detection of the virus in seabirds admitted for rehabilitation in South Africa has continued in 2023 (D.G. Roberts, pers. comm 2023).

2.2.2.3. Europe

In the Northern Hemisphere prior to 2022, seabirds were not widely affected by the HPAI panzootic despite outbreaks in 2014, 2016 and an outbreak commencing in 2020 (Xie et al. preprint; EFSA et al. 2023), despite significant mortalities of shorebirds and waterfowl as exemplified by the death of 16,000 barnacle geese (*Branta leucopsis*) in the United Kingdom in November/December 2021, which represented nearly 10% of the species' Svalbard population (Wille & Barr 2022; Calinendo et al. 2022a). Estimates of barnacle geese mortalities migrating from Greenland in the same season are estimated to be up to 5,000 birds (NatureScot 2023). A high degree of enterotropism in the intestinal tract of wild ducks and geese, including barnacle geese, was seen, in addition to the typical respirotropism and neurotropism of HPAI (Caliendo et al., 2022b). This adaptation could enhance the virus's ability for faecal-oral transmission in wild birds, a mechanism more common in LPAI (Caliendo et al. 2020, Caliendo et al. 2022b).

With the emergence of 2.3.4.4b H5N1, high mortality has occurred in seabirds across Europe and North America, with large outbreaks in gannets, terns, gulls, and skuas (NatureScot 2023; EFSA et al. 2023; Knief et al. preprint; Lane et al. preprint). For example, in 2022, approximately >22,000 sandwich terns were reported dead in Europe in a two-month period, comprising >17% of the

breeding population. In some instances, more than 60% of individuals in a colony died (Pohlmann et al. 2023), and France is believed to have lost 10% of their breeding sandwich tern population over a one-week period with almost 100% mortality of chicks observed (Knief et al. preprint). In monitored northern gannet (*Morus bassanus*) colonies in the North Atlantic, the earliest HPAI reports occurred in Iceland, followed by the islands of Scotland and a southward spread throughout the 2022 summer breeding season (Lane et al. preprint). Only one colony did not have increased mortality associated with HPAI. Over 5,000 carcasses were recorded at Bass Rock, United Kingdom, and many more birds died unobserved at sea, with a documented 75% decline in occupied nests (Lane et al. preprint). In Ireland, 3,126 northern gannets were suspected to have died during the 2022 outbreak (Oriol et al. 2023).

Predatory and scavenging seabirds such as skuas and gulls were also heavily affected. For example, in 2021, 10% of great skuas (*Stercorarius skua*) on some islands off the United Kingdom died due to HPAI (Banyard et al 2022). In 2022, following the death of 1,400 skuas, it was estimated that 60-70% of territories were unoccupied on the island of Foula, Scotland, (Camphuysen & Gear 2022). Gulls have similarly been heavily affected, with reports of continued outbreaks in black-headed gulls (*Larus ridibundus*), including substantial outbreaks in France, Belgium, Germany and the Netherlands (Dutch Wildlife Health Centre, 2023). Since 15 January 2023, almost 2,000 dead gulls, predominantly black-headed gulls, have been reported in the Netherlands (Dutch Wildlife Health Centre, 2023). While large HPAI outbreaks affecting single species are noteworthy, most outbreaks were not isolated to individual taxa, instead affecting entire seabird assemblages. For example, data from Scotland indicate mortality due to HPAI occurred concurrently across skuas, gannets, gulls, terns, guillemots, fulmars, shearwaters, shags and eiders (Falchieri et al. 2022).

2.2.2.4 Americas

High pathogenic avian influenza virus emerged in North America in November 2021 with the first wild bird detections in great black-backed gulls (*Larus marinus*) in Newfoundland, Canada (Alkie et al. 2022). Tens of thousands of seabirds, including gannets and murres, are reported to have died due to HPAI in Canada in 2022 (Harvey et al. 2023), and it was reported that many seabirds were absent from colonies in Alaska (Andy Ramey, pers. comm 2022). As seen in Europe, terns were heavily affected with 62% of caspian terns (*Hydroprogne caspia*) in Lake Michigan reported to have died due to HPAI in 2022 (Harvey et al. 2023). Due to COVID-19 restrictions, most seabird

colonies in the Arctic were not visited by researchers in 2022 (Gregory Robertson, pers. comm 2022), such that the true scale of this outbreak is unknown.

In 2022, the virus emerged in South America spreading from Colombia to southernmost Chile in less than six months, travelling over 6,000 km. Unlike in other regions, outbreaks of HPAI in South America have been heavily biased toward seabirds, with relatively few reports in other wild birds, for example in waterfowl, which have been heavily affected in other regions. By July 2023, the deaths of 519,541 seabirds had been reported by the Peruvian Government; which included 36% of the Peruvian pelican population (*Pelecanus thagus,* n=57,335), 229,554 Peruvian boobies (*Sula variegata*), 201,047 guanay cormorants (*Leucocarbo bougainvillei*), and 5,573 inca terns (*Larosterna inca*) (Ariyama et al. preprint; Leguia et al. 2023; Perú Ministerio de Salud 2023). Gulls, including kelp gulls (*Larus dominicanus*) have also tested positive for HPAI in Peru and Chile (Azat et al. preprint; Jimenez-Bluhm et al. 2023). HPAI has now emerged in penguin populations in South America, with 3,157 humboldt penguins (*Spheniscus humboldti*) and 460 magellanic penguins (*Spheniscus magellanicus*) found dead in Peru and Chile during the 2022-2023 suspected of HPAI infection, with four (4) humboldt penguins testing positive to HPAI in Chile (Chile Servicio Agricola y Ganadero, 2023).

2.2.2.5 Oceania & Antarctica

As of 1 September 2023, HPAI strains of clade_2.3.4.4b were not detected in wildlife or poultry in Oceania (including Australia and New Zealand) or Antarctica (Rafique et al. 2023).

2.3. Epidemiology, pathology, and clinical signs of disease

Avian species have differing levels of susceptibility to disease caused by HPAI infection. For example, 58% of mallards (*Anas platyrhynchos*) experimentally infected with HPAI 2.3.4.4b H5N1 have subclinical infections, that is, an absence of clinical signs (Spackman et al. 2023), and mallards can migrate while infected with 2.3.4.4b H5N8 (Lv et al. 2022). In contrast, seabirds such as gannets, terns and kittiwakes are highly susceptible to clinical disease (Lane et al. preprint; Knief et al. preprint; Rijks et al. 2022; Oriol et al. 2023).

Despite genetic and associated epidemiological changes, specific clinical signs, gross lesions, and microscopic lesions caused by HPAI are consistent with those previously reported for other HPAI H5Nx viruses. Importantly, clade 2.3.4.4b HPAI is highly neurotropic, such that numerous reports of sick birds and South American sea lions include neurological signs due to the

predilection of the virus for the nervous system; approximately 20% developed neurological signs and 18% developed corneal opacity (Spackman et al. 2023). Sick and dying Sandwich Terns were reported to have abnormal posture with wings outstretched, and opisthotonos (severe hyperextension and backward arching of the neck) characterised by backward arching of the head, neck, and spine caused by muscle contraction (Rijks et al. 2022), example video at this link https://wwwnc.cdc.gov/eid/article/28/12/22-1292-vid1.

Stumbling, walking in circles or without coordination were additional clinical signs noted in infected great skuas, while some other birds were reported to have a drooping head and opisthotonos was reported in several species (Camphuysen et al. 2022). Ocular abnormalities are not limited to experimentally infected mallards, for example, northern gannets which recovered from HPAI infection had altered iris colouration (Lane et al. preprint) and while the latter was reportedly widespread across northern gannet colonies, it is unclear if this has been observed in other species.

In Humboldt penguins and South American sea lions' tremors and seizures were frequently observed in individuals suffering from HPAI in Chile and Argentina. Difficulties in locomotion and partial paralysis has also been observed in affected sea lions in Chile and Argentina (Victor Manuel Neira Ramírez, pers comm.; Ralph Eric Thijl Vanstreels pers obs.). Other more subtle clinical signs of infection can include fever and lethargy (Noh et al. 2021). In wild populations of griffon vultures (*Gyps fulvus*) exposed to HPAI, reduced mobility was noted (Duriez et al. 2023).

Viral shedding occurs in both faeces (cloacal) and respiratory (oropharyngeal) secretions, suggesting several routes of transmission. While the faecal-oral route is considered most important for LPAI (Roche et al. 2009; Lambrecht et al. 2016) recent evidence suggests that oral viral shedding might play a central role in the transmission of HPAI 2.3.4.4b strains among seabirds (Camphuysen et al. 2022). Colonial nesting seabirds and pinnipeds could be at greater risk of disease spread due to the close proximity of individuals and nests over a long period of time during the breeding season which will increase direct contact rates among individuals and/or increase direct contact with infected faeces and water.

2.4 Avian Influenza in Mammals

While previous lineages of HPAI H5Nx rarely infected mammals, thousands of mammals have now been infected with HPAI H5N1 2.3.4.4b. Mammalian species affected are predominantly carnivores, such as felids (domestic cats, lions, tigers, lynx), canids (coyotes, foxes, domestic dogs), mustelids (otters, minks, skunks, polecats), and bears (EFSA et al. 2023; Leguia et al. 2023; WOAH 2023; FAO 2023). The majority of infections were recorded in predators and scavengers, which were presumably infected through consumption of infected birds or carcasses. With the exception of a viral outbreak in farmed minks (Agüero et al. 2023; De Vries et al. 2023), no onward transmission chains in felids, canids, mustelids, or bears have been established.

This, however, is not the case for infections in marine mammals which are a diverse group comprising pinnipeds (true seals, fur seals, sea lions and walruses), cetaceans (whales, dolphins and porpoises), sirenians (manatees and dugongs), and marine fissipeds (polar bears and otters). The largest number of marine mammal deaths attributed to HPAI to date has been reported in South American sea lions with 9,314 deaths as of August 2023 in Peru (Gamarra-Toledo et al. preprint). In Chile, 16,856 pinniped mortalities have been recorded, predominantly South American sea lions but including small numbers of South American fur seals (*Arctocephalus australis*) although these mortalities have not been confirmed as being due to HPAI (Chile Servicio Nacional de Pesca y Acuicultura, 2023). In addition to 39 marine otters (*Lontra felina*), 106 cetaceans and two Southern elephant seals (*Mirounga leonina*), although only a small subset of these carcasses were tested for HPAI (Chile Servicio Nacional de Pesca y Acuicultura, 2023). Cases in South American sea lions along the Argentinean coast commenced in August 2023, with cases detected in all major rookeries from South to North, including in Rio Grande (Tierra del Fuego), Punta Loyola (Santa Cruz), Puerto Piramides (Chubut), Punta Bermeja (Rio Negro), Mar del Plata and Quequén (Buenos Aires) (SENASA, 2023a, 2023b). However, this recent mortality of sea lion in Argentina has not been associated with significant seabird mortality, raising the possibility that direct transmission between sea lions may have played a central role in the spread.

In Phocidae (true seals), first infection with 2.3.4.4b H5N8 was initially implicated in the death of a Grey Seal (*Halichoerus grypus*) in the Baltic Sea in 2017, although at this time, there was a lack of evidence to suggest high pathogenic of the virus to pinnipeds (Shin et al. 2019). In 2022, both Grey and Harbour seals (*Phoca vitulina*) died in an unusual mortality event in the Northeastern states of the USA concurrent with a second wave of avian infections, with infection considered to

be transmitted from wild birds to seals via environmental transmission of the virus (Puryear et al. 2023). Seals demonstrated both respiratory and neurological clinical signs prior to death and seal-to-seal transmission was not considered to be the primary route of transmission (Puryear et al. 2023). Grey and Harbour seals sampled in Scotland, USA, Canada, and the UK, and Caspian seals (*Pusa caspica*) in Russia have also tested positive for HPAI (EFSA et al. 2023). To date no phocid seals have been reported as positive in South America; two southern elephant seals were reported to have died in Chile during the outbreak, but no testing was conducted (Chile Servicio Nacional de Pesca y Acuicultura, 2023).

Finally, both dolphins and porpoises have tested positive for HPAI. In 2022, Harbour Porpoises (*Phocoena phocoena*) in Sweden and the UK (EFSA et al. 2023), a Bottlenose Dolphin (*Tursiops truncatus*) in Florida, USA (Thorsson et al. 2023; Carey 2022), and a White-sided Dolphin (*Lagenorhynchus acutus*) in Canada (EFSA et al. 2023) stranded and tested positive for HPAI. In South America, mortality has been attributed to HPAI infection in 12 Chilean Dolphins (*Cephalorhynchus eutropia*) (Chile Servicio Nacional de Pesca y Acuicultura, 2023) and in 16 Burmeister's Porpoises (*Phocoena spinipinnis*) (Chile Servicio Nacional de Pesca y Acuicultura, 2023) in Chile, and in a Common Dolphins (*Delphinus delphis*) in Peru (Leguia et al. 2023). All marine mammal numbers, and particularly those of cetaceans, are likely to be vast underestimates of the true situation, as only animals on land can be counted and tested for HPAI infection.

2.5 Risk to Human Health

The World Health Organization (WHO) states that the risk for human infection remains low but is greater for people occupationally exposed to infected birds. The UK joint Human Animal Infections and Risk Surveillance (HAIRS) group indicated the probability of human infection to the general population was "very lUSA< ow", and the probability of infection would be considered "low" for those exposed to infected live or dead non-avian wildlife (HAIRS 2023). However, the WHO also indicates that the zoonotic risk is elevated (Wille & Barr 2022).

There have been 863 human cases of HPAI H5N1 with 456 deaths between 2003–2021 (Wille & Barr 2022), although the vast majority of human cases occurred prior to 2008 and were therefore caused by HPAI H5N1 lineages that are now largely extinct. Since the onset of the current panzootic in 2020, there have been confirmed cases of HPAI H5N6 (China, Laos), H5N8 (Russia)

and H5N1 (China, Vietnam, Cambodia, UK, USA, Ecuador, Chile). In addition to confirmed cases, there have been an additional seven cases (USA, UK, Spain, Nigeria) of “environmental carriage” of HPAI H5N1 in humans, that is, these individuals returned a positive test for HPAI H5N1, but they had no clinical signs or indication of true infection (Aznar et al. 2023; Wille & Barr 2022). In all instances but one, a direct link to infected or dead birds has been reported. Overall, there have been few human infections despite the enormous widespread viral burden in poultry and wild birds. Indeed, in 4,000 occupationally exposed poultry workers in the USA, only a single individual returned a positive HPAI test, and this was later confirmed to be an environmental carrier rather than a true infection (Kniss et al. 2023).

The overall mortality rate in humans, when considering all HPAI H5Nx infections, is high (~50%), however, the mortality rate of human infections with clade 2.3.4.4 HPAI H5Nx is lower, with 40% reported (across all cases in China) (BNO News 2021). To date there have been no examples of human-to-human HPAI H5Nx transmission detected (Wille & Barr 2022). However, there are several instances of suspected mammal-to-mammal transmission, including within a mink farm (Agüero et al. 2023; De Vries et al. 2023), and between marine mammals in South America (Leguia et al. 2023). A recent laboratory study also demonstrated direct transmission between ferrets of viruses isolated from birds (Kobasa et al. preprint).

3. Expected Pathways of HPAI Arrival to sub-Antarctic and Antarctica

Table 1. Suspected pathways of HPAI into the sub-Antarctic and Antarctica

Pathway	**Likelihood**	**Species potentially involved**	**Evidence**
Directly from South America (Tierra del Fuego, or Patagonia) via birds	High	kelp gull, brown skua, southern giant petrel, snowy sheathbill, all waterfowl, and shorebirds	LPAI connectivity data. Known and suspected movements between Tierra del Fuego/Patagonia
Directly from South America via pinnipeds	Medium	Antarctic fur seals, southern elephant seals, leopard seals.	There are few records of individuals moving between Patagonia and Falklands (Islas Malvinas) to Antarctica.
From South America, via the Falkland Islands (Islas Malvinas)	Medium	sheathbills, giant petrels, skuas	Spring/Winter migrations of sheathbills and giant petrels from South America to Falklands (Islas Malvinas) to Antarctica
From infected continents, via sub-Antarctic islands (other than South America to Falklands (Islas Malvinas))	Low	Foraging seabirds, and potentially vector species above.	The virus has been in Africa for years and never made the jump.
Directly from Africa via birds	Low		The virus has been in Africa for years and never made the jump
From Oceania	Low	Birds and leopard seals, southern elephant seals	No HPAI in Oceania

Highly pathogenic avian influenza 2.3.4.4b H5Nx is causing outbreaks in seabirds of southern Africa and as of April 2023, HPAI 2.3.4.4b H5N1 had been detected in black necked swans (*Cygnus melancoryphus*) and South America sea lions in Tierra del Fuego, Chile (EFSA 2023;

Chile Servicio Nacional de Pesca y Acuicultura, 2023). This virus has not been detected in Australia and New Zealand as of 1 September 2023. As such, the highest risk of viral incursion to the sub-Antarctic and Antarctica in 2023 is from South America.

Species such as kelp gulls and brown skuas which transit between South America and the Antarctic Peninsula, are suspected to be important vectors for the introduction of HPAI into the sub-Antarctic south of South America and Antarctic Peninsula. Based on previous LPAI studies, southern giant petrels, another species known to transit between these regions (Figure 2), may also play a role in viral dispersal (de Souza Peterson et al. 2017).

Evidence for the putative role of these species also comes from studies of LPAI. For example, genetic connectivity of viruses from brown skuas and southern giant petrel between Antarctica and South America has been demonstrated (de Seixas et al. 2022; de Souza Petersen et al. 2017) and antibodies against LPAI have been detected in kelp gulls sampled in Kerguelen but have not been detected in other species suggesting these kelp gulls could have been infected outside of Kerguelen (Boulinier et al. unpublished). Finally, all three species are scavengers, and data from the Northern Hemisphere indicates scavenging is likely a major route of infection, with a large diversity of raptors and gulls affected to date.

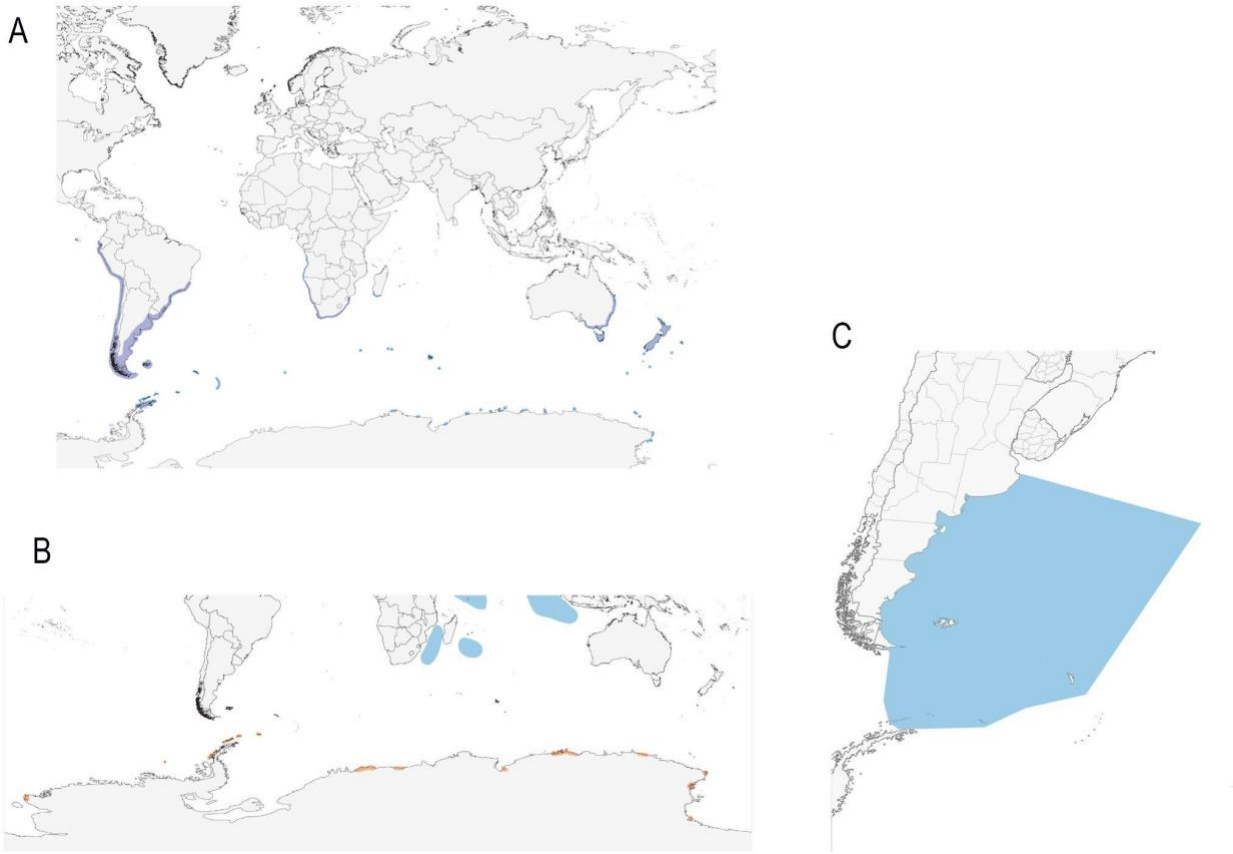

Figure 1. Map of distribution of a) kelp gulls, b) south polar skuas and c) brown skuas during breeding (orange) and non-breeding (blue) seasons and year-round distribution which may contribute to virus dispersal and introduction to Antarctica. Range maps from https://birdsoftheworld.org/.

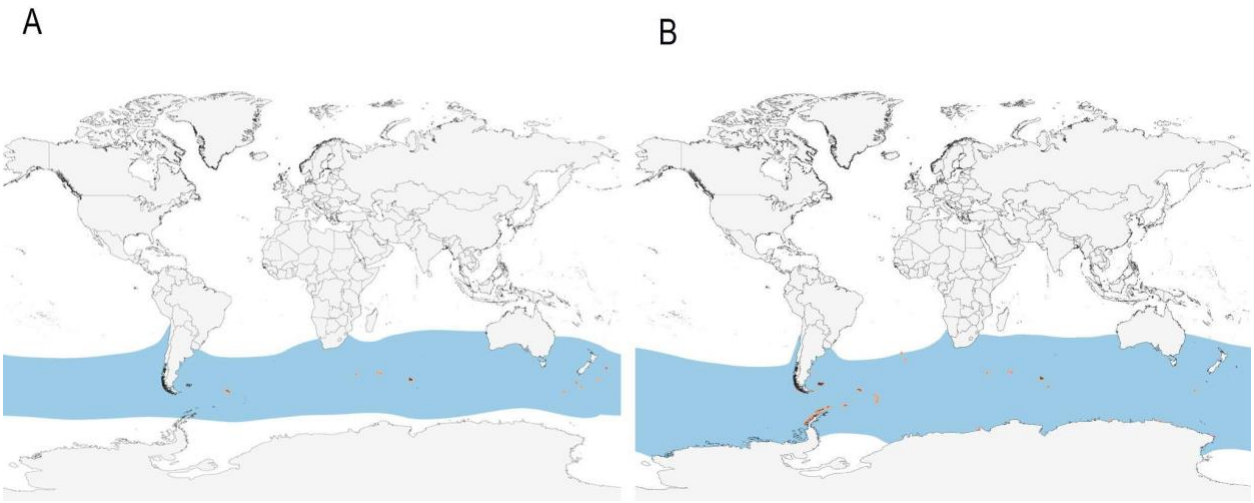

Figure 2. Map of distribution map of a) northern giant petrels and b) southern giant petrels during breeding (orange) and non-breeding (blue) seasons and year-round distribution which may contribute to virus dispersal and introduction to Antarctica. Range maps from https://birdsoftheworld.org/

3.1 Movement of HPAI into and within sub-Antarctic and Antarctica

The Pacific Americas flyway and the Mississippi–American flyways have been identified as an important pathway for the entry of Avian Influenzas into the Antarctic Peninsula, with some LPAI strains of American and Eurasian lineage found in brown skuas, southern giant petrels and chinstrap penguins (Barriga et al. 2016; de Seixas et al. 2022; de Souza Petersen et al. 2017). With current cases in the Pacific significantly increasing throughout 2023 and the rapid movement of the virus in South America, the most likely entry of HPAI into the sub-Antarctic and Antarctica will be via the Falklands (Islas Malvinas), the Scotia Arc and Antarctic Peninsula.

Given this is the first time HPAI will enter the Southern Ocean and the limited data that is available for the movement and spread of pathogens in the region, it is difficult to predict how it will move within the sub-Antarctic and Antarctic regions. However, highly mobile species and prospecting juveniles, such as black-browed albatrosses (*Thalassarche melanophris*) that move between colonies, are likely to be major spreaders of the virus once it arrives (Campioni et al. 2017; Boulinier 2023).

3.2 Potential Vector and Spreader Species

Past detection of LPAI strains in southern giant petrels, snowy sheathbills, and brown skuas along the Antarctic Peninsula, along with their migratory and scavenging behaviour, make these species potential vectors for HPAI incursion into the sub-Antarctic and Antarctic Peninsula (Barriga et al. 2016; de Souza Petersen et al. 2017; de Seixas et al. 2022; Petersen et al. 2017). In addition to these species, other species considered to be potential vectors due to their scavenging behaviour, past infections and migratory patterns include kelp gulls (high proportion of seropositive individuals indicating previous exposure to LPAI; Boulinier et al unpublished), south polar skua and Arctic terns, the latter having been heavily impacted by H5N1 outbreak in the Northern Hemisphere (EFSA et al. 2023). Shorebirds and waterfowl moving between South America, and the Falklands (Islas Malvinas) might also act as potential vectors for HPAI incursion into the sub-Antarctic, although there is little data on the movements of, or on AIV epidemiology, in those populations. This includes for example, species migrating between South America and the Falklands (Islas Malvinas) such as the white-rumped sandpiper (*Calidris fuscicollis*). It also includes species such as the two-banded plover (*Charadrius falklandicus*) or the upland goose (*Chloephaga picta*) which are present both in South America and in the Falklands (Islas Malvinas), however there is limited information on the connectiveness of these populations (Scherer et al 2013; Shirihai 2002; Summers 1983).

After introduction of the virus to the sub-Antarctic, those species could also contribute to its spread. Prospecting juveniles and sub-adults of all seabird species are also considered to pose a significant risk for transmission of HPAI between colonies. For species such as the black-browed albatross, daily movement of prospecting individuals between colonies can be as much as 10-20% of the sub-adult population (Campioni et al. 2017).

4. Disease Risk Assessment for sub-Antarctic and Antarctica

The Antarctic Wildlife Health Network (AWHN) has developed a risk assessment to evaluate the risk of HPAI to wildlife in the sub-Antarctic and Antarctica. To ensure this risk assessment remains up to date, the AWHN will be developing a monitoring database for recording HPAI and suspected HPAI outbreaks. Based on information in the database the risk assessment will be automatically updated and accessible to all operators for use in risk assessments for their region and can be used to inform their response plans. To ensure information is up to date and accurate in the

database, the AWHN would encourage all operators to input any data on suspected and confirmed HPAI outbreaks and negative results from surveillance programs.

The risk assessment was conducted in two stages, one focused on the wildlife groups and the other focused on geographical areas.

4.1 Risk Assessment for Wildlife Groups

For the wildlife groups, three risk variables (known susceptibility, risk behaviour, and population connectivity) were scored in a semi-quantitative scale (1 to 5) for each of the 30 wildlife groups. Table 2 presents the criteria used to score these variables and Table 3 presents the scores assigned to each wildlife group. The three scores can be multiplied by one another, then scaled to a range from 0 to 100 (subtract minimum, divide by range, and then multiply by 100), resulting in the Group Vulnerability Score (GVS) for each wildlife group (Figure 3).

For the risk assessment for wildlife groups, ‘Antarctic fauna’ was defined as all birds and mammals breeding on land in the geographical areas in addition to cetaceans whose normal distribution range includes at least part of Antarctica, with a total of 168 species in five categories: 79 seabirds, 24 freshwater aquatic birds, 32 terrestrial birds, 11 pinnipeds, and 22 cetaceans. According to their taxonomy and relevant biological characteristics, these species were assigned to 30 wildlife groups (Table 2). Information about the taxonomy and the geographic distribution was derived from the literature and public databases (Berta 2015; BirdLife International 2019; International Union for Conservation of Nature and Natural Resources 2019; Lepage 2023).

Table 2. Variables and definitions of the scores used to quantify risk for wildlife groups.

Variable	Score	Definition
A) Known susceptibility Is the group capable of being infected by HPAI? (Scoring considers available information at the family/order level)	1	Susceptibility is unlikely. There are no reports of AIV infection in these species nor in closely related taxa, and there has been extensive surveillance effort.
	2	Susceptibility is unknown. There are no reports of AIV infection in these species (nor in closely related taxa - to be consistent with 1), however surveillance effort has been minimal.
	3	Susceptibility is probable. There are no reports of HPAI infection in these species (closely related taxa), however LPAI infection has been detected.
	4	Susceptibility is known but impacts have been limited. There are reports of HPAI infection in these species, but no mass mortality events have been attributed to HPAI.
	5	Susceptibility is known and impacts are significant. Mass mortality events of these species have been attributed to HPAI.
B) Risk behaviours Does the group present behaviours that might put it at an increased risk of exposure/transmission of HPAI?	1	Predominantly solitary (intra-species interactions limited to pair formation and parental care) and specialized feeding habits (diet relying on plants, invertebrates or fishes).
	2	Solitary with occasional gregariousness (intra-species interactions usually limited to feeding frenzy events or

		territorial defense) and specialized non-predatory feeding habits (diet relying on plants, invertebrates, or fishes).
	3	Moderate gregariousness (congregation in low-density roosters or crepuscular/nocturnal rafts, surface nesting in low-density colonies, burrow-nesting, seasonal pods/social groups at sea, colonial (pinnipeds)) or generalist feeding habits (flexible diet that may occasionally include kleptoparasitism/predation/scavenging of birds or mammals).
	4	High gregariousness (congregation in high-density roosters, foraging in mixed-species flocks or pods, surface nesting in high-density colonies, year-round pods/social groups at sea, colonial (pinnipeds)) or frequent predatory feeding habits (heavy reliance on kleptoparasitism, predation or scavenging of birds or mammals).
	5	High gregariousness (congregation in high-density roosters, foraging in mixed-species flocks or pods, surface nesting in high-density colonies, year-round pods/social groups at sea, colonial (pinnipeds)) and frequent predatory feeding habits (heavy reliance on kleptoparasitism, predation or scavenging of birds or mammals).
C) Population connectivity Do the individuals of this group frequently interact with those of other island groups, continents, or oceans?	1	Predominantly endemic to a specific island group or region within Antarctica.
	2	Movement of individuals is largely limited to specific regions or relatively close island groups within Antarctica, with scarce evidence of long-distance gene flow or movements.

	3	Breeding is restricted to Antarctica/sub-Antarctic, but individuals often engage in circumpolar movements in the Southern Ocean, with frequent at-sea or land-based (pinnipeds) sightings in temperate/subtropical waters. Alternatively, breeding occurs at temperate/subtropical pelagic waters.
	4	Breeding distribution comprises other large landmasses and/or temperate/subtropical islands, with occasional movement of individuals from these regions to Antarctica/sub-Antarctica.
	5	Breeding distribution comprises other large landmasses, with frequent movement of individuals from these regions to Antarctic/sub-Antarctica.

Based on the variables in Table 2, a group vulnerability score was developed for each Antarctic Fauna group (score for individual criteria can be seen in Table S1 and individuals in each wildlife group are listed in S2 in supplementary file). According to the analysis, the most vulnerable avian group are the gulls and skuas, followed by birds of prey (e.g., hawks, caracaras), terns and shorebirds. For mammals, otariids (fur seals and sea lions) are considered the most vulnerable groups followed by phocids (excluding southern elephant seals) and dolphins (Figure 3).

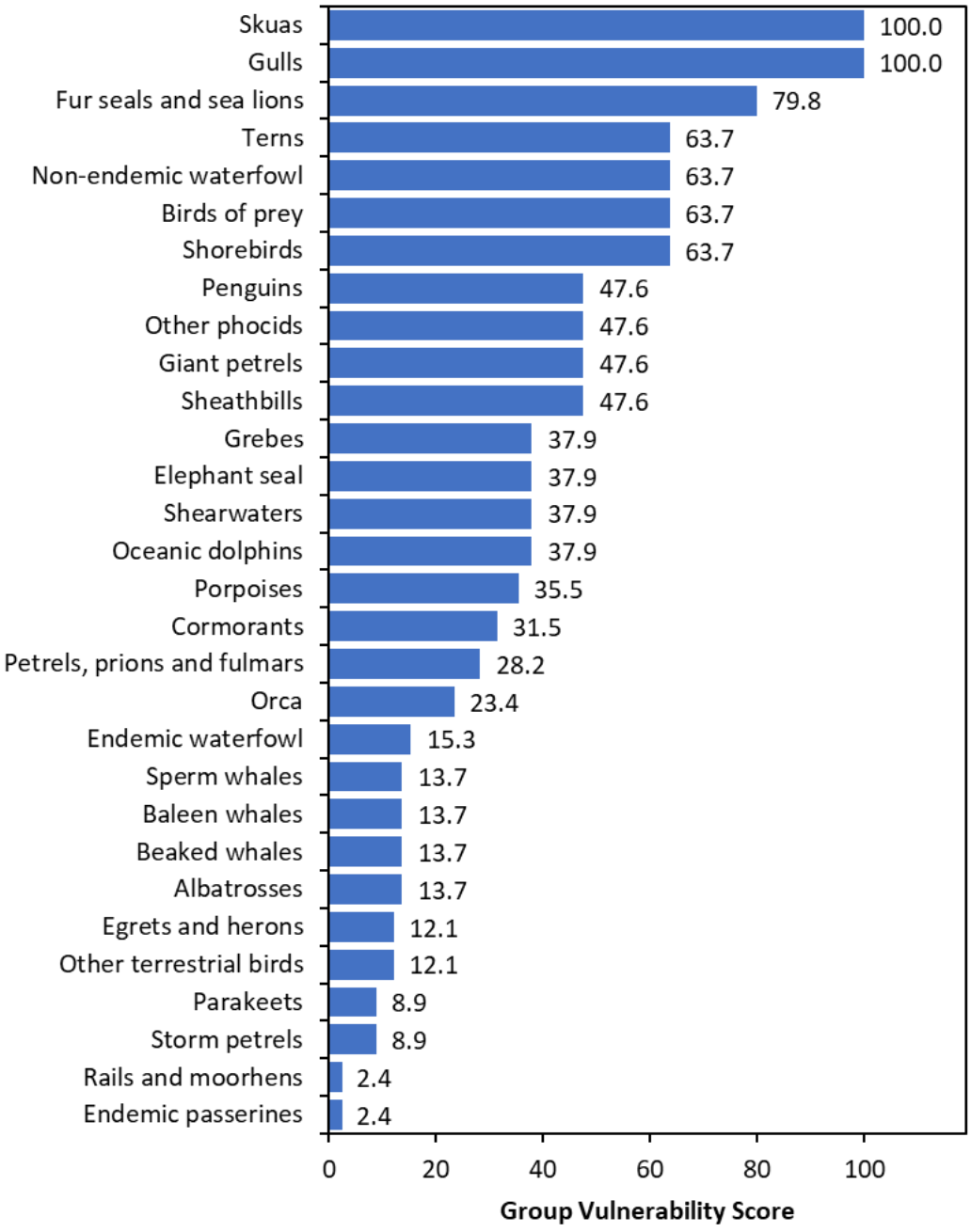

Figure 3. Group Vulnerability Score for Antarctic fauna determined via evaluation of known HPAI susceptibility, host risk behaviours and population connectivity.

4.2 Risk Assessment for Geographical Areas

There are conflicting definitions for the Antarctic territory; for the purpose of this assessment, we refer to the area of the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR), which comprises mainland Antarctica and several sub-Antarctic islands. This region was subdivided into 20 geographical areas (Figure 4 and Table S3; adapted from Vanstreels et al. 2020). Additionally, another eight island groups in the sub-Antarctic/subtropical region were also considered in this assessment: Falklands (Islas Malvinas), Tristan da Cunha Islands, Gough Island, Amsterdam and St Paul Islands, Macquarie Island, Auckland Islands, Campbell Island, Antipodes, and Bounty Islands (Figure 4). As a result, this assessment considered 28 geographical areas (Figure 4 and Table S3).

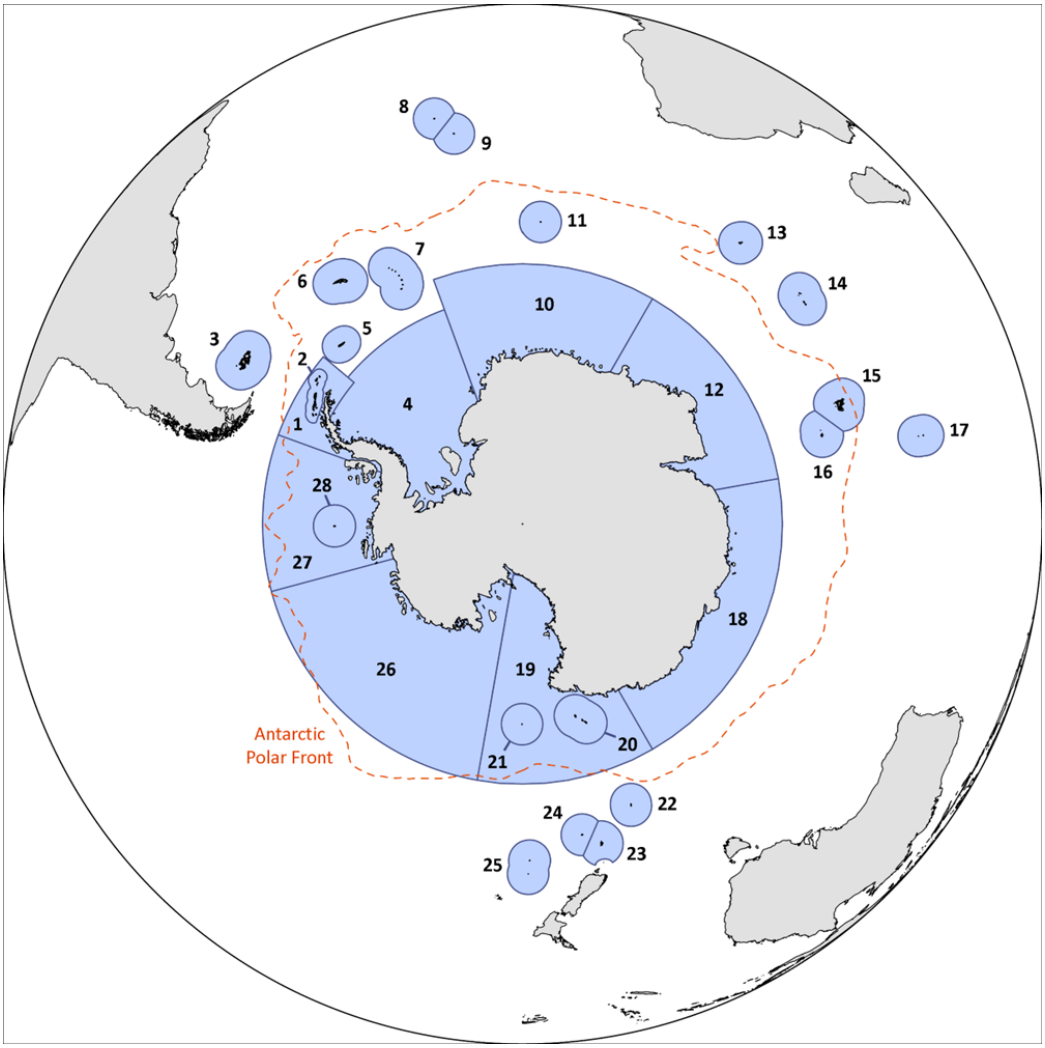

Figure 4. Geographical areas of interest for the biological risk assessment.

Note: (1) Antarctic Peninsula and Palmer Archipelago, (2) South Shetland Islands, (3) Falklands (Islas Malvinas), (4) Antarctica Weddell Sea sector, (5) South Orkney Islands, (6) South Georgia Island, (7) South Sandwich Islands, (8) Tristan da Cunha Islands, (9) Gough Island, (10) Antarctica Atlantic Ocean sector, (11) Bouvet Island, (12) Antarctica Indian Ocean West sector, (13) Prince Edward Islands, (14) Crozet Islands, (15) Kerguelen Islands, (16) McDonald and Heard Islands, (17) Amsterdam and St Paul Islands, (18) Antarctica Indian Ocean East sector, (19) Antarctica Ross Sea sector, (20) Balleny Islands, (21) Scott Island, (22) Macquarie Island, (23) Auckland Islands, (24) Campbell Island, (25) Antipodes and Bounty Islands, (26) Antarctica Pacific Ocean West sector, (27) Antarctica Pacific Ocean East sector, (28) Peter I Island.

For geographical regions, three risk variables (proximity and wildlife exchange, reservoir hosts, and human presence) in a similar semi-quantitative scale (1 to 5) for each of the 28 geographical areas have been evaluated. The objective of this stage of the risk assessment was to compare the characteristics of each area that could predispose to increased vulnerability to HPAI incursions and/or infection persistence. Table 3 presents the criteria used to score these variables and Table S4 presents the scores assigned to each geographical area. The three scores can be multiplied by one another, then scaled to a range from 1 to 100 (subtract minimum, divide by range, and then multiply by 100), resulting in the Area Vulnerability Score (AVS) for each geographical area (Figure 5).

Table 3. Variables and definitions of the scores used to quantify risk for geographical areas.

Variable	Score	Definition
A) Proximity How geographically isolated is the area relative to other large landmasses (South America, Africa, Australia, or New Zealand)?	1	Extremely remote (≥2500 km) and separated by the Antarctic Polar Front.
	2	Extremely remote (≥2500 km) but not separated by the Antarctic Polar Front.
	3	Very remote (1500 – 2500 km).
	4	Remote (750 – 1500 km).
	5	Relatively close (<750 km).
B) Reservoir hosts Is there a substantial population of poultry or waterfowl in the area?	1	Occurrence of Anseriformes or Charadriiformes is limited to vagrants/visitors.
	2	No resident population of Anseriformes, but there is a breeding population of gulls, skuas or sheathbills.
	3	Small resident population of wild Anseriformes (<1000 pairs).
	4	Large resident population of wild Anseriformes (≥1000 pairs).

	5	Presence of poultry or domestic Anseriformes.
C) Human presence How frequent/intense is human presence in the area?	1	Sporadically visited by research expeditions.
	2	Intermittently occupied research station and minimal tourism intensity.
	3	Permanently occupied research station and minimal tourism intensity.
	4	Permanently occupied research station and substantial tourism intensity.
	5	Inhabited by civilian population.
D) Distance to current outbreak Is the area close to regions where HPAI has been detected in the last 12 months?	1	Extremely remote (≥2500 km) relative to recent HPAI detections.
	2	Very remote (1500 - 2500 km) relative to recent HPAI detections.
	3	Remote (750 - 500 km) relative to recent HPAI detections.
	4	Close (200 - 750 km) to recent HPAI detections.
	5	Very close (< 200 km) and/or contiguous to a geographical area with recent HPAI detections.

According to the area vulnerability analysis, the most vulnerable regions to HPAI incursion are the sub-Antarctic Islands between the southernmost tip of South America and the Antarctic Peninsula, with the Falklands (Islas Malvinas) at most risk (Figure 5). This is due to their geographical proximity to other landmasses with current HPAI outbreaks combined with high migratory wildlife exchange. Additionally, this region also houses some of the most vulnerable wildlife groups based on the group vulnerability score. Other sub-Antarctic Islands considered at high risk are South Georgia, Tristan da Cunha and Crozet Islands, South Shetland Islands, followed by the Antarctic Peninsula (Figure 5). In terms of immediate risk, the Falklands (Islas Malvinas) is the region with the most significant immediate risk with a score of 60, due to their proximity to existing outbreaks in South America, especially Tierra del Fuego (Figure 6).

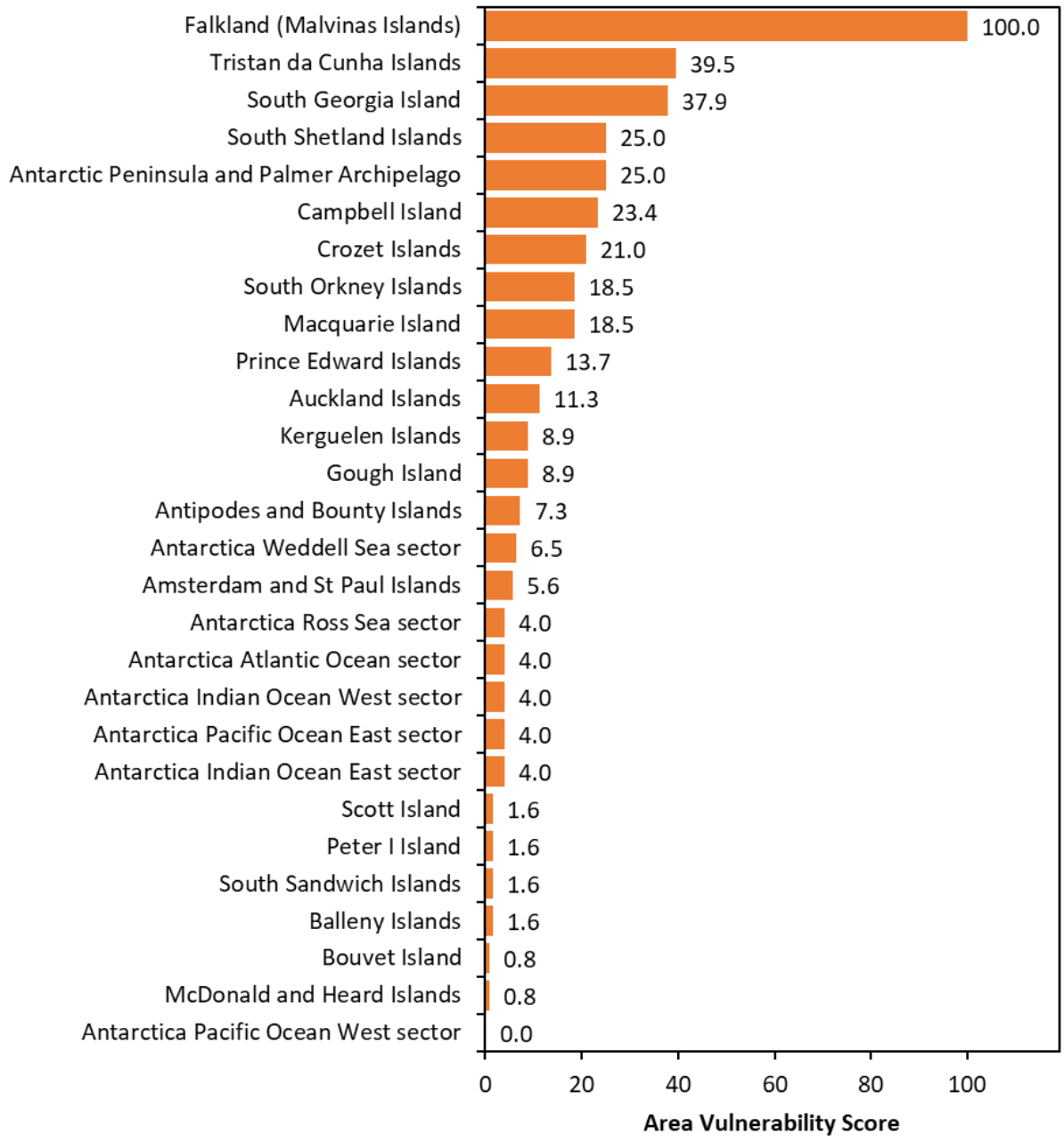

Figure 5. Area Vulnerability Score for Antarctica and sub-Antarctic determined by evaluation of proximity and wildlife exchange, presence of reservoir host and human presence. These correspond to the 28 areas previously identified.

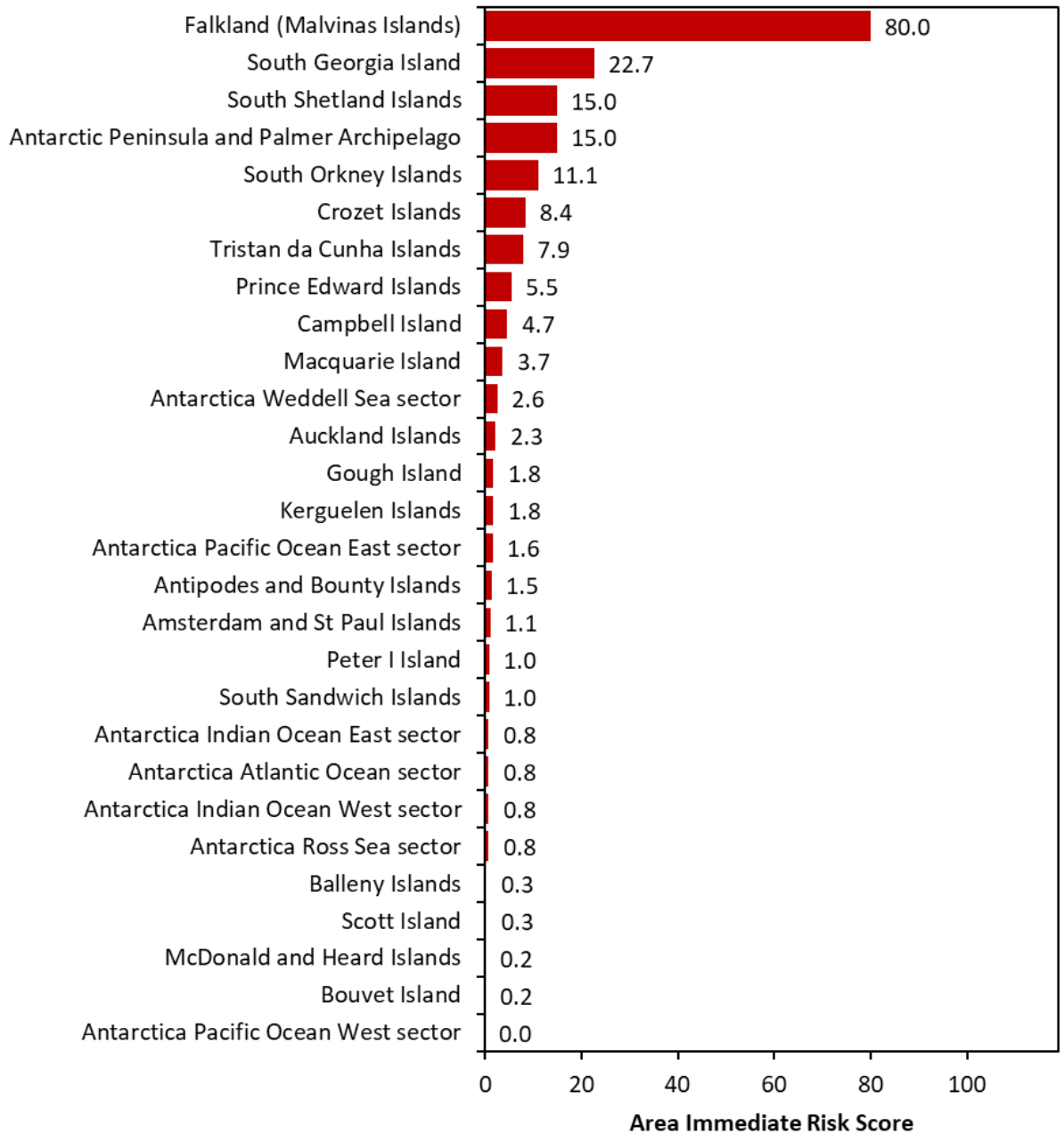

Figure 6. Immediate Risk Score for Antarctica and sub-Antarctic determined by evaluation of (1) proximity and wildlife exchange, (2) presence of reservoir host (3) human presence and (4) distance to current outbreaks. These correspond to the 28 areas previously identified.

5. Recommendations

Although the Antarctic Peninsula and nearby sub-Antarctic islands are at high risk for HPAI this Austral summer, normal research activities can continue providing that (a) basic biosecurity measures are adopted across the board when visiting/working at colonies including wearing of N95 masks, protective glasses (or sunglasses) and gloves, washing and disinfection of boots and clothes after fieldwork, (b) assessment of colonies for signs of sick animals or unusual numbers of carcasses in the area before entering and beginning fieldwork/tourism activity, (c) if there are signs of possible HPAI activity, abort handling of animals, but continue with monitoring at a distance (e.g. counts with binoculars/cameras/drones) and (d) notify disease experts and NAP/IAATO authorities.

5.1 Recommended HPAI Surveillance and Response by National Antarctic Programmes

Under ideal circumstances, the following process is the recommended approach for coordinated surveillance and monitoring for HPAI and LPAI in the region and recommended study species for surveillance purposes.

There are several challenges when it comes to Avian Influenza and infectious disease surveillance that must be taken into consideration. These challenges include: (1) appropriate Personal Protective Equipment (PPE) (sections 5.2.1 and 5.2.2.1.1), (2) collection kits and required preservation media for samples (3) trained personnel (see guidelines in section 6), (4) appropriate facilities if testing is on base, (5) cold chain and shipping, (6) sample collection, export and import permits, (7) capacity and willingness of state/national laboratories to analyse collected samples, and (8) capacity to provide medical care in the event of zoonotic infection and (9) budgetary considerations. Most of this information is covered in the guidelines in section 6.

Please note that recommendations, and importantly, regulations/restrictions of dealing with a class 3 pathogen will differ from country to country. Therefore, before committing to a surveillance or monitoring program, it is paramount that you confirm with your local/national government regulatory authority the rules and regulations regarding working with and importing class 3 pathogens. There are also restrictions on the shipment and importation of genomic material of class 3 pathogens which will differ depending on location of sampling and local/national government regulations.

5.1.1 Surveillance, Monitoring and Baseline testing for HPAI.

Information on whether wildlife populations have been exposed to avian influenza (both LPAI and HPAI) is extremely important information for understanding risk, predicting movement of the virus, and to inform epidemiological investigations.

- Collection of faecal samples during the season is a valuable minimally invasive method for surveying for the presence of AIV in a seemingly healthy population. Fresh faeces can be collected from the ground within a wildlife colony and stored in either DNA/RNA Shield or RNA Later (both media can be stored at room temperature for up to 1 month but will require freezing at – 20 °C or - 80 °C degrees for longer term storage) for genomic testing or in Virus Transport Media (requires specific transport/cold chain). RT-PCR, RT-qPCR or High Throughput Sequencing can be used to detect the presence of avian influenza in a population, although age class and other demographics are unknown. Please note, if using RT-PCR or RT-qPCR for surveillance of the current H5Nx clade 2.3.4.4b, it is imperative to ensure primers used are specific for this clade or the H5 subtype, as these methods will only provide absence/presence based on primer used and cannot differentiate between subtypes if using a general HPAI primer pair.
    - High throughput sequencing such as RNAseq using Illumina or MinION RNA, or cDNA sequencing will provide more in-depth information on the virus detected. These methods can be more time consuming and require specialised equipment/labs but are preferred methods for non-rapid results.
- Testing for antibodies against AIV (or specifically, against H5 strains) pre-, during and post-outbreak is a priority for baseline and surveillance data if possible. Please note, antibody testing requires the collection of blood and should only be collected by trained wildlife biologists or veterinarians and will require animal ethics and permits.
    - *Prior to an outbreak*, information on the antibody status of the target population can inform risk assessment. Indeed, prior exposure to AIV and subsequent antibody response can protect against HPAI infection. Protection levels are difficult to predict as it depends on many factors such as antibody levels (Swayne et al. 2015) and cross-reactivity between subtypes (notably, homotypic exposure confers higher protection levels than heterotypic exposure

(Fereidouni et al. 2009; Berhane et al. 2010; Costa et al. 2010). However, it is safe to assume that a fully naive population (that is, a population that has never been exposed to any AIV) is more likely to be susceptible to HPAI.

- *During or after a suspected outbreak*, as anti-AIV antibodies can likely persist for several months or few years (with variations across populations), the detection of antibodies in a previously antibody-negative population, or detection of antibodies in young individuals (after maternal antibodies have waned) is strong evidence that a pathogen has circulated in a population, even in the absence of pathogen detection or absence of clinical signs (which can be detectable only over a short time-window and therefore could be missed). After an outbreak, the detection of antibody-positive individuals can provide information on the numbers of individuals who survived exposure, informing case-fatality rates and potential for protection against future exposure events.

Biobanking of samples prior to an outbreak is an invaluable resource for risk assessment studies and epidemiological investigations. Such biobanks can build upon existing sample repositories such as avisample.net or be constituted *de novo* by collecting new samples when needed.

Priority species for surveillance studies include potential vector and/or sentinel species, such as predators/scavengers (e.g., skuas, giant petrels, and sheathbills). Visual inspections for signs of higher than usual mortality and unusual behaviour/clinical signs can bring valuable information even when sample collection is not available. As for samples, data collection pre-, during, and post-outbreaks are all valuable. Non-invasive monitoring/surveillance of HPAI via use of drone/visual inspection from distance of colony can also be invaluable where permitted.

5.1.2 Responding to Suspected HPAI Event by National Antarctic Programmes

The information below provides information on how to respond to suspected HPAI events before laboratory confirmation of virus in the region. Please note, no personnel should enter a site with suspected HPAI without appropriate training and PPE to protect personnel from zoonotic infection. Biosecurity around suspected sites should also be increased following the recommendations below to reduce the risk of spreading the virus throughout the infected colony and to other colonies/regions.

5.1.2.1 Report & Communication of HPAI.

Recording and reporting of suspected outbreaks in a timely manner is of upmost importance to ensure a rapid and coordinated response to suspected outbreaks. All suspected outbreaks should be reported as per your organisations “communication plan and chain of command.”

When HPAI is strongly suspected or confirmed in the Antarctic, timely and clear reporting, and communications “up” and out of Antarctica to national contact points and sharing information through to other COMNAP national Antarctic programs and IAATO is important. Establishing reporting and communications lines early so they can assist with decision points and implementation of heightened levels of response is essential. It is recommended that when a suspected case is detected you should.

- Report suspected case through the station Manager up through to national points of contact.
- Activate your programs’ communication plan (COMNAP Communications Plan)

Please keep in mind that WOAH stresses the importance of reporting outbreaks of avian influenza in unusual hosts, as the virus has been increasingly detected in mammals in recent months, a situation that should be monitored. Global monitoring should include any reported changes in the level of avian flu virus pathogenicity to humans. WOAH notes that “High quality of information is key to support early detection and rapid response to potential threats to both animal and public health.” (WOAH HPAI Situation Report 23 June to 13 July 2023, page 5).

5.1.2.2 Suspected HPAI Event

5.1.2.2.1 Personal Protective Equipment

For surveillance and baseline sample collection all personnel should wear appropriate PPE to protect themselves from contamination. Personal Hygiene is also extremely important. Eye wear such as sunglasses should be worn, and N95 masks should be worn if coming into close contact with wildlife.

When responding to suspected disease outbreaks including HPAI, all personnel should wear:

- N95 Face mask or N95 Respirator
- Gloves
    - Double gloving is best.

        - Assists with removal of PPE and reduce self-contamination whilst removing PPE)
    - Gloves must extend over wrist to cover coverall cuff.
        - Use tape to ensure there are no spaces between gloves and coverall.
- Impermeable Coveralls made with fabric/plastic and seams/closures.
- Protective eyewear
- Rubber/washable boots (e.g., muck boots) should be worn.

Please note, PPE must be donned correctly in the proper order before entering a suspected/confirmed site. Removing used PPE is a high-risk process that requires a structured procedure. PPE must be removed slowly and deliberately in the correct sequence to reduce the possibility of self-contamination and disposed of as biohazardous waste.

The World Health Organisation provides online training for correctly donning and removing PPE and it is strongly recommended that all team members complete this training at a minimum before using PPE. Link to Training - https://openwho.org/courses/IPC-PPE-EN?locale=en

5.1.2.2.2 Biosecurity

Biosecurity measures should be increased to prevent the spread of the virus to other wildlife colonies. This includes closure of any sites with suspected outbreaks with access only permitted for the collection of samples to confirm HPAI outbreak or post-mortem examination (carcass must be triple bagged for removal/transport) by highly trained veterinarian.

Increased cleaning and scrubbing of clothing, boots and vehicles following visitation to all wildlife colonies in the vicinity of an outbreak area.

5.1.2.2.3. Collection of samples and storage

During suspected HPAI events, collection of samples should be conducted by trained wildlife biologists or veterinarians to reduce stress on wildlife being sampled and the risk of infection/bite of untrained personnel handling wildlife. For guidelines on sample collection and analysis refer to section 6.

5.1.2.2.4 Confirmation of HPAI

Where possible, we recommend the establishment of certified testing at Antarctic gateway cities with appropriate facilities to safely handle and test biological samples.

If testing facilities are available and approval is granted (check with local authority) to test onsite, the use of genomic testing such as RT-PCR and RT-qPCR are recommended.

5.1.2.2.5 Continued monitoring of a suspected outbreak.

If signs of HPAI are suspected, it is advisable to get an estimate on the number of individuals affected, the coverage of the area and to continue monitoring the site remotely (for example, via binoculars or drone) to monitor for expansion in the number of individuals infected. Drones can be used to monitor colonies from a safe distance and can eliminate the risk of spread of the virus throughout the colony due to absence of humans and can also be used to identify the best path to take if entry to the colony is required. Drone use for such monitoring his highly recommended if highly trained (experience flying in sub-Antarctic/Antarctica and around wildlife colonies), licenced and permitted pilots are available. Guidelines on how to conduct drone surveys are provided in section 6.

5.1.2.2.6. Removal of carcases and culling

Based on information gathered from the extensive outbreak of HPAI throughout the United Kingdom and Europe and guidelines provided by Pearce-Higgins and British Trust for Ornithology, carcasses should only be removed for post-mortem analysis for confirmation of infection or if they are in close proximity to human dwellings. This is to minimise the risk of disturbance and abandonment at the colony and haul out sites to collect carcasses, the spread of virus by moving carcasses, and minimise human movement. There are also increased risks to human health from handling carcasses If carcasses are removed from site and they should be incinerated after post-mortem analysis (conducted using requisite PPE) to ensure complete virus destruction. Another challenge for the sub-Antarctic and Antarctica is the limited resources for appropriate disposal. Carcasses cannot be buried or composted in Antarctica; therefore, they must be incinerated. Due to the lack of incineration facilities in the region, carcass removal should only be considered if appropriate facilities are available and only if carcasses are in close proximity to human dwellings where the risk of human infection is increased. If carcass removal is to be undertaken refer to the guidelines set out in the FAOs Scientific Task Force on Avian Influenza and wild birds' statement (https://www.fao.org/3/cc6936en/cc6936en.pdf).

Culling of wild bird populations in an attempt to control the spread of the virus throughout the region has been shown to have no benefit in wild birds and therefore should not be considered in the region as a method to prevent the spread to other colonies (FAO, 2023).

5.1.3 Recording HPAI outbreaks

The Antarctic Wildlife Health Network will be developing an online database for recording suspected and confirmed outbreaks and surveillance results to enable real time monitoring of HPAI in the sub-Antarctic and Antarctica. The inputted data will be displayed on a map and will be connected to the live risk assessment document to provide up to date information to all operators on the current risk of a HPAI incursion into the different bioregions identified in Figure 4 to assist operators to update their own risk assessments and response plans.

5.1.5 Response Plan

The AWHN recommends a phased response plan be developed for conducting research and tourism activities in relation to potential HPAI this season. A good example plan developed by the British Antarctic Survey can be found here: https://www.comnap.aq/s/United-Kingdom-ATCM45-IP039-2023.pdf

5.2 Recommendations for Visitation at Wildlife Colonies

The recommendations below are for any operator conducting visits to colonies for tourist/non-scientific purposes including base staff from National Antarctic Programmes, Tourism operators, fisheries vessels, or private yachts.

More detailed recommendations for surveillance and responding to potential HPAI outbreaks in the sub-Antarctic and Antarctica can be found in Dewar et al. (preprint).

5.2.1 Before visiting a colony.

- Ensure all equipment (including boots, backpacks, camping equipment markers, bags, and field gear etc.) is cleaned of any soiled material (i.e., soil, faeces) and disinfected before disembarking the boat/leaving base and every time after visiting a colony.
- A trained or experienced guide/research team should survey the colony via Zodiac for coastal colonies or via an elevated position or drone (if permitted) for in-land colonies to look for signs of HPAI, unusual behaviour or clusters of dead individuals.
- If a suspected HPAI event is detected or large numbers of dead individuals are observed (unusual mortality event), all landings in the area should be abandoned and reported to your National Competent Authority and/or IAATO (depending on whether the visit is via members of a National Program or Tourism operator).

- Tourism operators should follow IAATO's guidelines on responding to unusual mortality events.
- Members of National Programs should follow their competent authorities' response plan and communicate suspected sightings to neighbouring bases. IAATO should also be informed if suspected cases are near tourist sites.
- The site should be closed for a minimum of 24 - 48 hrs. After the minimum closure period, the site should be observed from a distance or surveyed via drone (if fully trained and permitted pilot and drone are available). If signs of disease/unusual mortality are still evident or have expanded, the site should be closed for the remainder of the season.
- All base staff/expeditioners should follow their National Antarctic Programmes Avian Influenza Response Plan.

5.2.2 During a Visit

- All visitors should strictly adhere to ATCM general guidelines, IAATO guidelines or any other local requirements (https://documents.ats.aq/recatt/att483_e.pdf), unless permitted for research purposes.
- Avoid sitting/laying down on the ground within a wildlife colony, especially areas that contain faeces and potentially contaminated soil/water.
- **Visitors must never touch wildlife, dead or alive unless they have a permit that allows the handling of wildlife.**

5.2.3 Post-Visit/Biosecurity

- Boot cleaning - all visible faecal and soil material must be removed from boots and all boots cleaned with either 70-90% ethanol, Virkon™ S, F10 or 10% bleach (sodium hypochlorite) and soap following recommendations of WOAH and IUCN 2022 and FAO 2023 guidelines.
- Any clothing with visible soil or faecal contamination must be cleaned off and then disinfected using Virkon S.
- Any gear taken to a site that could have come in contact with the ground, rocks, beach, etc and with visible soil or faecal contamination must be cleaned with soap and disinfected using, Virkon™ S, F10 or 10% bleach (sodium hypochlorite) following recommendations of WOAH and IUCN 2022 and FAO 2023 guidelines until removed.

6. Guidelines for Scientific Surveillance, Testing and Monitoring

6.1. Recommended Training for Personnel for HPAI surveillance and Testing.

Due to HPAI being a class 3 pathogen, the Antarctic Wildlife Health Network recommends National Antarctic Programs establish 'surveillance/monitoring teams' of expeditioners with either skill in the biological sciences, veterinary science, wildlife ecology/biology, microbiology/virology and molecular sciences to collect and analyse samples from Antarctic/sub-Antarctic wildlife for surveillance of HPAI. All members of these teams must have undergone training in the following areas:

- Correct use and removal of appropriate PPE - Training can be obtained from the World Health Organisation (https://openwho.org/courses/IPC-PPE-EN?locale=en) or Local Government Infectious Disease Response Team
- Sample collection techniques
    - How to collect faecal samples (aseptic collection of faeces from the ground - identification of suitable material, and collection), oral and cloacal swabs (please note damage can be done to animals' intestines if done incorrectly, so training is imperative).
    - Collection of samples using aseptic technique
- Movement around colonies with signs of infectious disease to prevent spread.

We recommend National Antarctic Programmes contact their local government Veterinary authorities or Agricultural departments responsible for investigating and managing animal disease outbreaks and biosecurity for assistance with training.

6.2. Sample Collection and Detection for HPAI

6.2.1. HPAI Surveillance

6.2.1.1. Permits & Jurisdiction

All scientific activities involving wildlife will require animal ethics approval and scientific permits for the collection of samples which can include non-invasive collection of samples (for example, substrate sampling). Please check requirements for your jurisdiction with your local Authorising organisation.

Export and import permits may be required for samples collected. As HPAI is a class 3 pathogen, restrictions on its shipping, export and importation are likely to apply, including for DNA/RNA/cDNA. In some jurisdictions, the importation of HPAI or suspected HPAI samples will either be prohibited or require specialised permits. Before collecting samples, you must contact your permitting body and local customs/quarantine authority and arrange all requisite permits. Similarly, if you are wanting to send samples to a certified laboratory, prior arrangements will need to be in place before sending samples. It is advisable to make these arrangements before the start of the season to ensure any samples collected will be tested.

6.2.1.2 Items for Sample collection

We recommend genomic screening for surveillance of HPAI; therefore, samples should be stored in RNA stabilisation agents such as Zymo DNA/RNA Shield or Sigma RNALater. Both preservation **media deactivate the virus** and preserve the RNA for short periods of time at room temperature (~ 1 month). For long term storage (> 2 months) samples should be stored at -20 or -80 degrees Celsius. Please note, ethanol will not preserve RNA or deactivate the virus and is not a suitable storage media for samples to be tested for HPAI. Additionally, when sending samples to a certified laboratory for testing, the laboratory could require samples to be stored in Virus Transport Media which enriches and keeps the virus alive for in-vitro studies. This media has very specific cold chain requirements and must be shipped as soon as possible. For these reasons, Virus Transport Media are not recommended for surveillance programs but for confirmation of outbreaks if using a certified laboratory for HPAI testing.

Items required.

- Preservation media (Recommended media include Zymo DNA/RNA Shield and Sigma RNALater)
- Sterile DNase/RNase free 2 mL screw cap tubes (cryovials for long term storage are preferred)
- Sterile tips or swabs for collection of faeces/scats (preferably individually wrapped swabs and pre-sterilised DNase/RNase free tips)
- Permanent marker pen for recording collection details on tubes (date of collection, location of collection, species sampled (including for substrate sampling)

6.2.1.3 Sample Collection

For surveillance, non-invasive sample collection methods can be used, including collection of fresh scats from the ground, soil and water samples. Place 1 mL of preservation media into the screw cap tubes prior to collecting samples.

For collection of scats, using either a sterile pipette tip or swab, collect fresh scats from the ground and place into a 2 mL tube with 1 mL of preservation media and shake to ensure the sample is covered by preservation media. Record details of collection on the side of the tube with a permanent marker pen.

Note. Do not fill the tube with faecal material, around 1g is sufficient, if you overfill the tube the sample will not be preserved as the sample needs to be immersed in preservation media. Refer to manufacturer's instructions on preservation media to sample ratio. Samples can then be transported back to base at room temperature. RNA can be stored at room temperature for approximately one month and DNA for one year. For longer storage, samples need to be stored at - 20 °C or - 80 °C degrees Celsius.

6.2.1.4. Genomic Screening

For absence/presence studies, RT-qPCR or RT-PCR can be used to detect the virus. For more in-depth studies and identification of subtypes, clade, variant, etc. high throughput sequencing is required.

6.2.1.4 Antibody Testing

Antibody testing is usually conducted on serum samples stored frozen, dried or on filter paper. Such samples are obtained from blood samples collected on heparin- or EDTA-rinsed syringes or needles. Ideally, about 200 microliters of plasma (500 microliters of whole blood) should be collected to allow for subtype specific antibody screening HI. Smaller samples can be used for pan-AIV ELISA (targeting the conserver nucleocapsid protein).
Detection of AIV genetic material by RT-PCR can provide additional information, especially if genetic data can subsequently be obtained.

6.2.2. Detection of HPAI during Suspected Outbreak

6.2.2.1 Items for Sample Collection Kit

Sampling requirements will depend on the method used for sample analysis and available storage facilities (availability of cold chain). However, it is recommended that all bases have the following items available for sample collection and storage.

- Virus Transport Media
    - For maintenance of live virus for testing by Certified Laboratories
- RNA preservation agent
    - Either Zymo DNA/RNA Shield or Sigma RNA Later for genomic screening
- Sterile pipette tip or flocked Swabs
- 2 mL screw cap tubes
- Boxes for storage/transport of tubes

If sending samples to a certified laboratory outside of the Antarctic/sub-Antarctica, samples need to be stored in Virus Transport Media and shipped as soon as possible to ensure survival of the virus for testing. For samples that cannot be shipped as soon as possible after collection and therefore need to be stored for longer periods of time, or for samples collected for genomic testing, these samples should be stored in an RNA preservation media (refer to 6.2.1.2).

6.2.2.2. Collection of Samples

Reminder: Only trained personnel in full PPE should be entering an affected wildlife colony and handling wildlife. All direct sampling of live animals should only occur under appropriate scientific permits (including approved animal ethics permits) and conducted by fully trained staff.

For seabirds, cloacal and oropharyngeal swabs should be collected from live individuals showing signs of infection with swabs placed in preservation media immediately after swabbing. For pinnipeds, nasal swabs should be collected. For live cetaceans, blow samples can be collected by drone using the method outlined in Pirotta et al. 2017. Blow can then be stored in 2 mL tubes with at least 1 mL of preservation media.

If carcasses of seabirds are present, oropharyngeal and cloacal swabs should be collected from as many individuals as possible and post-mortem analysis conducted on a subset of individuals with tissue samples including the brain and lungs collected for testing along with cloaca/rectal

swab and oral swabs. For post-mortem analysis of seabirds, carcasses should be triple bagged on site to prevent spillage of virus. Carcasses should only be collected for post-mortem if a suitable BSL-2 facility is available. Tissue and biological samples should be stored in the appropriate media outlined above depending on required testing.

Post-mortem analysis on cetaceans and pinnipeds will not be possible, unless conducted in the field by trained personnel with experience with post-mortem procedures in cetaceans and pinnipeds. Note. Brain is the best tissue for the detection of HPAI and should be collected, if possible, in conjunction with swabs for mammals (and even dead birds) as the virus is highly neurotropic. However, collection of brain tissues requires experience and expertise particularly when collecting from larger animals. For this reason, unless personnel have experience in post-mortem technique, the recommendation for dead mammals is the collection of respiratory (nasal) and rectal swabs.

6.2.2.3. Detection of Virus

For samples being sent to a certified laboratory outside of Antarctica/sub-Antarctic, samples need to be stored in Virus Transport Media. Prior to shipping, please contact a certified laboratory to make arrangements for receipt of samples and check importation requirements for the region.

For onsite testing, we recommend the following genomic screening methods for HPAI.

RNA extractions

There are a variety of commercial extraction kits available. The appropriate kit will depend on the preservation media used and the type of sequencing being conducted, but viral RNA kits are usually best to target viruses.

RT-PCR/RT-qPCR Based Detection of HPAI

For rapid detection of HPAI presence, we recommend either using RT-PCR or RT-qPCR detection.

6.3. Drone (remotely piloted aircraft) Survey Guidelines

Drones offer an exceptional opportunity to remotely and minimally-invasively survey and monitor populations with suspected and active HPAI outbreaks. Provided that operators have the right

permits and risk assessment in flight, an overflight would give a much wider perspective of the area than from the ground and provides the capability to monitor large or inaccessible areas. An important benefit regarding the use of drones enables operators to remotely monitor active outbreaks without direct contact with infected animals and therefore avoiding exposure of humans to a possible zoonotic pathogen, and/or possibly contributing to the spread of virus. Especially useful would-be videos to share with national permitting authorities as to mass mortality or behaviours. Other benefits include less disturbance to the animals as this is a less invasive approach, is a faster and less expensive method to survey the area and can be conducted even if conditions are too rough to land at a site (Dickens et al 2021; Harris et al 2019). Development of a program of drone surveys can also enable the collection of repeated observations and thereby contribute knowledge to significant gaps in our understanding on rate of spread and movement of virus throughout a colony and the impact on a population. During the 2022 outbreak in the UK, researchers were able to use drones to survey a remote gannet colony from a boat (see link to Dr Hart's Presentation below). From the video footage the team were able to differentiate between healthy and infected individuals and clearly identify carcasses from live birds. Dr Tom Hart's presentation on the use of cameras and drones for seabird monitoring at the Seabird Conference can be viewed at this link https://youtu.be/M_lVvySSFRE, as well as a guide to best practice for drone use around seabirds (Edney et al, in press).

If HPAI is suspected, it is important to be especially cautious in flying as in the event of a crash, trying to recover an aircraft would be inadvisable due to biosecurity concerns. The variability of weather conditions can make it difficult to plan exact flight times ahead of time.

As of 2018, several NAPs have published guidelines for the operation of RPAs in the Antarctic Treaty Area (ATA) alongside the handbook produced by COMNAP (2022). These include Germany, Poland, New Zealand, the US (Harris et al. 2019) and IAATO (2018). Since then, Australia and the UK also have produced guidelines. An infographic showing the guidelines used by Penguin Watch for the use of drones around seabird colonies is shown in Figure 7. Any mission using RPAs should only be conducted under the strictest conditions applying best practice policies and always using a precautionary approach keeping in mind the differences in wildlife responses (SCAR 2019). In 2018, the Committee for Environmental Protection (CEP) recommended that the

Antarctic Treaty Consultative Meeting (ATCM) adopt Environmental Guidelines for operation of Remotely Piloted Aircraft Systems (RPAS) in Antarctica (Resolution 4 (2018) Annex). The Guidelines are targeted at small to medium-sized drones (≤25 kg in weight) and aim to assist in undertaking Environmental Impact Assessments for activities involving drones and to aid decision making for use of drones through provision of guidance. These Guidelines address issues to be considered in the planning, operational and post-flight phases of using drones in Antarctica. Please note, the flying of drones in the sub-Antarctic and Antarctica are strictly prohibited and cannot be conducted without appropriate licences, permits and training. All permits are issued by your Competent Authority.

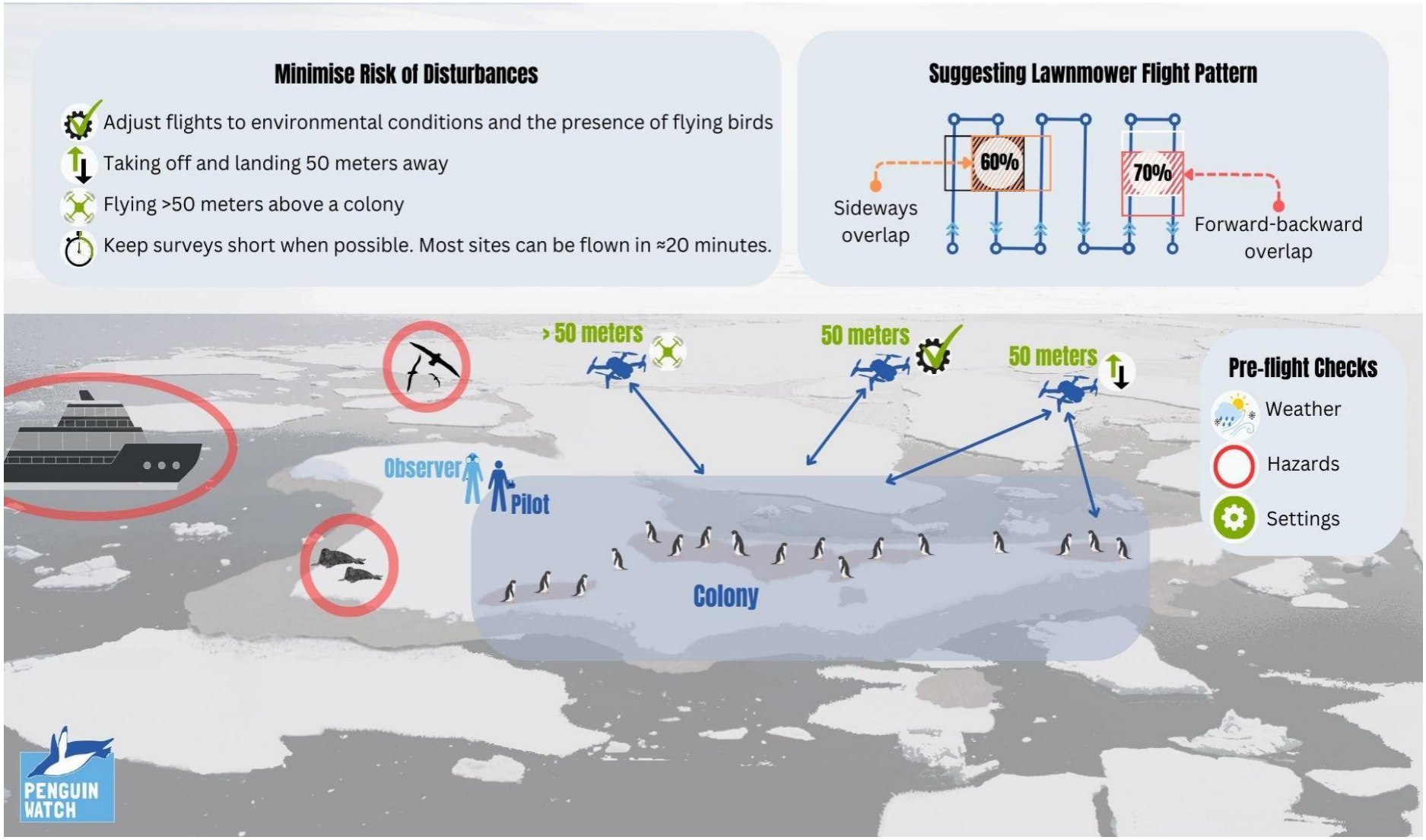

Figure 7. Guidelines for drone surveys designed by Penguin Watch

7. Recommendations for HPAI Research

There is limited data available on the effects, spread and movement of HPAI in wild seabirds and mammals, especially in the polar regions. Key knowledge gaps and areas for research include (but not limited to) the following:

- Identification of susceptible, vector and reservoir species
- Pathophysiology and Histopathology of the virus in seabirds and mammals
- Increased research into the movements of vector species and potential spreaders.
    - Limited data is available on potential vector and spreader species and age classes. Increased research into the movement of these species (such as skuas, giant petrels, sheathbills, and prospecting juveniles) is essential for understanding the pathways for introduction of this virus and other emerging pathogens and the potential spread between colonies (Boulinier 2023).
- Virulence
- Pathways into the sub-Antarctic/Antarctica
- Movement within and between wildlife colonies
- Evidence of past LPAI infections and current presence of HPAI
- Genomic lineage and origin

It is recommended that coordinated scientific studies in these areas be established where possible to learn more about this virus and future emerging infection disease outbreaks.

In addition, ongoing disease surveillance programs should be established to identify new and emerging pathogens into the region as increased infectious disease in wildlife are predicted to increase globally due to the effects of climate change (Cohen et al 2020).

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9. Appendices and Supplementary Material

Table S1. Scoring of wildlife groups (according to the criteria defined in Table 2).

Wildlife group	A) Known susceptibility	B) Risk behaviours	C) Population connectivity	Group Vulnerability Score (GVS)
Albatrosses	2	3	3	13.7
Giant petrels	3	5	4	47.6
Shearwaters	4	4	3	37.9
Petrels, prions and fulmars	4	3	3	28.2
Storm petrels	2	2	3	8.9
Penguins	5	4	3	47.6
Gulls	5	5	5	100
Terns	5	4	4	63.7
Skuas	5	5	5	100

Sheathbills	3	5	4	47.6
Shorebirds	5	4	4	63.7
Cormorants	5	4	2	31.5
Endemic waterfowl	5	4	1	15.3
Non-endemic waterfowl	5	4	4	63.7
Grebes	4	3	4	37.9
Egrets and herons	4	1	4	12.1
Rails and moorhens	4	1	1	2.4
Birds of prey	5	4	4	63.7
Endemic passerines	4	1	1	2.4
Parakeets	4	3	1	8.9
Other terrestrial birds	4	1	4	12.1

Fur seals and sea lions	5	5	4	79.8
Elephant seal	3	4	4	37.9
Earless seals	5	4	3	47.6
Baleen whales	2	3	3	13.7
Sperm whales	2	3	3	47.6
Beaked whales	3	2	3	37.9
Orca	2	5	3	28.2
Oceanic dolphins	5	4	3	8.9
Porpoises	5	3	3	47.6

Table S2. List of species in each wildlife group.

Wildlife group	Species
Albatrosses	*Diomedea epomophora, Diomedea exulans, Phoebetria fusca, Phoebetria palpebrata, Thalassarche carteri, Thalassarche chlororhynchos, Thalassarche chrysostoma, Thalassarche melanophris, Thalassarche salvini*
Giant petrels	*Macronectes giganteus, Macronectes halli*
Shearwaters	*Ardenna carneipes, Ardenna gravis, Ardenna grisea, Puffinus assimilis, Puffinus elegans*
Petrels, prions and fulmars	*Aphrodroma brevirostris, Daption capense, Fulmarus glacialoides, Halobaena caerulea, Pachyptila belcheri, Pachyptila crassirostris, Pachyptila desolata, Pachyptila macgillivrayi, Pachyptila salvini, Pachyptila turtur, Pachyptila vittata, Pagodroma nivea, Pelecanoides georgicus, Pelecanoides urinatrix, Procellaria aequinoctialis, Procellaria cinerea, Procellaria conspicillata, Pterodroma incerta, Pterodroma lessonii, Pterodroma macroptera, Pterodroma mollis, Thalassoica antarctica*
Storm petrels	*Fregetta tropica, Garrodia nereis, Oceanites oceanicus*

Penguins	*Aptenodytes forsteri, Aptenodytes patagonicus, Eudyptes chrysocome, Eudyptes chrysolophus, Eudyptes filholi, Eudyptes moseleyi, Eudyptes schlegeli, Eudyptes sclateri, Megadyptes antipodes, Pygoscelis adeliae, Pygoscelis antarcticus, Pygoscelis papua*
Gulls	*Larus dominicanus, Larus maculipennis, Larus novaehollandiae, Larus scoresbii*
Terns	*Sterna hirundinacea, Sterna striata, Sterna virgata, Sterna vittata*
Skuas	*Catharacta antarctica, Catharacta maccormicki*
Sheathbills	*Chionis albus, Chionis minor*
Shorebirds	*Charadrius bicinctus, Charadrius falklandicus, Charadrius modestus, Coenocorypha aucklandica, Gallinago magellanica, Haematopus ater, Haematopus leucopodus*
Cormorants	*Leucocarbo atriceps, Leucocarbo campbelli, Leucocarbo colensoi, Leucocarbo magellanicus, Leucocarbo purpurascens, Leucocarbo ranfurlyi, Leucocarbo verrucosus*
Endemic waterfowl	*Anas aucklandica, Anas eatoni, Anas flavirostris, Anas nesiotis*

Non-endemic waterfowl	*Anas georgica, Anas superciliosa, Chloephaga hybrida, Chloephaga picta, Chloephaga rubidiceps, Coscoroba coscoroba, Cygnus melancoryphus, Lophonetta specularioides, Mareca sibilatrix, Spatula cyanoptera, Spatula versicolor, Tachyeres brachypterus*
Grebes	*Podiceps occipitalis, Rollandia rolland*
Egrets and herons	*Ardea alba, Bubulcus ibis, Nycticorax nycticorax*
Rails and moorhens	*Gallinula comeri, Laterallus rogersi, Lewinia muelleri*
Birds of prey	*Asio flammeus, Caracara plancus, Cathartes aura, Falco novaeseelandiae, Falco peregrinus, Geranoaetus polyosoma, Phalcoboenus australis*
Endemic passerines	*Anthus antarcticus, Nesospiza acunhae, Nesospiza questi, Nesospiza wilkinsi, Rowettia goughensis, Turdus eremita*
Parakeets	*Cyanoramphus auriceps, Cyanoramphus hochstetteri, Cyanoramphus novaezelandiae, Cyanoramphus unicolor*

Other terrestrial birds	*Anthornis melanura, Anthus correndera, Anthus novaeseelandiae, Cinclodes antarcticus, Cistothorus platensis, Hirundo neoxena, Leistes loyca, Melanodera melanodera, Muscisaxicola maclovianus, Petroica macrocephala, Prosthemadera novaeseelandiae, Spinus barbatus, Troglodytes cobbi, Turdus falcklandii, Urodynamis taitensis*
Fur seals and sea lions	*Arctocephalus australis, Arctocephalus forsteri, Arctocephalus gazella, Arctocephalus tropicalis, Otaria flavescens, Phocarctos hookeri*
Southern elephant seal	*Mirounga leonina*
Other Phocids	*Hydrurga leptonyx, Leptonychotes weddellii, Lobodon carcinophaga, Ommatophoca rossii*
Baleen whales	*Balaenoptera bonaerensis, Balaenoptera borealis, Balaenoptera musculus, Balaenoptera physalus, Caperea marginata, Eubalaena australis, Megaptera novaeangliae*
Sperm whales	*Physeter macrocephalus*
Beaked whales	*Berardius arnuxii, Hyperoodon planifrons, Mesoplodon bowdoini, Mesoplodon grayi, Mesoplodon layardii, Tasmacetus shepherdi, Ziphius cavirostris*

Orca	*Orcinus orca*
Oceanic dolphins	*Cephalorhynchus commersonii, Globicephala melas, Lagenorhynchus cruciger, Lagenorhynchus obscurus, Lissodelphis peronii*
Porpoises	*Phocoena dioptrica*

Table S3. Summary of geographical areas of interest and their corresponding CCAMLR subareas.

Region	Geographical area	CCAMLR
Antarctic (south of 60°S)	Antarctic Peninsula and Palmer Archipelago	48.1
	South Shetland Islands	48.1
	South Orkney Islands	48.2
	Antarctica Weddell Sea sector	48.5
	Antarctica Atlantic Ocean sector	48.6
	Antarctica Indian Ocean West sector	58.4.2–4
	Antarctica Indian Ocean East sector	58.4.1
	Antarctica Ross Sea sector	88.1
	Antarctica Pacific Ocean West sector	88.2
	Antarctica Pacific Ocean East sector	88.3
	Balleny Islands	88.1
	Scott Island	88.1

	Peter I Island	88.3
Subantarctic sensu stricto (south of Antarctic Polar Front)	South Georgia Island	48.3
	South Sandwich Islands	48.4
	Bouvet Island	48.6
	Kerguelen Islands	58.5.1
	McDonald and Heard Islands	58.5.2
Subantarctic sensu lato	Prince Edward Islands	58.7
	Crozet Islands	58.6
	Falklands (Islas Malvinas)	not applicable
	Tristan da Cunha Islands	
	Gough Island	
	Amsterdam and St Paul Islands	
	Macquarie Island	
	Auckland Islands	
	Campbell Island	

Antipodes and Bounty Islands

Table S4. Scoring of geographical areas (according to the criteria defined in Table 4).

Geographical area	A) Proximity and wildlife exchange	B) Reservoir hosts	C) Human presence	D) Distance to current outbreak*	Area Vulnerability Score (AVS)	Area Present Risk Score (APRS)*
Antarctic Peninsula	4	2	4	3	25.0	15.0
South Shetland Islands	4	2	4	3	25.0	15.0
South Orkney Islands	4	2	3	3	18.5	11.1
Antarctica Weddell Sea sector	3	1	3	2	6.5	2.6
Antarctica Atlantic Ocean sector	1	2	3	1	4.0	0.8
Antarctica Indian Ocean West sector	1	2	3	1	4.0	0.8
Antarctica Indian Ocean East sector	1	2	3	1	4.0	0.8

Antarctica Ross Sea sector	1	2	3	1	4.0	0.8
Antarctica Pacific Ocean West sector	1	1	1	1	0.0	0.0
Antarctica Pacific Ocean East sector	3	2	1	2	4.0	1.6
Balleny Islands	3	1	1	1	1.6	0.3
Scott Island	3	1	1	1	1.6	0.3
Peter I Island	3	1	1	3	1.6	1.0
South Georgia Island	3	4	4	3	37.9	22.7
South Sandwich Islands	3	1	1	3	1.6	1.0
Bouvet Island	1	2	1	1	0.8	0.2
Kerguelen Islands	1	4	3	1	8.9	1.8
McDonald and Heard Islands	1	2	1	1	0.8	0.2
Prince Edward Islands	3	2	3	2	13.7	5.5
Crozet Islands	3	3	3	2	21.0	8.4

Falklands (Islas Malvinas)	5	5	5	4	100.0	80.0
Tristan da Cunha Islands	2	5	5	1	39.5	7.9
Gough Island	2	2	3	1	8.9	1.8
Amsterdam and St Paul Islands	2	2	2	1	5.6	1.1
Macquarie Island	4	2	3	1	18.5	3.7
Auckland Islands	5	3	1	1	11.3	2.3
Campbell Island	5	3	2	1	23.4	4.7
Antipodes and Bounty Islands	5	2	1	1	7.3	1.5